# The GABA Excitatory/Inhibitory Shift in Brain Maturation and Neurological Disorders

Yehezkel Ben-Ari, Ilgam Khalilov, Kristopher T. Kahle and Enrico Cherubini

*Neuroscientist* published online 30 April 2012
DOI: 10.1177/1073858412438697

# The GABA Excitatory/Inhibitory Shift in Brain Maturation and Neurological Disorders

The Neuroscientist
XX(X) 1–20

DOI: 10.1177/1073858412438697
http://nro.sagepub.com
SAGE

**Yehezkel Ben-Ari[1], Ilgam Khalilov[1], Kristopher T. Kahle[2], and Enrico Cherubini[3]**

## Abstract

Ionic currents and the network-driven patterns they generate differ in immature and adult neurons: The developing brain is not a "small adult brain." One of the most investigated examples is the developmentally regulated shift of actions of the transmitter GABA that inhibit adult neurons but excite immature ones because of an initially higher intracellular chloride concentration $[Cl^-]_i$, leading to depolarizing and often excitatory actions of GABA instead of hyperpolarizing and inhibitory actions. The levels of $[Cl^-]_i$ are also highly labile, being readily altered transiently or persistently by enhanced episodes of activity in relation to synaptic plasticity or a variety of pathological conditions, including seizures and brain insults. Among the plethora of channels, transporters, and other devices involved in controlling $[Cl^-]_i$, two have emerged as playing a particularly important role: the chloride importer NKCC1 and the chloride exporter KCC2. Here, the authors stress the importance of determining how $[Cl^-]_i$ is dynamically regulated and how this affects brain operation in health and disease. In a clinical perspective, agents that control $[Cl^-]_i$ and reinstate inhibitory actions of GABA open novel therapeutic perspectives in many neurological disorders, including infantile epilepsies, autism spectrum disorders, and other developmental disorders.

## Keywords

GABA, excitation/inhibition, neonatal neurons, epilepsies, bumetanide, NKCC1, KCC2, phenobarbital, diazepam, GABA-acting antiepileptic drugs

The aims of this review are to provide a background on the roles of GABAergic signaling in brain maturation and in neurological disorders. GABA, the major inhibitory transmitter, plays a crucial role in controlling neuronal excitability and a wide range of behaviorally relevant oscillations in the brain. GABAergic signaling is unique in that the polarity of its actions depends in part on the intracellular concentration of chloride $[Cl^-]_i$ that is highly labile, leading to depolarizing and even excitatory actions in certain conditions. The initial thrive to these studies was the discovery that during brain maturation, $[Cl^-]_i$ levels are intrinsically higher than in adult neurons with a developmental shift of the actions of GABA. Curiously, a return to an immature state in terms of $[Cl^-]_i$ occurs after seizures, spinal cord lesions, and other pathological conditions. The mechanisms underlying chloride accumulation in immature and pathological neurons are beginning to be unravelled with a different efficacy of chloride cotransporters—notably, NKCC1 and KCC2—that respectively import and export chloride. If GABA depolarizes and excites pathological neurons, then agents that reduce $[Cl^-]_i$ may also be of therapeutic value. Here, we discuss these issues in parallel, stressing the importance of the dynamic plasticity of intracellular chloride levels. We also review the therapeutic issues, relying on experimental and clinical evidence on actions of diuretics in infantile epilepsies but also in autism spectrum disorders (ASDs).

## Developmental Sequence of GABA Actions

### *GABA Depolarizes and Excites Immature Neurons*

Upon binding, GABA triggers conformational changes in $GABA_A$ receptors ($GABA_ARs$), which function as ligand-gated chloride channels, to facilitate the passive

[1]INMED, INSERM unit 901 and Neurochlore, Marseille, France
[2]Department of Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
[3]International School for Advanced Studies (SISSA), Trieste, Italy

**Corresponding Author:**
Yehezkel Ben-Ari, Founder and Honorary Director of INMED & CEO Neurochlore, INMED campus scientifique de Luminy, 163 route de luminy, 13273 Marseilles Cedex 09, France
Email: ben-ari@inmed.univ-mrs.fr

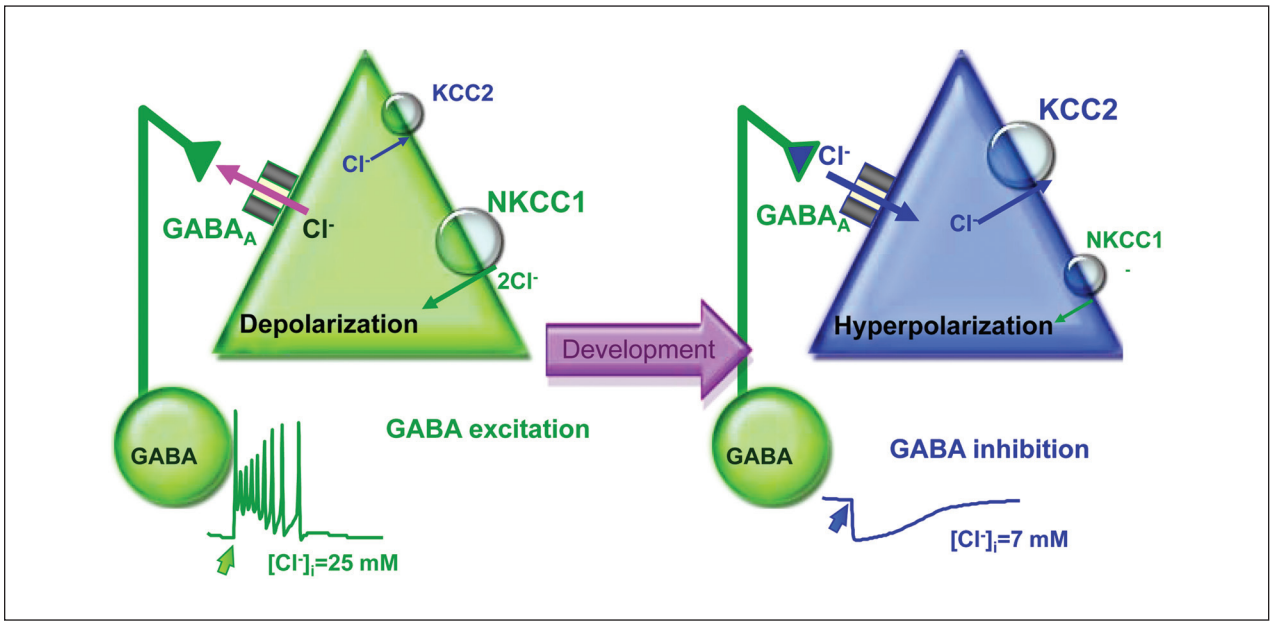


**Figure 1.** The GABA shift of actions is determined in part by a sequential development of two major chloride cotransporters, NKCC1 and KCC2. The former, which imports chloride, but not the latter, which exports it, is present in utero already. In more adult neurons, KCC2 fully operates, whereas NKCC1 is less active, leading to a higher accumulation of chloride in immature neurons (Ben-Ari and others 2007; also see Yamada and others 2004).

inflow or outflow of $Cl^-$ ions depending on the neuron's equilibrium potential for chloride ($E_{Cl-}$). In adult neurons, the level of intracellular chloride $[Cl^-]_i$ is relatively low, with the reversal potential for $Cl^-$ near the resting membrane potential ($V_m$); thus, minor changes in $[Cl^-]_i$ can significantly affect the strength and even polarity of GABA's effects. When $[Cl^-]_i$ is high, $E_{Cl-}$ is positive relative to $V_m$; $GABA_AR$ activation results in net $Cl^-$ efflux and neuronal depolarization. When $[Cl^-]_i$ is low, $E_{Cl}$ is negative relative to $V_m$, and $GABA_AR$ activation triggers $Cl^-$ influx and hyperpolarization (Figure 1). The likelihood of action potential generation is increased with depolarization and decreased with hyperpolarization. Generally, low $[Cl^-]_i$ facilitates GABA-mediated *inhibition*, whereas high $[Cl^-]_i$ facilitates GABA-mediated *excitation*. However, GABA can depolarize and still inhibit targeted cells via its shunting action (Mohajerani and Cherubini 2005; Banke and McBain 2006).

GABAergic signals operate early, depolarize, and increase intracellular calcium, acting as a major source of excitatory drive. Thus, when neurons are still migrating and before synapses have been formed, GABA is released in a calcium- and *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)–independent way by growth cones and astrocytes and diffused away to activate in a paracrine fashion extrasynaptic receptors (Demarque and others 2002) and modulate neuronal migration (Demarque and others 2002; Komuro and Rakic 1993; Manent and others 2006; Wang DD and others 2003; Young and others 2010). The absence of an efficient uptake system enables GABA to accumulate in the extracellular space and reach a concentration that is sufficient to exert its depolarizing and excitatory effects on distal neurons. Blocking this depolarizing action in utero heavily affects migration and circuit formation, indicating that GABA modulated these and many other functions at an early stage (Manent and others 2005; Cancedda and others 2007; Wang DD and Kriegstein 2010). In the hippocampus of rodents and primates (in utero), GABAergic signals mature and operate before glutamatergic ones, and this correlates with the level of dendritic arborization (Tyzio and others 1999; Khazipov and others 2001; Ben Ari and others 2007). Three stages of develoment can be identified: 1) silent neurons with no apical dendrites without functional synapses, 2) GABA-only neurons with small apical and not basal dendrites that express GABA but not glutamate postsynaptic currents (PSCs), and 3) GABA and glutamate neurons with extensive apical and basal dendrites that generate both GABA and glutamate PSCs (Figure 2). This sequence is also respected in GABAergic interneurons, albeit earlier and thus in utero, indicating that interneurons provide all the activity at an early stage (Ben Ari and others 2004; Gozlan and Ben Ari 2003). The vast majority of interneurons have already fully operative synapses at a time when most pyramidal cells are silent (Ben

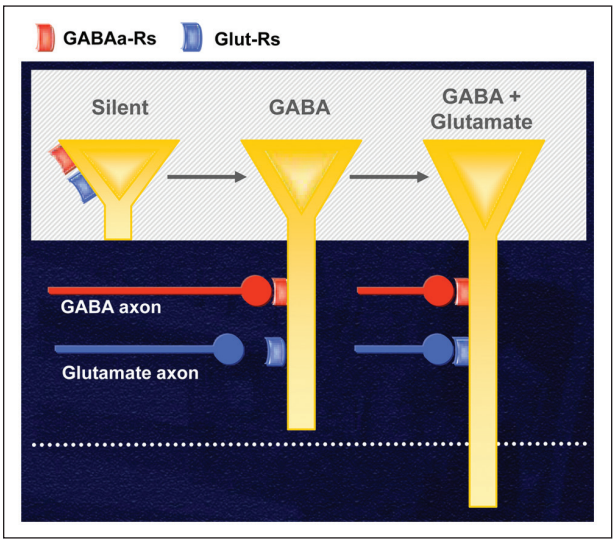


**Figure 2.** The GABA glutamate developmental sequence in the hippocampus pyramidal neurons were patch-clamp recorded at P0, their GABA and glutamatergic postsynaptic currents (PSCs) recorded, and the neurons subsequently reconstructed morphologically after intracellular injection of biocytine. Note that at the same age, most pyramidal neurons (80%) are silent with no GABA and glutamate PSCs and no dendritic arbor. Approximately 10% of neurons have a small apical dendrite and GABA but not glutamate PSCs, and the remaining neurons have both GABA and glutamate PSCs and large apical dendrites that reach the distal lacunosum moleculare region. Clearly, GABAergic synapses are first formed as soon as neurons have an apical dendrite, with the formation of glutamatergic ones requiring apical dendrites that have reached the upper part of the molecular layer. Modified from Tyzio and others (1999); similar observations were made in both GABAergic interneurons (Hennou and others 2002) and primate hippocampal neurons in utero (R. Khazipov, unpublished data, 2001).

Ari and others 2007). Therefore, GABAergic neurons provide the early source of activity in otherwise silent networks.

*How are the excitatory actions of GABA generated?* In many brain structures, $E_{GABA}$ is below the spike threshold, requiring other devices to trigger sodium spikes. GABAergic PSCs trigger the activation of voltage-gated currents that enable reaching the spike threshold (Valeeva and others 2010; Cherubini and others 2011). As the spike threshold differs in different types of immature neurons, GABA will depolarize some neurons and excite others (Rheims and others 2008; also see Banke and McBain 2006). GABA can also trigger calcium currents and remove the voltage-dependent magnesium block from *N*-methyl-d-aspartate (NMDA) receptors (Leinekugel and others 1997; Ben-Ari and others 1997), thus allowing calcium entry and activation of second messengers and a wide range of trophic actions (Leinekugel and others 1997; Garaschuk and others 2000; Yuste and others 1995). It has been suggested that this sequence enables neurons at very different development stages to fire together because of the long-lasting duration of the currents generated by GABA and NMDA receptors in immature neurons (Ben-Ari 2001, 2002; Cherubini and others 2011).

## *A Unique Polarity Shift during Delivery*

The dramatic and transient hyperpolarizing shift during delivery constitutes a particularly striking illustration of the biological relevance of the GABA developmental sequence (Tyzio and others 2006; see Figure 3). Using single-channel recordings to determine $E_{GABA}$ from embryonic to postnatal periods, a dramatic reduction of $[Cl^-]_i$ has been observed shortly before and after delivery. This shift, which is mediated by oxytocin receptors that triggers labor, is abolished by a selective oxytocin receptor antagonist. Oxytocin-mediated reduction of $[Cl^-]_i$ exerts neuroprotective actions, reducing the severity of anoxic episodes (Tyzio and others 2006). It also exerts analgesic actions, increasing the threshold of pain reactions and reducing electrical signals in pain pathways (Mazzuca and others 2011). Both effects are mediated by alterations of $[Cl^-]_i$ and mimicked by diuretics that reduce $[Cl^-]_i$. Interestingly, vaginal-delivered babies are less sensitive to pain than babies delivered by cesarean section (Bergqvist and others 2009; see Figure 4). These observations have important clinical implications, considering the importance of pain and anoxic episodes in long-term sequels of delivery-related insults. They also suggest that the regulation of GABA polarity during delivery is an important biological signal.

## *Early Correlated Network Activity in the Immature Hippocampus*

Correlated neuronal activity occurring at late embryonic, early postnatal stages of development represents a hallmark of developmental circuits, well preserved during evolution (Ben-Ari and others 2007). It can be detected in almost every brain structure, including the retina (Feller and others 1997; Galli and Maffei 1988), neocortex (Yuste and Katz 1991; Owens and others 1996; Dammerman and others 2000; Maric and others 2001), hippocampus (Ben-Ari and others 1989), hypothalamus (Chen and others 1996), cerebellum (Eilers and others 2001), spinal cord (Ritter and others 1999; Wang J and others 1994), and many other brain regions (Ben Ari and others 2007). This early synchronized activity, which may differ in its specific pattern among different brain regions, is crucial for synaptic wiring and refinement of local neuronal circuits according to the Hebbian rule that "neurons that fire together wire together."

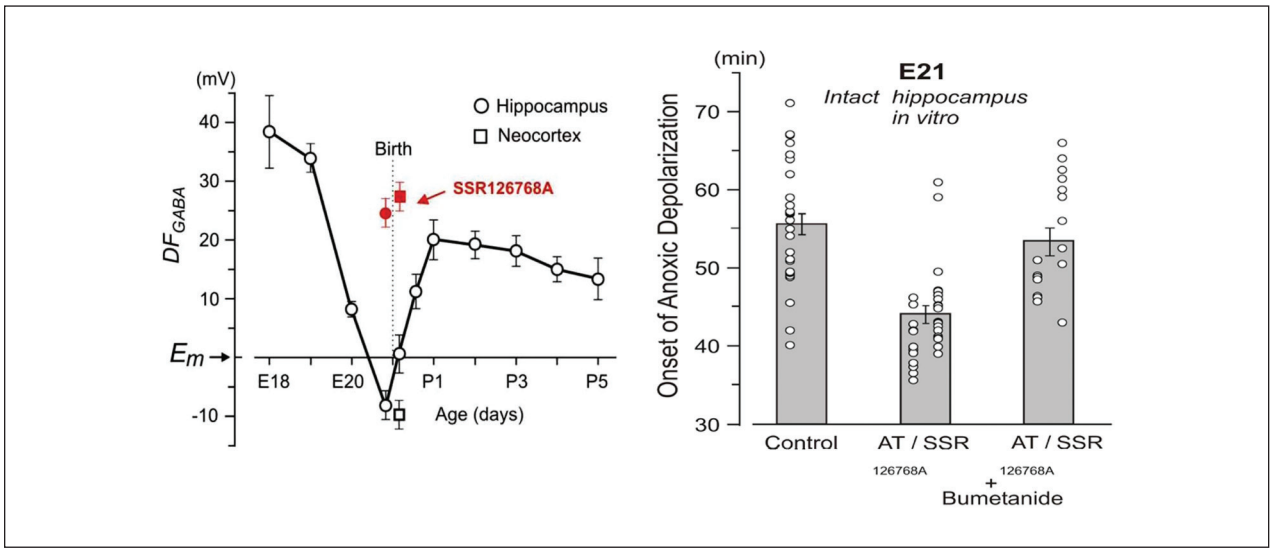


**Figure 3.** A dramatic transient shift of GABA actions during the delivery period. (A) The alterations of the driving force of GABA (DF GABA) determined with single *N*-methyl-d-aspartate (NMDA) and GABA channel recordings are depicted on the ordinates and the age in the abscissa. Note the transient abrupt loss of intracellular chloride to very low values that are never observed afterward. Note that administration of an antagonist of oxytocin receptors alleviates this shift, indicating that this is mediated by oxytocin signals; in keeping with this, the antagonist has no effects two days later when endogenous oxytocin levels are low. (Tyzio and others 2006). (B) The onset of anoxic depolarization obtained by long-lasting anoxic episodes on intact hippocampi in vitro is faster when oxytocin receptors have been blocked. Therefore, oxytocin, by reducing ongoing activity and intracellular chloride levels, also acts as a neuroprotective agent. Adapted from Tyzio and others (2006).

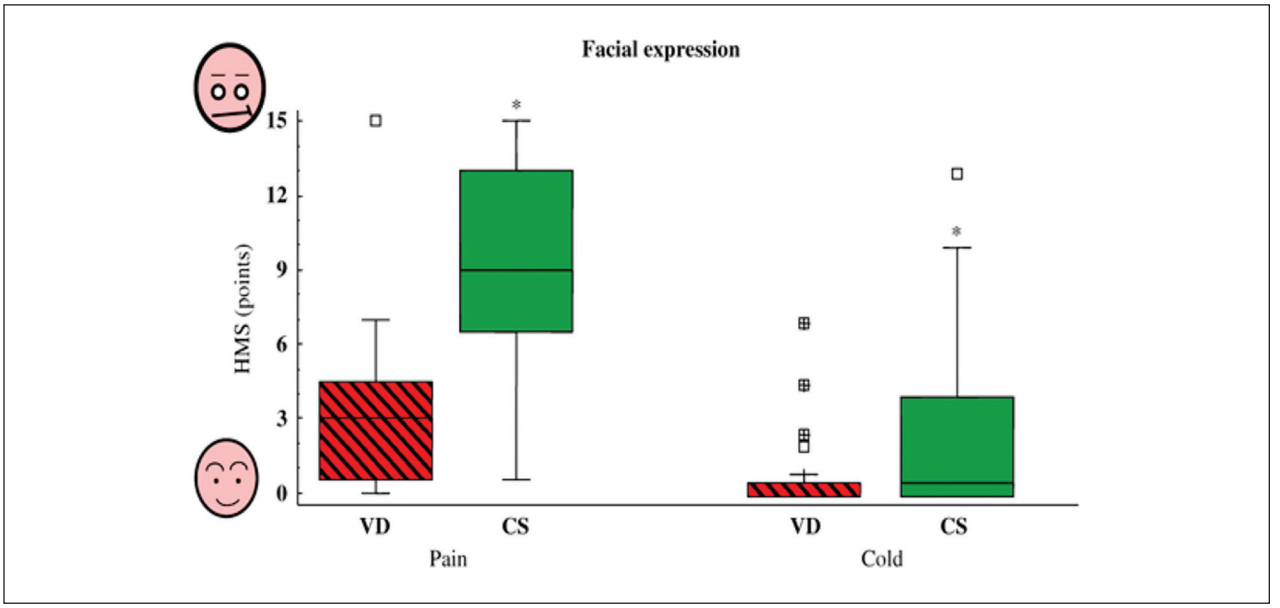


**Figure 4.** Facial reaction to painful cold stimuli of newborns differs in babies delivered vaginally (VD) and by cesarean section (CS). The reactions are significantly more severe in babies delivered by cesarean section than in babies delivered vaginally. Adapted from Bergqvist and others (2009). * = significant difference.

In the hippocampus, during the first week of postnatal life, the so-called giant depolarizing potentials or GDPs (Figure 5; Ben-Ari and others 1989) constitute a primordial form of synchrony between neurons, preceding more organized forms of activity such as theta and gamma rhythms (Buzsaki and Draguhn 2004). They have been proposed to be the in vitro counterpart of "sharp waves" recorded in rat pups in vivo during immobility periods,

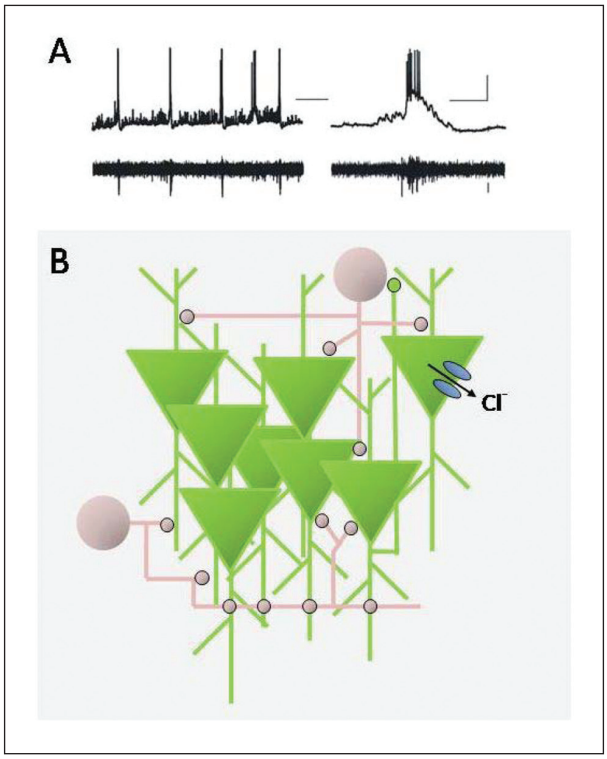


**Figure 5.** Giant depolarizing potentials (GDPs) are generated within a local network by the interplay between GABAergic and glutamatergic neurons. (A) Concomitant gramicidin-perforated patch clamp (upper trace) and field potentials recordings (lower trace) from a P6 CA3 pyramidal cell. On the right, a GDP is shown on an expanded time scale. (B) Schematic drawing showing interconnected pyramidal cells (green) and GABAergic interneurons (pink). GABA, released from GABAergic interneurons, binds and opens $GABA_A$ receptors (in blue), leading to an outwardly directed flux of chloride, which depolarizes principal cells.

sleep, and feeding (Leinekugel and others 2002). GDPs are characterized by long-lasting recurrent depolarizations up to 50 mV in amplitude, giving rise to bursts of action potentials and separated by silent periods. This discontinuity pattern is highly reminiscent of the "tracé discontinu" first described by Dreyfus-Brisac in electroencephalograms of immature babies (Stockard-Pope and others 1992). A similar pattern of activity also has been detected in the hippocampus of fetal macaque during the second half of gestation (R. Khazipov, unpublished data, 2001).

The appearance of GDPs is preceded by a well-defined sequence of events. At late embryonic stages of development, uncorrelated spontaneous activity consisting of calcium action potentials occurs in cortical structures. Synchronous activity emerges at birth: This is generated first by spontaneous plateau assemblies or SPAs and later by synaptic-driven events (GDPs and early network oscillations or ENOs). SPAs are nonsynaptic membrane oscillations generated by the activation of intrinsic membrane conductances in small groups of neurons coupled by gap junctions (Crepel and others 2007). They occur primarily during delivery and are modulated by oxytocin, in parallel with the transient shift of GABA actions (Tyzio and others 2006). As the network matures, SPAs are down-regulated possibly via CREB signaling and the activation of NMDA receptors that reduce connexins (Arumugam and others 2005). The down-regulation of SPA coincides with the appearance of GDPs. These are generated by the synergistic action of glutamate and GABA, which, as already mentioned, orchestrates neuronal ensembles via its depolarizing and excitatory action (Cherubini and others 2011; Ben-Ari 2002). GDPs disappear toward the end of the second postnatal week in concomitance with the shift of GABA from the depolarizing to the hyperpolarizing direction (Ben-Ari and others 1989). ENOs, initially thought to constitute the cortical counterpart of hippocampal GDPs, have been shown to precede and coexist during a restricted period of time with GDPs (Allene and others 2008; Allene and Cossart 2010b). ENOs are low-frequency oscillations dysplaying slow kinetics that gradually involve the entire network, whereas GDPs are recurrent oscillations that repetitively synchronize local neuronal assemblies. ENOs have different reversal potentials than GDPs—with a more substantial participation of NMDA receptor-driven currents (Figure 6; Allene and Cossart 2010a)—and are facilitated by anoxic conditions, raising the possibility that they may be relevant to pathological situations (Allene and Cossart 2010b). Interestingly, the maturation of NMDA signaling that controls immature patterns may recruit AMPA receptors, thereby converting postsynaptically silent connections into active ones (Voronin and Cherubini 2004). Interestingly, when both SPAs and GDPs are present at intermediary developmental stages, GDPs switch off SPAs (Crepel and others 2007).

## *How Are GDPs Generated?*

GDPs require the synchronous activation of a relatively small number of cells. At least in the CA3 area, they can be still detected in small islands comprising hundreds of neurons, isolated from the rest of the hippocampus (Khazipov and others 1997; Garaschuk and others 1998; Bolea and others 1999). They involve the activation of both principal cells and interneurons, which, by releasing GABA and glutamate at resting membrane potential, activate $GABA_A$, NMDA, and AMPA receptors (Ben-Ari 2002; Bonifazi and others 2009). At the network level, however, GDPs express both components, although the magnitude of the GABAergic conductance exceeds that of the glutamatergic one (GDP reversal is close to $E_{GABA}$) (Ben-Ari and others 1989; Bolea and others 1999). The

glutamatergic component can be unveiled by blocking the GABAergic one by loading the cell with an intracellular solution containing potassium fluoride, which only poorly permeates $GABA_A$ receptor channels. In this condition, GDPs reverse polarity at a membrane potential close to the equilibrium potential for AMPA receptor-mediated responses ($E_{AMPA}$) (Bolea and others 1999). The two components of GDPs can be examined by holding two neighboring pyramidal neurons at $E_{GABA}$ (fixed at –70 mV) and $E_{AMPA}$ (~0 mV), respectively. With this procedure, it becomes clear that the GABAergic component always precedes the glutamatergic one by several milliseconds, suggesting that principal cells are driven by GABAergic interneurons (Mohajerani and Cherubini 2005). Furthermore, using network dynamics imaging, online reconstruction of functional connectivity, and targeted whole-cell recordings from immature hippocampal slices, it was recently demonstrated that GABAergic interneurons with large axonal arborizations operate as functional hubs able to synchronize large ensembles of cells (Bonifazi and others 2009). These neurons appear also to have been generated earlier than other interneurons (Picardo and others 2011), suggesting that the developmental sequence includes a specific timing of leader and follower cells with subtypes of GABAergic interneurons programmed to control the generation of network activities.

In analogy with the synchronized activity generated in the disinhibited hippocampus (De la Prida and others 2006), GDPs emerge when a sufficient number of cells fire and the excitability of the network attains a certain threshold within a restricted time window. Simultaneous recordings from pairs of CA3 pyramidal neurons have shown a concurrent increment in the instantaneous firing frequency previous to GDP onset, which correlates with an increased frequency of spontaneously occurring synaptic events (De la Prida and Sanchez-Andres 1999). Although the entire hippocampal network possesses the capacity to generate GDPs, the CA3 area is particularly well equipped because of its extensive glutamatergic recurrent collaterals and spontaneous intrinsic bursts that can drive other neurons to fire (Sipila and others 2005; Safiulina and others 2008). In addition, the activation of extrasynaptic $GABA_A$ receptors by ambient GABA generates a tonic $GABA_A$-mediated conductance that contributes to depolarizing the neurons (Sipila and others 2005) and enhancing cell excitability and glutamatergic drive to principal cells (Marchionni and others 2007). An additional contributing factor is the low expression of Kv7.2 and Kv7.3 channels responsible for the non-inactivating, low-threshold M current ($I_M$) that in adulthood controls spike after-depolarization and burst generation (Yue and Yaari 2004). The low density of $I_M$ at birth contributes to produce intrinsic bursts that, in comparison to adults, are more robust, last longer, and recur more regularly (Safiulina and others 2008).

In neonates, thalamocortical oscillations that occur at a faster pace than GDPs are generated by an interplay of depolarizing and hyperpolarizing conductances and notably the slow hyperpolarization-activated cation current $I_h$, carried by HCN channels (Pape 1996). $I_h$, however, is not essential for GDP generation since synchronous oscillations occur also in neurons lacking HCN channels (Bender and others 2005). GDPs typically terminate by a slow after-hyperpolarization (AHP), lasting a few seconds and mediated by calcium-activated potassium conductances (Ben-Ari and others 1989; Sipila and others 2006). In addition to AHP, other factors may contribute to the observed periodicity, including a delayed activation of $GABA_B$ receptors by GABA (De la Prida and others 2006; McLean and others 1996; Fiorentino and others 2009).

## The Cation-Chloride Cotransporters NKCC1 and KCC2 Affect GABA Signaling via Regulation of Neuronal Chloride Homeostasis

Clearly, the level of $[Cl^-]_i$ is crucial for proper GABAergic signaling. What regulates neuronal $[Cl^-]_i$? The cation-chloride cotransporters (CCCs) are intrinsic membrane proteins that transport $Cl^-$ ions, together with $Na^+$ and/or $K^+$ ions, in an electroneutral manner due to the stoichiometric coupling and directionality of translocated ions. Therefore, members of this family are prime regulators of neuronal $[Cl^-]_i$.

The Na-(K)-Cl CCCs (NCC, NKCC1, and NKCC2), by using the large inward gradient for $Na^+$ across cell membranes, load $Cl^-$ to raise $[Cl^-]_i$ above its electrochemical equilibrium. Four different K-Cl CCCs (KCC1, KCC2, KCC3, and KCC4), using the large outward gradient for $K^+$ across cell membranes, primarily mediate $Cl^-$ efflux, reducing $[Cl^-]_i$ below its equilibrium potential. The relative activities of the $Cl^-$-importing versus $Cl^-$-exporting CCCs determine the overall level of $[Cl^-]_i$ in numerous cell types, thereby playing key roles in the regulation of neuronal excitability, cell volume, epithelial solute, and water transport. In neurons, the ubiquitous bumetanide-sensitive NKCC1 and the neuronal-specific KCC2 are the primary $Cl^-$ importer and exporter, respectively (Figure 2).

In embryonic and early postnatal life, NKCC1 activity is robust in hippocampal neurons, which display minimal activity of KCC2, resulting in a positive $E_{Cl-}$ relative to $V_m$ such that the GABAR mediates an outwardly directed $Cl^-$ current, prompting neuronal depolarization. Neuronal

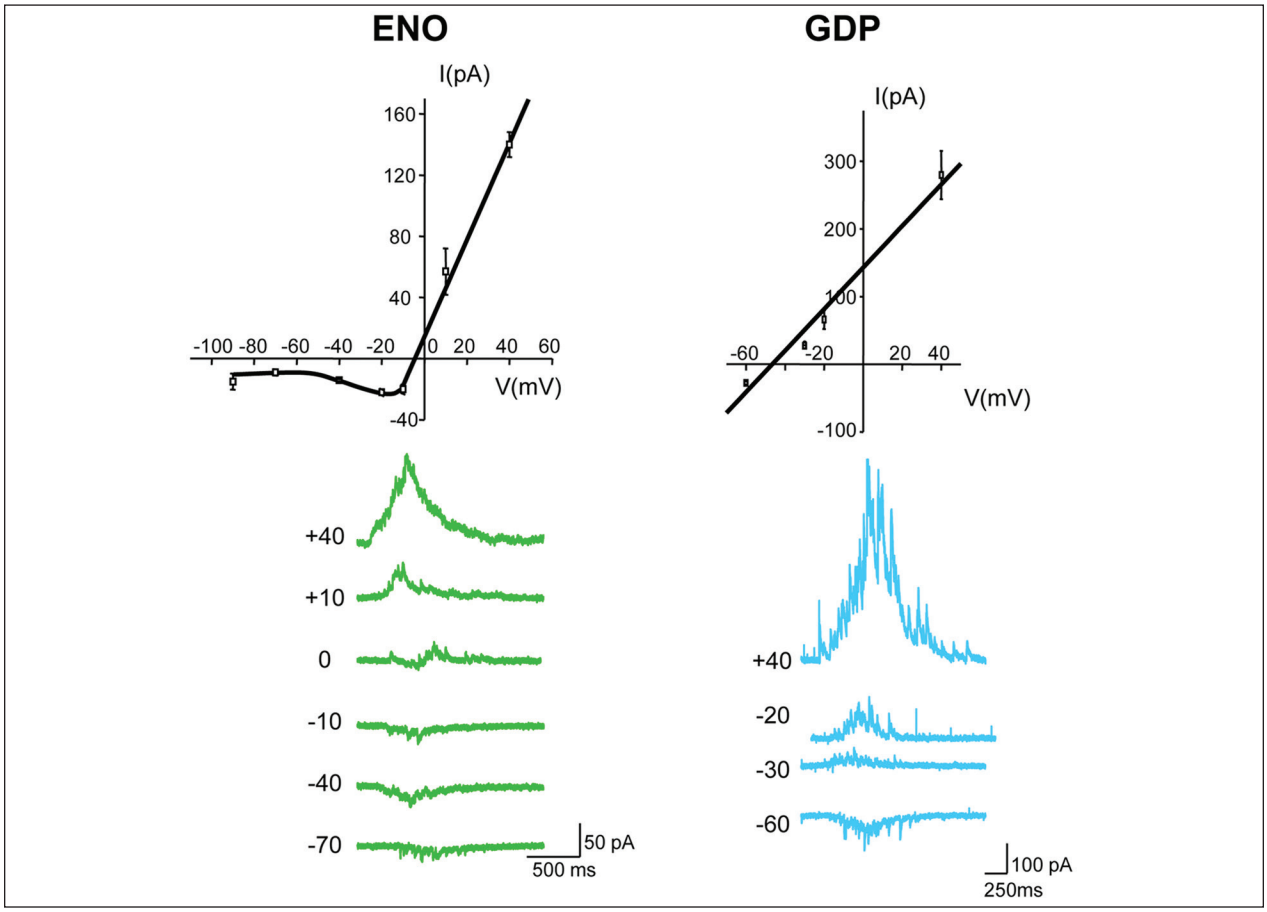


**Figure 6.** Major differences between the reversal potential of genuine giant depolarizing potentials (GDPs) and early network oscillations (ENOs). With patch-clamp recordings, the reversal potential was determined for GDPs and ENOs generated spontaneously by immature neocortical slices. Note that the GDPs have a linear I/V curve that reverses around 45 mV, whereas ENOs reverse at potentials that are closer to 0 mV, suggesting a higher implication of glutamatergic signaling in the latter than in the former. In keeping with this, the I/V curves are linear for GDPs and a typical plateau slope corresponding to the contribution of *N*-methyl-d-aspartate (NMDA) receptor-mediated currents for the ENOs. Adapted from Allene and others (2008) and Allene and Cossart (2010a).

$Cl^-$ efflux is mediated largely by KCC2, which is expressed at very low levels at birth, but in the adult is active in the retina, cortex, cerebellum, and the dorsal horn of the spinal cord. A negative shift in the GABA reversal potential ($E_{GABA}$) is paralleled by a robust increase in KCC2 expression near the end of the second postnatal week in rat cortical neurons (Rivera and others 1999). KCC2 expression in the human neocortex begins to increase at 40 weeks after conception and, coupled with a concurrent decrease in functional NKCC1 activity, results in an $E_{Cl-}$ that is negative relative to the neuron's $V_m$, thereby rendering GABAergic signals hyperpolarizing (Dzhala and others 2005). However, further physiological experiments are required to quantify the extent of functional reduction of NKCC1 activity in adults.

The Na-K-2Cl and K-Cl cotransporters are regulated in reciprocal fashion by serine-threonine phosphorylation/dephosphorylation. Cell swelling, high $[Cl^-]_i$, and protein phosphatases stimulate the KCCs but inhibit NKCC1 by promoting their net dephosphorylation, with an overall effect of decreasing $[Cl^-]_i$. By promoting cotransporter phosphorylation, cell shrinkage, low $[Cl^-]_i$, and protein phosphatase inhibitors activate NKCC1 and inhibit the KCCs, thereby increasing $[Cl^-]_i$. Protein phosphorylation is likely the predominant mechanism by which the *dynamic* modulation of CCC function takes place (Figure 7).

Recent studies have established the (WNK) serine-threonine kinases (members of which are mutated in two different Mendelian disorders: a syndrome of NaCl-sensitive hypertension termed pseudohypoaldosteronism type II and hereditary sensory and autonomic neuropathy type II [HSANII]) as central key $Cl^-$-sensitive regulatory kinases of the NKCC1 and KCC2 cotransporters (Kahle and others 2012). WNK kinases *activate* NKCC1 by

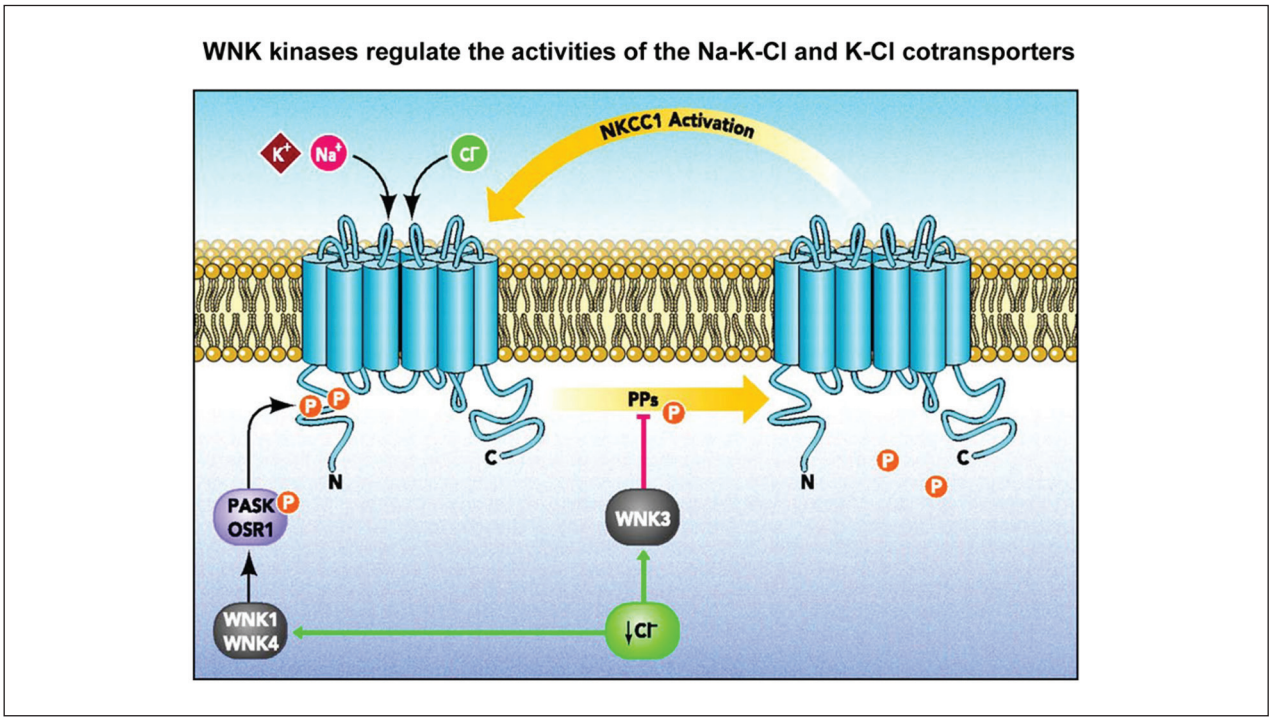


**Figure 7.** Dynamic modulation of $Cl^-$ homeostasis via regulatory phosphorylation of cation-chloride cotransport. Precise regulation of the intracellular concentration of chloride $[Cl^-]_i$ is necessary for proper cell GABA neurotransmission. The Na-K-2Cl (NKCCs) and K-Cl (KCCs) mediate cellular chloride influx and efflux, respectively, and are key determinants of $[Cl^-]_i$ in numerous cell types, including neurons. A chloride-sensitive kinase likely mediates the reciprocal but coordinated phosphoregulation of the NKCCs and the KCCs, and recent evidence suggests that the WNK (with no lysine = K) serine-threonine kinases, directly or indirectly via the downstream Ste20-type kinases SPAK/OSR1, are critical components of this signaling pathway. KCC2 is also regulated by tyrosine phosphorylation. Pathological alterations in the expression and/or phosphorylation of NKCC1 and KCC2 have been shown to underlie the $Cl^-$ derangements promoting GABAergic depolarization and excitation seen in several neurological disorders, such as epilepsy, neuropathic pain, and spasticity after spinal cord injury.

promoting its phosphorylation at Thr203/Thr207/Thr212 (human) by SPAK and OSR1 kinases (Vitari and others 2006). WNKs *decrease* KCC2 activity without modifying membrane trafficking by promoting KCC2 phosphorylation at T991/T1048 (Kahle and others 2005; Rinehart and others 2009). The principal site of phosphorylation for PKC within KCC2 is S940 in expression systems and neurons. Phosphorylation of S940 by PKC has been shown to increase cell surface stability of KCC2 and increase KCC2 transport activity, an effect independent of modified trafficking (Lee and others 2007). Tyrosine phosphorylation has also been shown to regulate KCC2 activity and stability at Y1081 and Y903 (Wake and others 2007). Therefore, an activity-dependent regulation of the operation and expression of chloride cotransporters provides a mechanistic substrate for the regulation by activity of the intracellular levels of chloride.

## Polarity of GABA Actions and Synaptic Plasticity

Like glutamatergic neurons, GABAergic synapses can undergo a variety of forms of synaptic plasticity, including long-term potentiation and depression of their glutamatergic inputs, leading to persistent modifications in the release probability of GABA from GABAergic interneurons. Persistent changes of synaptic efficacy in GABAergic synapses in both immature and adult neurons have been extensively documented. The underlying mechanisms include both pre- and postsynaptic mechanisms that differ according to the developmental age, the neuronal type, and other criteria (Sivakumaran and others 2009; Gaiarsa 2004). Several signaling molecules have been implicated in these alterations, including brain-derived neurotrophic factor (Gubellini and others 2005).

In addition, GABAergic signals are endowed with the unique possibility of altering in an activity-dependent manner the chloride gradient in postsynaptic targets, leading to a polarity shift that can convert the response to GABA from hyperpolarizing to depolarizing. This form of plasticity is restricted to GABAergic currents (Woodin and others 2003; Fiumelli and Woodin 2007). Thus, even brief synaptic stimulation or paired pre- and postsynaptic activity induces long-term synaptic plasticity at GABAergic synapses manifested by a depolarization shift of the reversal potential for GABA and a weakening of synaptic

inhibition (Balena and Woodin 2008). This is mediated by a decrease in the function of the neuron-specific $K^+$-$Cl^-$ cotransporter KCC2, leading to an increase of $[Cl^-]_i$. This pairing-induced depolarization of $E_{GABA}$ requires $Ca^{2+}$ influx through both the L-type voltage-gated $Ca^{2+}$ channels and *N*-methyl-D-aspartic acid receptors (Balena and Woodin 2008), confirming their relevance to more conventional modes of synaptic plasticity.

It is therefore important to determine the dynamic chloride regulation (DCR) in various conditions. This is by no means a simple task and raises several fundamental issues as it is age, neuronal type, and even neuronal compartment dependent (Yamada and others 2007; Foldy and others 2010). In addition, although little information is available at present, it is likely that the plethora of interneurons types with their different genetic fate and properties (Tricoire and others 2011; Butt and others 2005; Picardo and others 2011) have different dynamic chloride regulations. Discussing these fundamental issues is beyond the scope of this review, but a few remarks should be emphasized.

To determine DCR, it is necessary to load the cell with chloride and measure how fast chloride returns to control values. Usually, GABA is applied repetitively from a pipette localized close to the recorded neurons (in a perforated patch) at a holding potential corresponding to $E_{Cl-}$. Then a large depolarizing pulse (from $E_{Cl-}$ to 0 mV) is applied, and the time for GABA responses to reach $E_{Cl-}$ is determined. This approach shows that immature neurons require a longer period to return to control levels than adult ones, suggesting that the chloride cotransporters are not as efficient at early stages (Nardou and others 2011). In addition, the relatively specific KCC2 antagonist (DIOA) augments this duration, suggesting a less efficient KCC2 action. Additional factors and mechanisms are likely including more efficient chloride import and/or other undetermined mechanisms. Therefore, it is safe to conclude that the dynamic regulation of chloride is variable and strongly dependent on the ongoing activity generated by neurons, thereby limiting the intrinsic values of measures of the more static parameters of GABA reversal potential. In addition, extensive reviews have been published on the tonic GABAergic component, an important parameter present in immature neurons that also provides a substantial source of GABAergic activity (Sebe and others 2010; Mody and Pearce 2004; Glykys and Mody 2007; Farrant and Nusser 2005).

## Polarity of GABA Actions in Disease

### *High $[Cl^-]_i$ Levels and Depolarizing Actions of GABA in Epileptic Neurons*

If the polarity of GABA is heavily dependent on ongoing neuronal activity, pathological episodes of enhanced activity are likely to persistently alter the actions of GABA. Early in vivo (Ben-Ari and others 1979) and in vitro observations (Schwartzkroin and Prince 1980) suggest a high degree of fragility of GABAergic inhibition to seizures.

Using hippocampal slices from the temporal lobe of patients with epilepsy, Miles and coworkers have shown that GABA depolarizes about 30% of neurons, notably in the subiculum, where recurrent synchronized interictal-like activities are recorded in vitro and in vivo (Cohen and others 2002). Similar excitatory actions of GABA have been reported in vitro using a variety of convulsive agents and procedures (Dzhala and Staley 2003; Yamada and others 2004; Quilichini and others 2003). An important issue is the relevance of the lasting applications of drugs and conditions used to determine the "chronic" alterations of GABA polarity. Indeed, long-lasting (hours) applications on slices of high $K^+$ or 0 $Mg^{++}$ do not necessarily mimic the sequels of recurrent seizures and their chronic actions. Additional concerns are the use of bath applications of GABA analogues to determine GABAergic inhibition that produce strong shunting actions, chloride imaging techniques that have important limitations (notably pH sensitivity), and possible interactions between epileptic agents and tested antiepileptic agents. To avoid these issues, a triple chamber has been developed that enables one to apply a convulsive agent to one intact hippocampus placed in one chamber and determine the effects of propagated seizures on the other intact hippocampus that has not been subjected to long-term applications (Khalilov and others 1997). In addition, single GABA and NMDA channel recordings provide unambiguous measures of $DF_{GABA}$ and $V_{rest}$, respectively. In this preparation, recurrent seizures lead to the formation of an epileptogenic mirror focus (MF) that generates seizures spontaneously. In MF neurons that have never been subject to epileptic agents, GABA depolarizes and excites neurons and generates spontaneous seizures, indicating that the propagation of seizures to naive neurons is sufficient to persistently augment $[Cl^-]_i$ (Khalilov and others 2003; Khalilov and others 2005). This preparation is highly suited to determine the conditions required for seizures to beget seizures. It was found that recurrent seizures that propagate from one hippocampus to the other will trigger the formation of an MF only when high-frequency oscillations are included in the seizures. This in turn is conditioned by the presence of functional GABA and NMDA signals, indicating that GABA and NMDA receptor-driven currents converge to generate high-frequency oscillations required to induce the long-term effects of seizures (Khalilov and others 2003; Khalilov and others 2005; see Figure 8).

Dynamic phosphoregulation of KCC2 has recently been shown to be associated with pathophysiological glutamate release and seizures. For example, S940 phosphorylation regulates KCC2 functional expression under

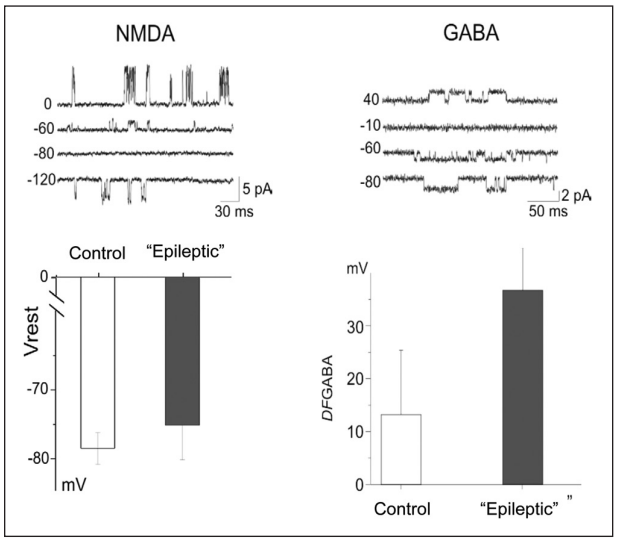


**Figure 8.** Single *N*-methyl-d-aspartate (NMDA) and GABA channel recordings from naive and epileptic neurons from mirror foci in the intact hippocampus. Note that NMDA channels are not different, whereas GABA channels have a significantly more depolarized $DF_{GABA}$, suggesting a stable depolarizing GABA in epileptic neurons with few changes in the resting membrane potential. Adapted from Khalilov and others (2003) and Khalilov and others (2005).

conditions of excessive neuronal activity (Lee and others 2011). NMDA receptor activity and $Ca^{2+}$ influx cause the protein phosphatase 1–dependent dephosphorylation of Ser940 in rat neurons, leading to a loss of KCC2 function that coincides with a deficit in hyperpolarizing GABAergic inhibition. Blocking dephosphorylation of Ser940 reduces glutamate-induced down-regulation of KCC2 and substantially improves the maintenance of hyperpolarizing GABAergic inhibition (Lee and others 2011). In addition, tyrosine phosphorylation at residues Y1081 and Y903 within KCC2 has been shown to reduce the stability of KCC2 by promoting lysosomal degradation and induction of status epilepticus in mice. Tyrosine phosphorylation regulates the membrane trafficking of the potassium chloride cotransporter KCC2 (Lee and others 2010). Collectively, these data demonstrate that alterations in both serine-threonine and tyrosine phosphorylation of KCC2 might play key roles in the losses of KCC2 function that underlie depolarizing GABA signaling in status epilepticus and other forms of epilepsy.

Although a wide range of mechanisms could mediate changes in $[Cl^-]_i$, usually these involve the chloride importer NKCC1 and the chloride exporter KCC2. Both an increased activity of NKCC1 and a down-regulation of KCC2 have been observed in experimental and human epileptic neurons. Thus, in neurons from human epileptic tissue, the depolarizing actions of GABA are associated with a reduced KCC2 immunolabeling, suggesting a reduction of KCC2 (Huberfeld and others 2007). Also, oocytes transfected with mRNA from the subiculum of epileptic patients exhibit an anomalous expression of both chloride cotransporters (Palma and others 2006; Eusebi and others 2009). Using various procedures to generate seizures and cotransporter blockers in slices, contradictory observations have been made, with some putting forward an up-regulation of NKCC1 and others a down-regulation of KCC2. Using long-lasting applications of 0 $Mg^{++}$, Dzhala and coworkers (2010) reported that bumetanide prevented chloride accumulation with recurrent seizures and reduced subsequent seizure severity. These authors also reported positive actions of the diuretic in vivo after injections of kainate, but the effects of the diuretic in vivo were quite limited and manifested primarily by a reduction of the power spectra of electroencephalogram (EEG) seizures and not by the clinical manifestations. Other studies in vitro provided contradictory observations on the effects of the diuretic in different models (Zhu and others 2008; Margineanu and Klitgaard 2006; Kilb and others 2007; Dzhala and others 2010; Dzhala and others 2005). In the triple chamber, chloride removal after loading is slowed down, and there is a loss and internalization of KCC2 in epileptic neurons of the mirror focus (Khalilov and others 2003, 2005; Nardou and others 2011). Although a contribution of NKCC1 cannot be excluded, NKCC1 is neither necessary nor sufficient for these shifts to take place since they are observed in NKCC1 KOs (Nardou and others 2011). Parallel studies have provided a mechanistic substrate to the alterations of chloride cotransporters. Thus, enhanced activity and recurrent seizures reduce KCC2, and this is mediated by a tyrosine phosphorylation that controls protein trafficking (Lee and others 2010; Rivera and others 2004; Rivera and others 2002). Similar regulatory sites for NKCC1 have been reported (Darman and others 2001) as well as alterations of NKCC1 mRNA (Okabe and others 2002; see also Sen and others 2007). Interestingly, in contrast to recurrent seizures, single events activate KCC2 activity and augment the efficiency of chloride removal (Khirug and others 2010), suggesting that the down-regulation and intracellular trafficking of KCC2 (also see below) require recurrent, not single, seizures, thereby differentiating seizures and epilepsy. Therefore, the plasticity of GABA polarity is clearly an important factor in the long-lasting sequels of seizures and how these beget further seizures, but the mechanisms underlying these alterations of chloride cotransporter regulation deserve more investigations.

## *GABA Excites Neurons in Other Pathological Conditions*

Chloride regulation is also perturbed in many other insults. Thus, dorsal root ganglion neurons isolated from axotomized sciatic nerves have elevated chloride levels due to an up-regulation of NKCC1 (Pieraut and others 2007). The depolarizing actions of GABA are suggested to

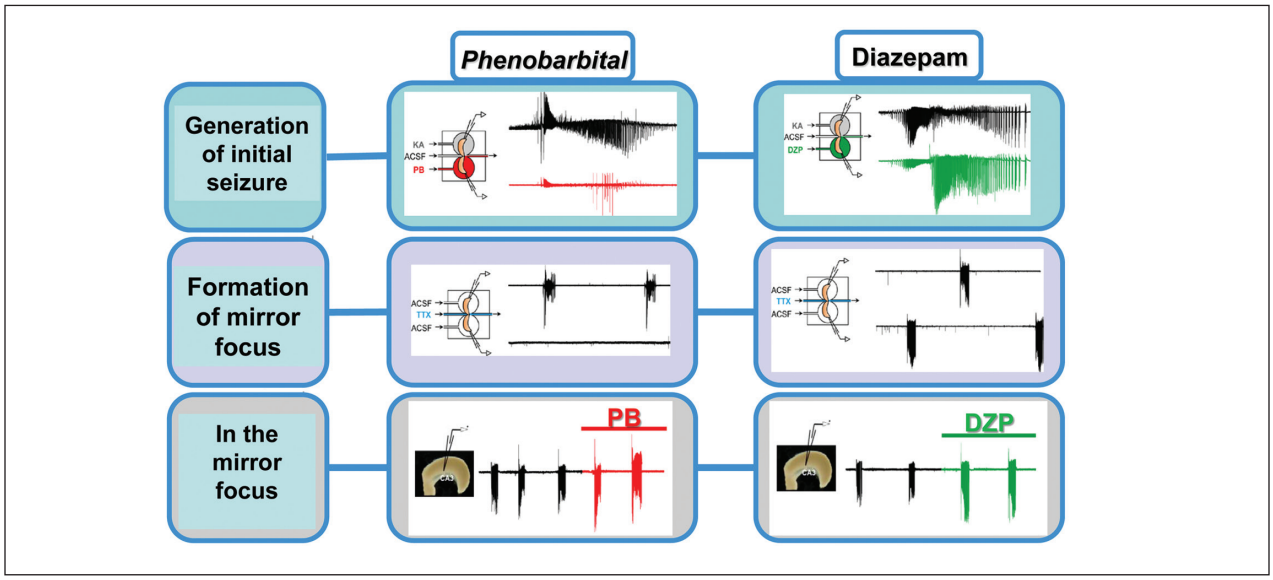


**Figure 9.** Comparison of diazepam (DZP) and phenobarbital (PB) on the triple chamber with the two intact hippocampi in vitro. Kainate was applied to one hippocampus and the consequences of the contralateral intact hippocampus that did not receive a convulsive agent determined. After recurrent seizures, the naive hippocampus became an epileptogenic mirror focus (MF) that generated ongoing seizures. Note that DZP aggravated initial seizures, failed to prevent the formation of an MF, and aggravated the seizures generated by the MF. In contrast, PB blocked the initial seizures and prevented at least transiently the formation of an MF but aggravated seizures in the MF. Adapted from Nardou and others (2011).

promote growth. A down-regulation of KCC2 and associated chloride shift has been reported after spinal cord lesions; these contribute to spasticity (Boulenguez and others 2010). Ischemic insults and brain trauma also lead to similar shifts (Shulga and others 2008; Papp and others 2008), suggesting that a variety of insults are associated with a similar response that possibly contributes to reactive plasticity. Furthermore, in transgenic mice chronically deprived of nerve growth factor (NGF) exhibiting a progressive neurodegenerative pathology resembling that observed in patients with Alzheimer disease (AD), GABA becomes depolarizing and excitatory. Real-time qRT-PCR and immunocytochemical experiments revealed a reduced expression of mRNA encoding for the *Kcc2* gene and of the respective protein (Lagostena and others 2010).

## Therapeutic Perspectives

### *Antiepileptic Drugs and Epilepsy Treatment*

The GABA-acting antiepileptic drug (AED) phenobarbital (PB) is the drug of first choice to treat neonatal seizures (Wheless and others 2007; Bassan and others 2008). Yet, these drugs often either fail to block seizures or even aggravate them (Boylan and others 2002; Guillet and Kwon 2007). These observations suggest that the excitatory actions of GABA mediate the paradoxical actions of GABA-acting AEDs and raise considerable interest in the possible therapeutic actions of diuretics that, by reducing $[Cl^-]_i$, facilitate the antiepileptic actions of PB and related drugs.

One study showed that PB blocked the first ictal event generated by 0 $Mg^{++}$ but not later ones, and bumetanide augmented the efficacy of PB actions of subsequent seizures (Dzhala and others 2008). These authors suggest that a combined use of PB and diuretics may improve the loss of efficacy of the former. In the triple chamber, bumetanide failed to prevent the formation of a mirror focus by seizures but reduced the severity of seizures spontaneously generated by an MF, suggesting that recurrent seizures overcome the protective actions of bumetanide on chloride. Bumetanide, however, had protective actions when applied at an early stage, confirming the need for rapid and aggressive treatment of seizures in the developing brain to avoid a down-regulation of KCC2 and a persistent accumulation of chloride (Nardou and others 2011; see Figure 9). Therefore, the history of seizures prior to bumetanide administration will condition its efficacy.

A large clinical trial on two-day-old babies with encephalopathy is presently being performed in Europe (www.nemo-europe.com). The inclusion criteria are phenobarbital-refractory epileptic encephalopathy in two-day-old babies with an indication to inject burinex in addition to phenobarbital. A somewhat similar trial has been initiated in the United States. These trials are important as they will confirm or refute the clinical relevance of NKCC1 antagonists in the treatment of epilepsies. Yet, as the timing of the administration appears to be instrumental, restricting the use of bumetanide to phenobarbital-resistant epilepsies may lead to negative results.

**Box 1.** Challenging the GABA Developmental Sequence

The developmental sequence of GABA has been challenged on the basis of three different arguments/experiments:

1. The depolarizing action of GABA is a slice artifact being mediated by the lesion that slice preparation entrains (Dzhala and others 2010). However, recordings from embryonic to adult slices, including from surface neurons using single GABA and *N*-methyl-d-aspartate (NMDA) channel recordings, show a clear-cut developmental sequence that cannot be explained by surface lesions (Tyzio and others 2003; Tyzio and others 2006). This argument cannot be reconciled with the inhibitory/hyperpolarizing actions of GABA on adult slices that are more vulnerable to lesions than immature ones with more neuronal ramifications that are cut by the procedure. In addition, this suggestion is at odds with the developmental sequence of GABA actions observed in chronic cultures, where this factor is eliminated, with the age, sex, and neuronal-type dependence of the shift (i.e., the depolarizing actions of GABA on initial segment of the axon) (Yamada and others 2007; Ben Ari and others 2007). These differences are therefore due to either technical issues (inadequate slice preparation and preservation) and/or limitations of the chlomeleon imaging technique that is highly sensitive to pH alterations that has been used extensively by this group.
2. Poor energetic levels of immature slices: Zilberter Holmgren and colleagues (Holmgren and others 2010) have reported that when ketone bodies and/or lactate/pyruvate are supplied, lower intracellular chloride levels are observed. The authors suggest that glucose does not faithfully mimic the physiological conditions of immature neurons and the slices ought to be perfused with these energy substrates. However, these observations have been inferred in a variety of brain structures and laboratories where physiological levels of β-hydroxybutyrate (BHB), lactate, and pyruvate did not alter $DF_{GABA}$ (Tyzio and others 2011; Kirmse and others 2010; see also Gomez-Lira and others 2011) (Figure 10). The assumptions on which this hypothesis is based are incompatible with extensive observations (Tyzio and others 2011). In addition, direct measures of mitochondrial pH have shown that the effects of lactate are due to pH, not to alterations of $[Cl^-]_i$ (Ruusuvuori and others 2010). Finally, the concentrations of pyruvate used by these authors, ~40-fold those present in the brain in physiological concentrations, are observed only in severe pathological conditions and bear no relevance to physiological ones. An extensive discussion of this issue can be found elsewhere (Tyzio and others 2011).
3. The need for in vivo recordings: It is logical to expect in vivo recordings to settle this issue. However, there are several problems to consider. First, although ethically acceptable, nonpainful chronic recordings are feasible in adults using an early implantation of a chronic support for electrodes, this is less obvious in newly born pups and, worse, in utero, necessitating the use of anaesthetic agents known to alter ongoing activity and GABA actions. The stress and pain produced in the absence of anaesthetic agents will unavoidably lead to the release of stress hormones and catecholamines that also alter GABA actions. Applications of large concentrations of GABA agonists to measure GABA actions can shunt neuronal activity, leading to inadequate conclusions as to $DF_{GABA}$. In addition, the effects of the blood and local inflammation produced by the pipettes are a problem to take into account, considering the actions these may have on local pH, intracellular chloride, and tonic release of GABA. Finally, as the response of GABA is age, neuronal fate, and even neuronal compartment dependent, recording from nonidentified neurons severely handicaps the conclusions. It is hoped that more appropriate chronic methods, coupled with imaging techniques, may allow better investigation of the postsynaptic effects of GABA.

## *Autism Spectrum Disorders*

Experimental and human observations suggest alterations of GABAergic signals in autism spectrum disorders (ASDs) (Zhang and others 2010; Gibson and others 2009; Dossche 2005; Pizzarelli and Cherubini 2011). Benzodiazepines exert paradoxical actions in patients with ASD, suggesting that chloride may be elevated (Marrosu and others 1987). In a pilot study, Lemonnier and Ben-Ari (2010) investigated the effects of long-term administrations of bumetanide (1 mg daily, three months) in infants with ASD and showed highly beneficial actions. The patients were more present, and the effects of the diuretic, which were highly significant with the Childhood Autism Rating Scale test, were associated with few side effects. Randomized double-blind studies are now being conducted. Interestingly, oxytocin has also been shown to transiently ameliorate visual

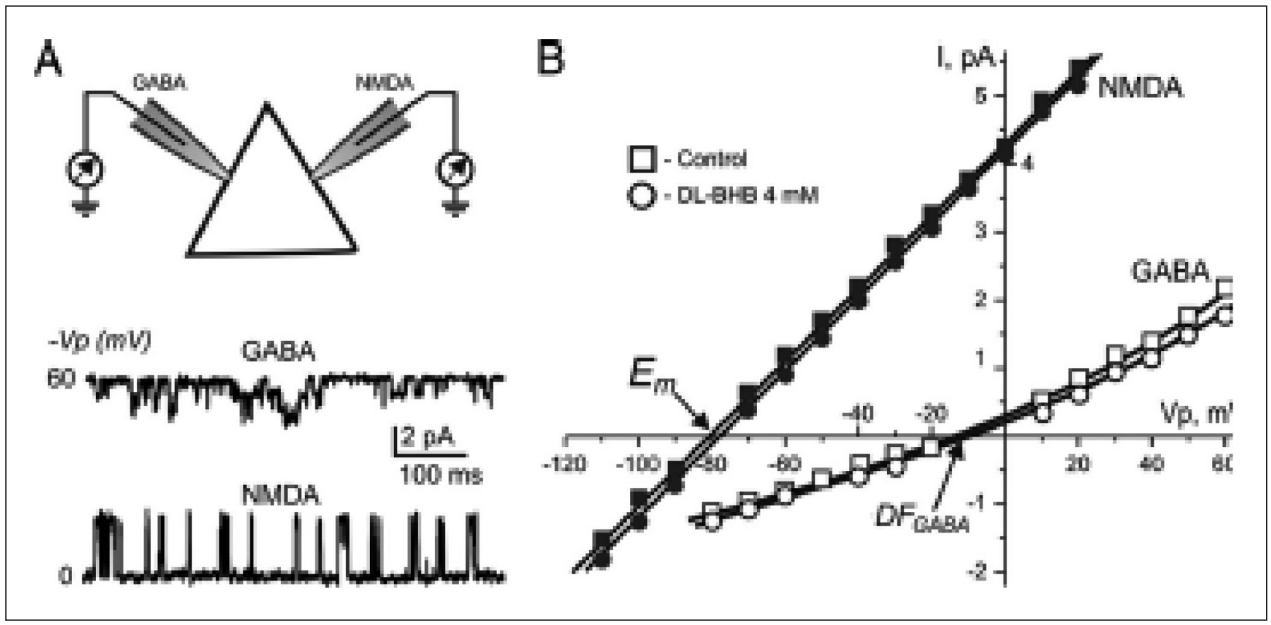


**Figure 10.** Lack of effects of ketone body metabolites on the polarity of GABA actions. Double single-channel recordings made form the same immature hippocampal pyramidal neuron. (A) Single *N*-methyl-d-aspartate (NMDA) and GABA channels were recorded with two independent patch-clamp electrodes to determine respectively the genuine resting membrane potential and the driving force of GABA and hence calculate $E_{GABA}$. (B) Note that the slopes of both currents were not altered by adding the ketone body metabolite β-hydroxybutyrate (4–5 mM). Adapted from Tyzio and others (2011).

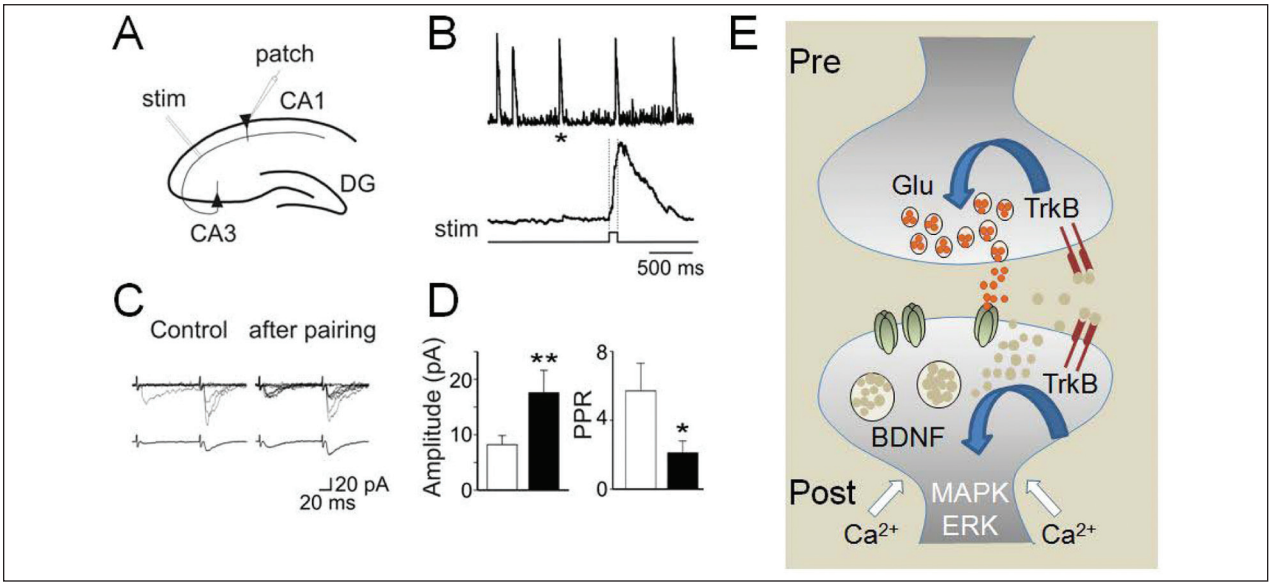


**Figure 11.** Pairing giant depolarizing potentials (GDPs) with Schaffer collateral stimulation persistently enhances synaptic efficacy at CA3-CA1 connections. (A) Schematic diagram of the hippocampus showing a CA1 pyramidal neuron receiving a synaptic input from a CA3 principal cell. The stimulating electrode (stim) was positioned in stratum radiatum. (B) In the upper trace, GDPs recorded from a CA1 principal cell in current-clamp mode. Below, the GDP with an asterisk is shown on an expanded time scale (spikes are blocked by QX-314 into the patch pipette). The rising phase of GDPs (between the dashed lines) was used to trigger synaptic stimulation (stim). (C) Individual responses (successes and failures) evoked by Schaffer collateral stimulation in control and 20 minutes after pairing are superimposed (upper traces). Average responses are below. (D) Each column represents the mean peak amplitude of synaptic responses (including failures) obtained before (white) and 20 minutes after pairing (black). PPR, paired pulse ratio. *$P < .05$. **$P < .01$. (E) Calcium flux through the voltage-dependent calcium channel (white arrows) opened by GDPs induces the release of brain-derived neurotrophic factor (BDNF) from the postsynaptic neuron. BDNF binds to tropomyosin-related kinase receptor B (TrkB) receptors localized on pre- and postsynaptic membranes. Activation of postsynaptic TrkB receptors causes the enhancement of glutamate release on the postsynaptic site, causing the activation of the MAPK/ERK signaling pathway. Modified from Mohajerani and others (2007).

**Box 2.** Giant Depolarizing Potentials (GDPs) Are Instrumental for Enhancing Synaptic Efficacy at Emerging GABAergic and Glutamatergic Synapses

According to the quantal theory, the synaptic efficacy $E$ can be defined as: $E = mQ$, where $m$ is the mean quantal content or mean number of quanta released per presynaptic action potential, and $Q$ is the quantal size or the mean amplitude of unitary EPSCs or IPSCs produced by a single quantum. $Q$ depends on both pre- and postsynaptic mechanisms, but $m$ is closely related to presynaptic factors—namely, to $N$, the number of release sites, and to $P$, the probability of releasing a single quantum. Thus, changes in any of these parameters lead to modifications in synaptic efficacy. Persistent modifications in $E$ such as those occurring during long-term potentiation (LTP) or long-term depression (LTD) are critical for information storage and for establishing new synaptic contacts during development. The immature brain is characterized by an elevated number of "silent" connections (Durand and others 1996; Gasparini and others 2000). These are synapses that do not conduct at rest either because the neurotransmitter is not released when the presynaptic terminal is invaded by an action potential (presynaptically silent) or because they are unable to detect the release of the neurotransmitter due to the lack of the respective receptors on the subsynaptic membrane (postsynaptically silent). Conversion of silent synapses into active ones represents the most common mechanism for LTP induction (Voronin and Cherubini 2004). It has been assumed that early calcium signals associated with GDPs may act as coincident detectors for enhancing $E$ at emerging synapses and for establishing new appropriate connections. To verify this hypothesis, a "pairing" protocol has been developed consisting of stimulating for a short period (five minutes) either the mossy fibers that in the immediate postnatal period release GABA (Safiulina and others 2006) or the Schaffer collateral with the rising phase of spontaneously occurring GDPs (Kasyanov and others 2004; Mohajerani and Cherubini 2006; Mohajerani and others 2007) (Figure 11). In such a way, GDP-associated calcium transients occur simultaneously with afferent inputs. This procedure led to a strong and persistent increase in synaptic strength and, in the case of presynaptically silent neurons (with $P$ close to 0), to synapse un-silencing (Kasyanov and others 2004; Mohajerani and Cherubini 2006; Mohajerani and others 2007). By introducing a delay between GDPs and afferent stimulation, synaptic responses progressively declined and regained the control level in about three seconds. In the absence of pairing, no significant changes in synaptic efficacy occurred. Pairing-induced synaptic potentiation could be prevented by loading the cell with the calcium chelator BAPTA or by adding nifedipine (but not D-APV) to the extracellular solution, indicating that calcium rise via voltage-dependent calcium channels, opened by the depolarizing action of GABA and glutamate during GDPs, is a common trigger for activity-dependent changes in synaptic efficacy. Interestingly, in previous reports (Caillard and others 1999; Gubellini and others 2001; Gubellini and others 2005), it was clearly shown that repeated bursts of action potentials, applied at low frequency to CA3 principal cells, are able to potentiate $GABA_A$-mediated synaptic currents in an NMDA-independent and brain-derived neurotrophic factor (BDNF)–dependent way. However, in these studies, the origin of GABAergic inputs was not identified.

Although the induction of LTP at both GABAergic and glutamatergic synapses is postsynaptic, its expression is presynaptic, as suggested by a pairing-induced decrease in failure rate, in paired pulse facilitation, and an increase in the inversed square of the coefficient of variation, all traditional indices of presynaptic changes in release probability. This implies a cross-talk between the post- and presynaptic sites of the synapse. It is likely that, at Schaffer collateral-CA1 synapses, BDNF, possibly secreted from the postsynaptic neuron during GDPs, is the retrograde signal. By binding to tropomyosin-related kinase receptor B (TrkB) localized on presynaptic neurons (Mohajerani and others 2007), BDNF increases the probability of glutamate release. In addition, BDNF can act also on postsynaptic TrkB receptors to activate the MAPK/ERK signaling pathway (Figure 11E). This would lead to transcriptional regulation and new protein synthesis required for the enduring forms of synaptic plasticity. Whether GDP-induced persistent changes in synaptic efficacy are associated with structural modifications necessary to rewire local neuronal circuits remain to be demonstrated.

communication in adults with ASD (Andari and others 2010). Oxytocin has also been found to rescue Prader Willi mice during the delivery period when these do not feed and many die within a few hours (Schaller and others 2010). Collectively, these observations point to the important actions of the GABA shift during delivery and to its possible implications in many early neurological and psychiatric disorders.

## Conclusions

Mounting evidence suggest that the levels of $[Cl^-]_i$ and associated polarity of GABA action are important parameters that reflect the level of excitability of neurons and networks and represent a signature of neuronal development. The plasticity of the polarity of GABA that is unique to this transmitter enables neurons to keep track of recent and less recent physiological and pathological events. As such, this provides a unique form of hysteresis and memory of recent ongoing activity. Long-term alterations of GABA actions are also a signature of insults and disorders that paves the way for novel therapeutic perspectives for diuretics and other drugs that, by reducing the levels of $[Cl^-]_i$, reinstate the hyperpolarizing action of GABA and behaviorally relevant oscillations that are strongly dependent on GABAergic networks.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

### Funding

The authors disclosed receipt of the following financial support for the research and/or authorship of this article: This work was supported by the French Medical Research council (INSERM), the French Medical Research (FRM) association, the French Federation of Brain Research (FRC), European Union grants (EU No), Ministero Università e Ricerca (MIUR, Italy), and telethon grant GGP11043.

### References

Allene C, Cattani A, Ackman JB, Bonifazi P, Aniksztejn L, Ben-Ari Y, and others. 2008. Sequential generation of two distinct synapse-driven network patterns in developing neocortex. J Neurosci 28:12851–63.

Allene C, Cossart R. 2010a. Early NMDA receptor-driven waves of activity in the developing neocortex: physiological or pathological network oscillations? J Physiol 588:83–91.

Allene C, Cossart R. 2010b. Early NMDA receptor-driven waves of activity in the developing neocortex: physiological or pathological network oscillations? J Physiol 588(Pt 1):83–91.

Andari E, Duhamel JR, Zalla T, Herbrecht E, Leboyer M, Sirigu A. 2010. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. Proc Natl Acad Sci U S A 107:4389–94.

Arumugam H, Liu XH, Colombo PJ, Corriveau RA, Belousov AB. 2005. NMDA receptors regulate developmental gap junction uncoupling via CREB signaling. Nat Neurosci 8:1720–6.

Balena T, Woodin MA. 2008. Coincident pre- and postsynaptic activity downregulates NKCC1 to hyperpolarize E(Cl) during development. Eur J Neurosci 27:2402–12.

Banke TG, McBain CJ. 2006. GABAergic input onto CA3 hippocampal interneurons remains shunting throughout development. J Neurosci 26:11720–5.

Bassan H, Bental Y, Shany E, Berger I, Froom P, Levi L, and others. 2008. Neonatal seizures: dilemmas in workup and management. Pediatr Neurol 38:415–21.

Ben Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. 2007. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol Rev 87:1215–84.

Ben Ari Y, Khalilov I, Represa A, Gozlan H. 2004. Interneurons set the tune of developing networks. Trends Neurosci 27:422–7.

Ben-Ari Y. 2001. Developing networks play similar melody. Trends Neurosci 24:354–60.

Ben-Ari Y. 2002. Excitatory actions of GABA during development: the nature of the nurture. Nat Rev Neurosci 3:728–39.

Ben-Ari Y, Cherubini E, Corradetti R, Gaïarsa J-L. 1989. Giant synaptic potentials in immature rat CA3 hippocampal neurones. J Physiol 416:303–25.

Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. 2007. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol Rev 87:1215–84.

Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaïarsa J-L. 1997. $GABA_A$, NMDA and AMPA receptors: a developmentally regulated 'ménage a trois'. Trends Neurosci 20:523–9.

Ben-Ari Y, Krnjevic K, Reinhardt W. 1979. Hippocampal seizures and failure of inhibition. Can J Physiol Pharmacol 57:1462–6.

Bender RA, Galindo R, Mameli M, Gonzalez-Vega R, Valenzuela CF, Baram TZ. 2005. Synchronized network activity in developing rat hippocampus involves regional hyperpolarization-activated cyclic nucleotide-gated (HCN) channel function. Eur J Neurosci 22:2669–74.

Bergqvist LL, Katz-Salamon M, Hertegard S, Anand KJ, Lagercrantz H. 2009. Mode of delivery modulates physiological and behavioral responses to neonatal pain. J Perinatol 29:44–50.

Bolea S, Avignone E, Berretta N, Sanchez-Andres JV, Cherubini E. 1999. Glutamate controls the induction of GABA-mediated giant depolarizing potentials through AMPA receptors in neonatal rat hippocampal slices. J Neurophysiol 81:2095–102.

Bonifazi P, Goldin M, Picardo MA, Jorquera I, Cattani A, Bianconi G, and others. 2009. GABAergic hub neurons orchestrate synchrony in developing hippocampal networks. Science 326:1419–24.

Boulenguez P, Liabeuf S, Bos R, Bras H, Jean-Xavier C, Brocard C, and others. 2010. Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. Nat Med 16:302–7.

Boylan GB, Rennie JM, Pressler RM, Wilson G, Morton M, Binnie CD. 2002. Phenobarbitone, neonatal seizures, and video-EEG. Arch Dis Child Fetal Neonatal Ed 86:F165–70.

Butt SJ, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, and others. 2005. The temporal and spatial origins of cortical interneurons predict their physiological subtype. Neuron 48:591–604.

Buzsaki G, Draguhn A. 2004. Neuronal oscillations in cortical networks. Science 304:1926–9.

Caillard O, Ben-Ari Y, Gaiarsa JL. 1999. Long-term potentiation of GABAergic synaptic transmission in neonatal rat hippocampus. J Physiol 518:109–19.

Cancedda L, Fiumelli H, Chen K, Poo M. 2007. Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. J Neurosci 27:5224–35.

Chen G, Trombley PQ, van den Pol AN. 1996. Excitatory actions of GABA in developing rat hypothalamic neurones. J Physiol 494(Pt 2):451–64.

Cherubini E, Griguoli M, Safiulina V, Lagostena L. 2011. The depolarizing action of GABA controls early network activity in the developing hippocampus. Mol Neurobiol 43:97–106.

Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. 2002. On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science 298:1418–21.

Crepel V, Aronov D, Jorquera I, Represa A, Ben-Ari Y, Cossart R. 2007. A parturition-associated nonsynaptic coherent activity pattern in the developing hippocampus. Neuron 54:105–20.

Dammerman RS, Flint AC, Noctor S, Kriegstein AR. 2000. An excitatory GABAergic plexus in developing neocortical layer 1. J Neurophysiol 84:428–34.

Darman RB, Flemmer A, Forbush B. 2001. Modulation of ion transport by direct targeting of protein phosphatase type 1 to the Na-K-Cl cotransporter 7. J Biol Chem 276:34359–62.

De la Prida LM, Huberfeld G, Cohen I, Miles R. 2006. Threshold behavior in the initiation of hippocampal population bursts. Neuron 49:131–42.

De la Prida LM, Sanchez-Andres JV. 1999. Nonlinear transfer function encodes synchronization in a neural network from the mammalian brain. Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topic 60:3239–43.

Demarque M, Represa A, Becq H, Khalilov I, Ben Ari Y, Aniksztejn L. 2002. Paracrine intercellular communication by a $Ca^{2+}$- and SNARE-independent release of GABA and glutamate prior to synapse formation. Neuron 36:1051–61.

Dossche DM. 2005. GABA in autism. Amsterdam: Academic Press.

Durand GM, Kovalchuk Y, Konnerth A. 1996. Long-term potentiation and functional synapse induction in developing hippocampus. Nature 381:71–5.

Dzhala VI, Brumback AC, Staley KJ. 2008. Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. Ann Neurol 63:222–35.

Dzhala VI, Kuchibhotla KV, Glykys JC, Kahle KT, Swiercz WB, Feng G, and others. 2010. Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. J Neurosci 30:11745–61.

Dzhala VI, Staley KJ. 2003. Excitatory actions of endogenously released GABA contribute to initiation of ictal epileptiform activity in the developing hippocampus. J Neurosci 23:1840–6.

Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, and others. 2005. NKCC1 transporter facilitates seizures in the developing brain. Nat Med 11: 1205–13.

Eilers J, Plant TD, Marandi N, Konnerth A. 2001. GABA-mediated $Ca^{2+}$ signalling in developing rat cerebellar Purkinje neurones. J Physiol 536:429–37.

Eusebi F, Palma E, Amici M, Miledi R. 2009. Microtransplantation of ligand-gated receptor-channels from fresh or frozen nervous tissue into Xenopus oocytes: a potent tool for expanding functional information. Prog Neurobiol 88:32–40.

Farrant M, Nusser Z. 2005. Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. Nat Rev Neurosci 6:215–29.

Feller MB, Butts DA, Aaron HL, Rokhsar DS, Shatz CJ. 1997. Dynamic processes shape spatiotemporal properties of retinal waves. Neuron 19:293–306.

Fiorentino H, Kuczewski N, Diabira D, Ferrand N, Pangalos MN, Porcher C, and others. 2009. $GABA_B$ receptor activation triggers BDNF release and promotes the maturation of GABAergic synapses. J Neurosci 29:11650–61.

Fiumelli H, Woodin MA. 2007. Role of activity-dependent regulation of neuronal chloride homeostasis in development. Curr Opin Neurobiol 17:81–6.

Foldy C, Lee SH, Morgan RJ, Soltesz I. 2010. Regulation of fast-spiking basket cell synapses by the chloride channel ClC-2. Nat Neurosci 13:1047–9.

Gaiarsa JL. 2004. Plasticity of GABAergic synapses in the neonatal rat hippocampus. J Cell Mol Med 8:31–7.

Galli L, Maffei L. 1988. Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. Science 242:90–1.

Garaschuk O, Hanse E, Konnerth A. 1998. Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. J Physiol 507: 219–36.

Garaschuk O, Linn J, Eilers J, Konnerth A. 2000. Large-scale oscillatory calcium waves in the immature cortex. Nature 3:452–9.

Gasparini S, Saviane C, Voronin LL, Cherubini E. 2000. Silent synapses in the developing hippocampus: lack of functional AMPA receptors or low probability of glutamate release? Proc Natl Acad Sci U S A 97:9741–6.

Gibson JR, Huber KM, Sudhof TC. 2009. Neuroligin-2 deletion selectively decreases inhibitory synaptic transmission originating from fast-spiking but not from somatostatin-positive interneurons. J Neurosci 29:13883–97.

Glykys J, Mody I. 2007. The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. J Physiol 582:1163–78.

Gomez-Lira G, Mendoza-Torreblanca JG, Granados-Rojas L. 2011. Ketogenic diet does not change NKCC1 and KCC2 expression in rat hippocampus. Epilepsy Res 96:166–71.

Gozlan H, Ben Ari Y. 2003. Interneurons are the source and the targets of the first synapses formed in the rat developing hippocampal circuit. Cereb Cortex 13:684–92.

Gubellini P, Ben-Ari Y, Gaïarsa JL. 2001. Activity- and age-dependent GABAergic synaptic plasticity in the developing rat hippocampus. Eur J Neurosci 14:1937–46.

Gubellini P, Ben-Ari Y, Gaïarsa JL. 2005. Endogenous neurotrophins are required for the induction of GABAergic long-term potentiation in the neonatal rat hippocampus. J Neurosci 25:5796–802.

Guillet R, Kwon J. 2007. Seizure recurrence and developmental disabilities after neonatal seizures: outcomes are unrelated to use of phenobarbital prophylaxis. J Child Neurol 22:389–95.

Hennou S, Khalilov I, Diabira D, Ben Ari Y, Gozlan H. 2002. Early sequential formation of functional $GABA_A$ and glutamatergic synapses on CA1 interneurons of the rat foetal hippocampus. Eur J Neurosci 16:197–208.

Holmgren CD, Mukhtarov M, Malkov AE, Popova IY, Bregestovski P, Zilberter Y. 2010. Energy substrate availability as a determinant of neuronal resting potential, GABA signaling and spontaneous network activity in the neonatal cortex in vitro. J Neurochem 112(4):900-12. Epub 2009 Nov 24.

Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, and others. 2007. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. J Neurosci 27:9866–73.

Kahle KT, Rinehart J, de los Heros P, Louvi A, Meade P, Vazquez N, and others. 2005. WNK3 modulates of $Cl^-$ transport in and out of cells: implications for control of cell volume and neuronal excitability. Proc Natl Acad Sci U S A 102:16783–8.

Kahle KT, Rinehart J, Lifton RP. 2012. Phosphoregulation of the Na-K-2Cl and K-Cl cotransporters by the WNK kinases. Biochim Biophys Acta 1802(12):1150–8.

Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E. 2004. GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. Proc Natl Acad Sci U S A 101:3967–72.

Khalilov I, Esclapez M, Medina I, Aggoun D, Lamsa K, Leinekugle X, and others. 1997. A novel in vitro preparation: the intact hippocampal formation. Neuron 19:743–9.

Khalilov I, Holmes GL, Ben Ari Y. 2003. In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. Nat Neurosci 6:1079–85.

Khalilov I, Le Van QM, Gozlan H, Ben Ari Y. 2005. Epileptogenic actions of GABA and fast oscillations in the developing hippocampus. Neuron 48:787–96.

Khazipov R, Esclapez M, Caillard O, Bernard C, Khalilov I, Tyzio R, and others. 2001. Early development of neuronal activity in the primate hippocampus in utero. J Neurosci 21:9770–81.

Khazipov R, Leinekugel X, Khalilov I, Gaïarsa J-L, Ben-Ari Y. 1997. Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices. J Physiol 498:763–72.

Khirug S, Ahmad F, Puskarjov M, Afzalov R, Kaila K, Blaesse P. 2010. A single seizure episode leads to rapid functional activation of KCC2 in the neonatal rat hippocampus. J Neurosci 30:12028–35.

Kilb W, Sinning A, Luhmann HJ. 2007. Model-specific effects of bumetanide on epileptiform activity in the in-vitro intact hippocampus of the newborn mouse. Neuropharmacology 53:524–33.

Kirmse K, Witte OW, Holthoff K. 2010. GABA depolarizes immature neocortical neurons in the presence of the ketone body β-hydroxybutyrate. J Neurosci 30:16002–7.

Komuro H, Rakic P. 1993. Modulation of neuronal migration by NMDA receptors. Science 260:95–7.

Lagostena L, Rosato-Siri M, D'Onofrio M, Brandi R, Arisi I, Capsoni S, and others. 2010. In the adult hippocampus, chronic nerve growth factor deprivation shifts GABAergic signaling from the hyperpolarizing to the depolarizing direction. J Neurosci 30:885–93.

Lee HH, Jurd R, Moss SJ. 2010. Tyrosine phosphorylation regulates the membrane trafficking of the potassium chloride cotransporter KCC2. Mol Cell Neurosci 45(2):173–9.

Lee HHC, Deeb TZ, Walker JA, Davies PA, Moss SJ. 2011. NMDA receptor activity downregulates KCC2 resulting in depolarizing $GABA_A$ receptor-mediated currents. Nat Neurosci 14:736–43.

Lee HHC, Walker JA, Williams JR, Goodier RJ, Payne JA, Moss SJ. 2007. Direct protein kinase C–dependent phosphorylation regulates the cell surface stability and activity of the potassium chloride cotransporter KCC2. J Biol Chem 282:29777–84.

Leinekugel X, Khazipov R, Cannon R, Hirase H, Ben Ari Y, Buzsaki G. 2002. Correlated bursts of activity in the neonatal hippocampus in vivo. Science 296:2049–52.

Leinekugel X, Medina I, Khalilov I, Ben-Ari Y, Khazipov R. 1997. $Ca^{2+}$ oscillations mediated by the synergistic excitatory actions of $GABA_A$ and NMDA receptors in the neonatal hippocampus. Neuron 18:243–55.

Lemonnier E, Ben-Ari Y. 2010. The diuretic bumetanide decreases autistic behaviour in five infants treated during 3 months with no side effects. Acta Paediatr 99:1885–8.

Manent JB, Demarque M, Jorquera I, Pellegrino C, Ben Ari Y, Aniksztejn L, and others. 2005. A noncanonical release of GABA and glutamate modulates neuronal migration. J Neurosci 25:4755–65.

Manent JB, Jorquera I, Ben Ari Y, Aniksztejn L, Represa A. 2006. Glutamate acting on AMPA but not NMDA

receptors modulates the migration of hippocampal interneurons. J Neurosci 26:5901–9.

Marchionni I, Omrani A, Cherubini E. 2007. In the developing rat hippocampus a tonic $GABA_A$-mediated conductance selectively enhances the glutamatergic drive of principal cells. J Physiol 581:515–28.

Margineanu DG, Klitgaard H. 2006. Differential effects of cation-chloride co-transport-blocking diuretics in a rat hippocampal slice model of epilepsy. Epilepsy Res 69:93–99.

Maric D, Liu QY, Maric I, Chaudry S, Chang YH, Smith SV, and others. 2001. GABA expression dominates neuronal lineage progression in the embryonic rat neocortex and facilitates neurite outgrowth via $GABA_A$ autoreceptor/$Cl^-$ channels. J Neurosci 21:2343–60.

Marrosu F, Marrosu G, Rachel M, Biggio G. 1987. Paradoxical reactions elicited by diazepam in children with classic autism. Funct Neurol 2(3):355–61.

Mazzuca M, Minlebaev M, Shakirzyanova A, Tyzio R, Taccola G, Janackova S, and others. 2011. Newborn analgesia mediated by oxytocin during delivery. Front Cell Neurosci 5:3.

McLean HA, Caillard O, Khazipov R, Ben-Ari Y, Gaïarsa J-L. 1996. Spontaneous release of GABA activates $GABA_B$ receptors and controls network activity in the neonatal rat hippocampus. J Neurophysiol 76:1036–46.

Mody I, Pearce RA. 2004. Diversity of inhibitory neurotransmission through GABAA receptors. Trends Neurosci 27:569–75.

Mohajerani MH, Cherubini E. 2005. Spontaneous recurrent network activity in organotypic rat hippocampal slices. Eur J Neurosci 22:107–18.

Mohajerani MH, Cherubini E. 2006. Role of giant depolarizing potentials in shaping synaptic currents in the developing hippocampus. Crit Rev Neurobiol 18:13–23.

Mohajerani MH, Sivakumaran S, Zacchi P, Aguilera P, Cherubini E. 2007. Correlated network activity enhances synaptic efficacy via BDNF and the ERK pathway at immature CA3 CA1 connections in the hippocampus. Proc Natl Acad Sci U S A 104:13176–81.

Nardou R, Yamamoto S, Chazal G, Bhar A, Ferrand N, Dulac O, and others. 2011. Neuronal chloride accumulation and excitatory GABA underlie aggravation of neonatal epileptiform activities by phenobarbital. Brain 134:987–1002.

Okabe A, Ohno K, Toyoda H, Yokokura M, Sato K, Fukuda A. 2002. Amygdala $Na^+$, kindling induces upregulation of mRNA for NKCC1, a $Na^+$, $K^+$-$2Cl^-$ cotransporter, in the rat piriform cortex. Neurosci Res 44:225–9.

Owens DF, Boyce LH, Davis MB, Kriegstein AR. 1996. Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. J Neurosci 16:6414–23.

Palma E, Amici M, Sobrero F, Spinelli G, Di Angelantonio S, Ragozzino D, and others. 2006. Anomalous levels of $Cl^-$ transporters in the hippocampal subiculum from temporal lobe epilepsy patients make GABA excitatory. Proc Natl Acad Sci U S A 103:8465–8.

Pape HC. 1996. From queer current to pacemaker: the hyperpolarization-activated cation current in thalamic neurones. J Physiol 491P:S12.

Papp E, Rivera C, Kaila K, Freund TF. 2008. Relationship between neuronal vulnerability and potassium-chloride cotransporter 2 immunoreactivity in hippocampus following transient forebrain ischemia. Neuroscience 154:677–89.

Picardo MA, Guigue P, Bonifazi P, Batista-Brito R, Allene C, Ribas A, and others. 2011. Pioneer GABA cells comprise a subpopulation of hub neurons in the developing hippocampus. Neuron 71:695–709.

Pieraut S, Laurent-Matha V, Sar C, Hubert T, Mechaly I, Hilaire C, and others. 2007. NKCC1 phosphorylation stimulates neurite growth of injured adult sensory neurons. J Neurosci 27:6751–9.

Pizzarelli R, Cherubini E. 2011. Alterations of GABAergic signaling in autism spectrum disorders. Neural Plast 2011: 297153.

Quilichini PP, Diabira D, Chiron C, Milh M, Ben-Ari Y, Gozlan H. 2003. Effects of antiepileptic drugs on refractory seizures in the intact immature corticohippocampal formation in vitro. Epilepsia 44:1365–74.

Rheims S, Minlebaev M, Ivanov A, Represa A, Khazipov R, Holmes GL, and others. 2008. Excitatory GABA in rodent developing neocortex in vitro. J Neurophysiol 100:609–19.

Rinehart J, Maksimova YD, Tanis JE, Stone KL, Hodson CA, Zhang J, and others. 2009. Sites of regulated phosphorylation that control K-Cl cotransporter activity. Cell 138:525–36.

Ritter A, Wenner P, Ho S, Whelan PJ, O'Donovan MJ. 1999. Activity patterns and synaptic organization of the ventrally located interneurons in the embryonic chick spinal cord. J Neurosci 19:3457–71.

Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, and others. 2002. BDNF-induced TrkB activation down-regulates the $K^+$-$Cl^-$ cotransporter KCC2 and impairs neuronal $Cl^-$ extrusion. J Cell Biol 159:747–52.

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, and others. 1999. The $K^+/Cl^-$ co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251–5.

Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipila S, and others. 2004. Mechanism of activity-dependent down-regulation of the neuron-specific K-Cl cotransporter KCC2. J Neurosci 24:4683–91.

Ruusuvuori E, Kirilkin I, Pandya N, Kaila K. 2010. Spontaneous network events driven by depolarizing GABA action in neonatal hippocampal slices are not attributable to deficient mitochondrial energy metabolism. J Neurosci 30:15638–42.

Safiulina VF, Fattorini G, Conti F, Cherubini E. 2006. GABAergic signaling at mossy fiber synapses in neonatal rat hippocampus. J Neurosci 26:597–608.

Safiulina VF, Zacchi P, Taglialatela M, Yaari Y, Cherubini E. 2008. Low expression of Kv7/M channels facilitates intrinsic and network bursting in the developing rat hippocampus. J Physiol 586:5437–53.

Schaller F, Watrin F, Sturny R, Massacrier A, Szepetowski P, Muscatelli F. 2010. A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. Hum Mol Genet 19:4895–905.

Schwartzkroin PA, Prince DA. 1980. Changes in excitatory and inhibitory synaptic potentials leading to epileptogenic activity. Brain Res 183:61–76.

Sebe JY, Looke-Stewart EC, Estrada RC, Baraban SC. 2010. Robust tonic GABA currents can inhibit cell firing in mouse newborn neocortical pyramidal cells. Eur J Neurosci 32:1310–8.

Sen A, Martinian L, Nikolic M, Walker MC, Thom M, Sisodiya SM. 2007. Increased NKCC1 expression in refractory human epilepsy. Epilepsy Res 74:220–7.

Shulga A, Thomas-Crusells J, Sigl T, Blaesse A, Mestres P, Meyer M, and others. 2008. Posttraumatic $GABA_A$-mediated $[Ca^{2+}]_i$ increase is essential for the induction of brain-derived neurotrophic factor-dependent survival of mature central neurons. J Neurosci 28:6996–7005.

Sipila ST, Huttu K, Soltesz I, Voipio J, Kaila K. 2005. Depolarizing GABA acts on intrinsically bursting pyramidal neurons to drive giant depolarizing potentials in the immature hippocampus. J Neurosci 25:5280–9.

Sipila ST, Huttu K, Voipio J, Kaila K. 2006. Intrinsic bursting of immature CA3 pyramidal neurons and consequent giant depolarizing potentials are driven by a persistent Na current and terminated by a slow Ca-activated K current. Eur J Neurosci 23:2330–8.

Sivakumaran S, Mohajerani MH, Cherubini E. 2009. At immature mossy-fiber-CA3 synapses, correlated presynaptic and postsynaptic activity persistently enhances GABA release and network excitability via BDNF and cAMP-dependent PKA. J Neurosci 29:2637–47.

Stockard-Pope J, Werner SS, Bickford RG. 1992. Atlas of neonatal electroencephalography. New York: Raven Press.

Tricoire L, Pelkey KA, Erkkila BE, Jeffries BW, Yuan XQ, McBain CJ. 2011. A blueprint for the spatiotemporal origins of mouse hippocampal interneuron diversity. J Neurosci 31:10948–70.

Tyzio R, Allene C, Nardou R, Picardo MA, Yamamoto S, Sivakumaran S, and others. 2011. Depolarizing actions of GABA in immature neurons depend neither on ketone bodies nor on pyruvate. J Neurosci 31:34–45.

Tyzio R, Cossart R, Khalilov I, Minlebaev M, Hubner CA, Represa A, and others. 2006. Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. Science 314:1788–92.

Tyzio R, Ivanov A, Bernard C, Holmes GL, Ben Ari Y, Khazipov R. 2003. Membrane potential of CA3 hippocampal pyramidal cells during postnatal development. J Neurophysiol 90:2964–72.

Tyzio R, Represa A, Jorquera I, Ben-Ari Y, Gozlan H, Aniksztejn L. 1999. The establishment of GABAergic and glutamatergic synapses on CA1 pyramidal neurons is sequential and correlates with the development of the apical dendrite. J Neurosci 19:10372–82.

Valeeva G, Abdullin A, Tyzio R, Skorinkin A, Nikolski E, Ben-Ari Y, and others. 2010. Temporal coding at the immature depolarizing GABAergic synapse. Front Cell Neurosci 4:17.

Vitari AC, Thastrup J, Rafiqi FH, Deak M, Morrice NA, Karlsson HKR, and others. 2006. Functional interactions of the SPAK/OSR1 kinases with their upstream activator WNK1 and downstream substrate NKCC1. Biochem J 397: 223–31.

Voronin LL, Cherubini E. 2004. 'Deaf, mute and whispering' silent synapses: their role in synaptic plasticity. J Physiol 557: 3–12.

Wake H, Watanabe M, Moorhouse AJ, Kanematsu T, Horibe S, Matsukawa N, and others. 2007. Early changes in KCC2 phosphorylation in response to neuronal stress result in functional downregulation. J Neurosci 27:1642–50.

Wang DD, Kriegstein AR. 2010. Blocking early GABA depolarization with bumetanide results in permanent alterations in cortical circuits and sensorimotor gating deficits. Cereb Cortex 21(3):574–87.

Wang DD, Krueger DD, Bordey A. 2003. GABA depolarizes neuronal progenitors of the postnatal subventricular zone via $GABA_A$ receptor activation. J Physiol 550: 785–800.

Wang J, Reichling DB, Kyrozis A, MacDermott AB. 1994. Developmental loss of GABA-induced and glycine-induced depolarization and $Ca^{2+}$ transients in embryonic rat dorsal horn neurons in culture. Eur J Neurosci 6:1275–80.

Wheless JW, Clarke DF, Arzimanoglou A, Carpenter D. 2007. Treatment of pediatric epilepsy: European expert opinion, 2007. Epileptic Disord 9:353–412.

Woodin MA, Ganguly K, Poo MM. 2003. Coincident pre- and postsynaptic activity modifies GABAergic synapses by postsynaptic changes in $Cl^-$ transporter activity. Neuron 39: 807–20.

Yamada J, Khirug S, Voipio J, Khiroug L, Kaila K. 2007. The GABAergic depolarization of the axon initial segment in cortical principal neurons is mediated by the Na-K-2Cl cotransporter NKCC1. Soc Neurosci Abstr 683.6.

Yamada J, Okabe A, Toyoda H, Kilb W, Luhmann HJ, Fukuda A. 2004. $Cl^-$ uptake promoting depolarizing GABA actions in immature rat neocortical neurones is mediated by NKCC1. J Physiol 557:829–41.

Young SZ, Platel JC, Nielsen JV, Jensen NA, Bordey A. 2010. $GABA_A$ increases calcium in subventricular zone astrocyte-like cells through L- and T-type voltage-gated calcium channels. Front Cell Neurosci 4:8.

Yue CY, Yaari Y. 2004. KCNQ/M channels control spike after depolarization and burst generation in hippocampal neurons. J Neurosci 24:4614–24.

Yuste R, Katz LC. 1991. Control of postsynaptic $Ca^{2+}$ influx in developing neocortex by excitatory and inhibitory neurotransmitters. Neuron 6:333–44.

Yuste R, Nelson DA, Rubin WW, Katz LC. 1995. Neuronal domains in developing neocortex: mechanisms of coactivation. Neuron 14:7–17.

Zhang C, Atasoy D, Arac D, Yang X, Fucillo MV, Robison AJ, and others. 2010. Neurexins physically and functionally interact with $GABA_A$ receptors. Neuron 66:403–16.

Zhu L, Polley N, Mathews GC, Delpire E. 2008. NKCC1 and KCC2 prevent hyperexcitability in the mouse hippocampus. Epilepsy Res 79:201–12.