Review Article

Spatial and Temporal Dynamics in the Ionic Driving Force for GABA$_A$ Receptors

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It is becoming increasingly apparent that the strength of GABAergic synaptic transmission is dynamic. One parameter that can establish differences in the actions of GABAergic synapses is the ionic driving force for the chloride-permeable GABA$_A$ receptor (GABA$_A$R). Here we review some of the sophisticated ways in which this ionic driving force can vary within neuronal circuits. This driving force for GABA$_A$Rs is subject to tight spatial control, with the distribution of Cl$^-$ transporter proteins and channels generating regional variation in the strength of GABA$_A$R signalling across a single neuron. GABA$_A$R dynamics can result from short-term changes in their driving force, which involve the temporary accumulation or depletion of intracellular Cl$^-$. In addition, activity-dependent changes in the expression and function of Cl$^-$ regulating proteins can result in long-term shifts in the driving force for GABA$_A$Rs. The multifaceted regulation of the ionic driving force for GABA$_A$Rs has wide ranging implications for mature brain function, neural circuit development, and disease.

1. Introduction

GABA$_A$ receptors (GABA$_A$Rs) are the principal mediators of fast synaptic inhibition in the brain. These receptors differ from most ligand-gated ion channels in that their reversal potential (E_{GABA}) is close to the resting membrane potential of neurons. Consequently, GABA$_A$Rs have the capacity to exhibit a different form of dynamics whereby small changes to the driving force of the underlying anionic currents can lead to significant changes in the nature and strength of GABA$_A$R-mediated transmission. For instance, if E_{GABA} is more negative than the resting membrane potential GABA$_A$R activation will result in membrane hyperpolarisation and inhibition. If E_{GABA} is more positive than the resting membrane potential however, stimulating GABA$_A$Rs will result in a combination of membrane depolarization and shunting inhibition. GABA$_A$R activation is only excitatory if E_{GABA} is positive enough to increase the probability of action potential generation.

The best described example of E_{GABA} modulation occurs during early development when neurons in the hippocampus and other brain structures have been shown to undergo a shift in the ionic driving force for GABA$_A$Rs from depolarising to hyperpolarising [1–4]. This change is the result of a developmental decrease in the levels of intracellular chloride ($[Cl^-]_i$), brought about by the increased contribution of the K$^+$-Cl$^-$ cotransporter, KCC2, which extrudes Cl$^-$, compared to the Na$^+$-K$^+$-2Cl$^-$ cotransporter, NKCC1, which normally functions to raise $[Cl^-]_i$ [5]. Changes to $[Cl^-]_i$ and GABA$_A$R-mediated currents have also been described as a result of neural trauma [6–17]. Since the reports that the Cl$^-$ driving force for GABA$_A$Rs is altered during development and in particular CNS disorders, there has been further careful examination of how neurons regulate $[Cl^-]_i$. This work confirms that $[Cl^-]_i$ and the associated ionic driving force for GABA$_A$Rs cannot be thought of as a fixed parameter. Rather, sophisticated mechanisms impact how Cl$^-$ is regulated in space and time, such that $[Cl^-]_i$ can vary between cells, within different parts of the same cell, and as a function of the history of the cell and the network in which it resides. Appreciating these mechanisms is important for understanding GABAergic signalling, not only in the mature nervous system, but also during neural circuit formation and in the context of CNS disorders. The diagram in Figure 1 provides an outline for this review by illustrating three ways in which the ionic driving force for GABA$_A$Rs may exhibit differences. We will focus on recent

work that has examined how spatial properties of neurons have been linked to differences in $[Cl^-]_i$ and how activity-dependent mechanisms can generate both short- and long-term changes in $[Cl^-]_i$. In doing so, we will also discuss the potential functional consequences of spatial and temporal differences in driving force for $GABA_ARs$.

2. Spatial Variations in E_{GABA}

Over recent years it has become increasingly apparent that the notion of universally hyperpolarising E_{GABA} in mature neurons of the CNS is a misleading one. E_{GABA} can vary across different types of neurons and this leads to different actions of $GABA_AR$ postsynaptic potentials (GPSPs) depending on the cell type in question [18–20]. For example, fast spiking inhibitory interneurons in the cortex and amygdala exhibit a considerably more depolarised E_{GABA} than neighbouring pyramidal cells, which may contribute to differences in the excitability of these two cell types [18]. What has also come to be appreciated is the fact that as well as intercellular variability, E_{GABA} can show *intracellular* differences. One of the most prominent examples involves the axon initial segment (AIS). Here, the E_{GABA} of inputs from axoaxonic (or Chandelier) cells tend to be significantly more positive than the E_{GABA} of separate GABAergic inputs targeting the soma [21–23] (see Figure 1). Axonal E_{GABA}, as determined in three studies, was found to be between 6 and 22 mV more positive than somatic E_{GABA} [21–23]. Such within-cell variations in E_{GABA} have been linked to the differential distribution of Cl^- cotransporter proteins. Immunogold labelling of KCC2 in hippocampal pyramidal and dentate gyrus cells has shown that the levels of this transporter are severalfold higher in the soma compared to the AIS, with local KCC2 densities at the plasma membrane of the AIS at around 6% the level of somatic KCC2 [23, 24]. NKCC1-null cells, or cells treated with bumetanide, do not exhibit axosomatic $[Cl^-]_i$ gradients, which indicates that NKCC1 is key to maintaining the higher E_{GABA} values recorded at the AIS [22].

The degree of differences in E_{GABA} between axon and soma may vary across different cell types and whether the resultant effect of an axoaxonic GABAergic input to a neuron is depolarising, hyperpolarising, inhibitory, or even excitatory is still not clear [29, 30]. The location of the AIS is close to the proposed site of action potential initiation and thus one might predict that if axoaxonic inputs are indeed depolarising these could help promote action potential initiation [21]. However, despite numerous studies [21–23, 30, 31] there is limited evidence that $GABA_AR$ synapses formed by axoaxonic cells at the AIS are able to trigger action potentials in the postsynaptic neuron. It is important to note that, even with depolarising driving forces, $GABA_AR$ synapses may still exert strong inhibitory effects via their shunting action upon excitatory currents [32]. Consequently, whether AIS $GABA_AR$ synapses are capable of evoking excitatory responses in pyramidal cells is still an open question and one that will be dependent on factors such as the number and relative timing of GABAergic and glutamatergic inputs, the magnitude of the $GABA_AR$

conductance and whether or not the depolarising actions persist beyond the shunting effect [33].

Local $[Cl^-]_i$ differences can also be found between the soma and dendrites of several types of neurons [22, 34, 35]. For example, $[Cl^-]_i$ has been shown to be higher and more depolarising in the dendrites than in the soma of certain ON-type retinal bipolar cells, a difference that underlies the receptive field properties of these neurons [34]. Numerous other studies, utilising a wide array of different techniques and preparations, have reported considerable variation in the strength and direction of somatodendritic Cl^- gradients [22, 35–39]. These differences can typically be explained by compartment specific expression of Cl^- transporter proteins regulated as a function of development, cell type, and brain region [34, 40]. However, it is worth remembering that because the degree of phasic and tonic $GABA_AR$ activity can itself influence $[Cl^-]_i$, and can also vary significantly between different experimental preparations, this may affect estimates of $[Cl^-]_i$ [41].

In a recent study, Földy et al. [42] discovered intracellular Cl^- regulation on an even more spatially refined scale and via a mechanism involving Cl^- regulators other than transport proteins. The authors examined the conductance and current-rectification properties of two types of GABAergic input onto the same perisomatic region of CA1 pyramidal neurons. Their recordings revealed that $GABA_AR$ currents at synapses receiving presynaptic input from parvalbumin-expressing fast-spiking basket cells (PVBCs) are selectively modulated by the voltage-gated Cl^- channel ClC-2. ClC-2 is found in the soma of pyramidal neurons and is an inwardly rectifying channel, which is activated by neuronal hyperpolarisation and allows Cl^- to flow out of the cell more easily than into it [43, 44]. ClC-2 activity was found to be strongly associated with PVBC synapses, in contrast to neighbouring synapses formed by cholecystokinin-expressing basket cells (CCKBCs). As a consequence, rates of Cl^- extrusion following intense $GABA_AR$ activity were found to be significantly faster at PVBC synapses. This is supported by Rinke et al. [45], who reported that neurons from mice lacking the ClC-2 channel show reduced rates of Cl^- removal and by the fact that the resting E_{GABA} at PVBC synapses is significantly lower than at CCKBC synapses [42]. The authors suggest that the presence of somatic CLC-2 and its contribution to Cl^- regulation could play an important role in preventing potentially detrimental increases in $[Cl^-]_i$ during periods of intense firing by soma targeting PVBCs [42]. As Földy et al. point out, their findings could be partly explained at a compartmental level, as the somatodendritic distribution of PVBC and CCKBC synapses does show some differences. Nevertheless, these recent studies have advanced our appreciation of Cl^- regulation by showing that, as well as being nonuniform across different neuronal compartments, E_{GABA} may vary between individual synapses within the same compartment. Thus, even assigning E_{GABA} to certain spatial regions of a cell may be an oversimplification and instead it could be more appropriate to consider E_{GABA} in terms of a particular input to a postsynaptic cell [46].
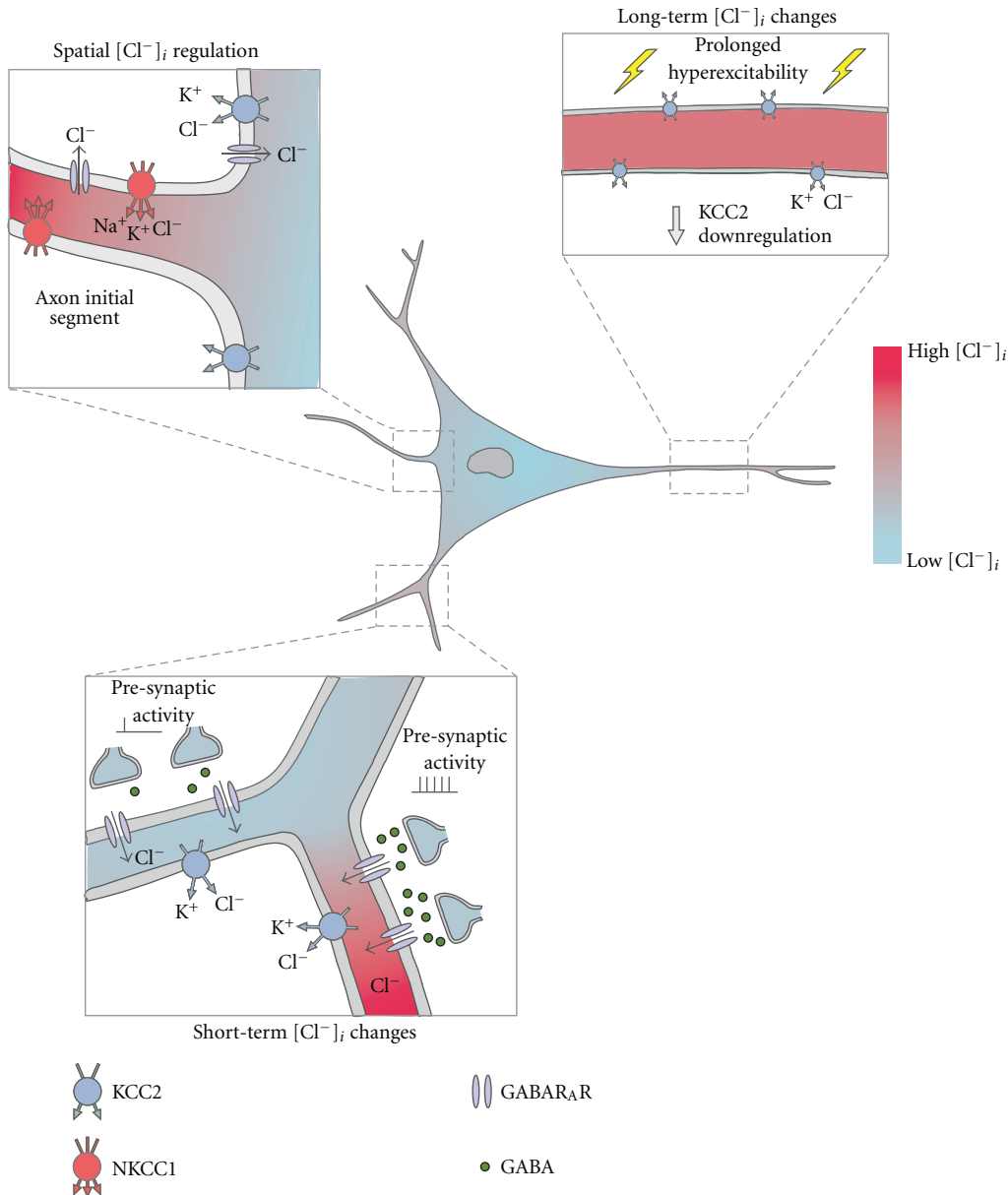
FIGURE 1: $[Cl^-]_i$ and the associated driving force for $GABA_A$Rs can be subject to spatial and activity-dependent temporal variations. The upper left panel shows an example of spatially regulated $[Cl^-]_i$. It has been reported that low levels of KCC2 expression within the axon initial segment enable NKCC1 to maintain relatively high levels of $[Cl^-]_i$ compared to the soma (indicated by the red colour inside the cell) [22–24]. This can generate a depolarising Cl^- driving force for $GABA_A$Rs within the axon [21–23]. The lower left panel shows an example of short-term $[Cl^-]_i$ loading within dendritic branches. Cl^- influx associated with low-level $GABA_A$R activity is dealt with by Cl^- regulation mechanisms (left-hand dendritic branch). However, during periods of intense $GABA_A$R activation, if E_{Cl^-} is hyperpolarised with respect to the membrane potential, high levels of Cl^- influx via $GABA_A$Rs can lead to localised increases in $[Cl^-]_i$ and consequently depolarising shifts in E_{GABA} (right-hand dendritic branch) [25, 26]. The upper right panel illustrates an example of long-term $[Cl^-]_i$ changes. Certain patterns of neural activity within mature neurons (e.g., repetitive coincidental pre- and postsynaptic spiking or prolonged postsynaptic spiking, interictal-like activity) can lead to a downregulation in KCC2 activity, resulting in long-term increases in $[Cl^-]_i$ [10, 27, 28].

3. Short-Term Temporal Changes in E_{GABA}

In addition to spatial variation, E_{GABA} can also show rapid temporal changes within individual cells (see Figure 1). It is well known that responses to intense $GABA_A$R activation can change from being hyperpolarising to depolarising in less than a second [36, 47, 48]. Such biphasic responses are now generally thought to represent a depolarising shift in E_{GABA}, caused by the differential collapse of the opposing concentration gradients of Cl^- and HCO_3^- [25, 26, 49]. $GABA_A$Rs are approximately five times more permeable to Cl^- than HCO_3^- [50]. Therefore at rest, E_{GABA} (typically -75 mV) is much closer to the very negative Cl^- reversal (E_{Cl^-}; typically -85 mV) than the considerably more positive

HCO_3^- reversal ($E_{HCO_3^-}$; typically -20 mV) [51]. During intense activation of $GABA_A$Rs however, rapid Cl^- influx exceeds Cl^- extrusion mechanisms and a breakdown in the Cl^- gradient occurs. An equivalent collapse of the HCO_3^- gradient is prevented by the activity of intra- and extracellular carbonic anhydrases, which use CO_2 as a substrate to rapidly regenerate intracellular HCO_3^-. As a result, with continued $GABA_A$R activation E_{GABA} shifts toward the more positive $E_{HCO_3^-}$, and this accounts for the depolarising phase of the biphasic response [25, 52]. Indeed, by blocking carbonic anhydrase with the drug acetazolamide, the depolarising response to strong $GABA_A$R activation is prevented [26]. Interestingly, a recent paper argues that this GABA elicited depolarisation is paradoxically accentuated by the activity of the electroneutral cotransporter KCC2 [53]. Following the $GABA_A$R—mediated accumulation of intracellular Cl^-, this leads to an accelerated extrusion of both Cl^- and K^+ by KCC2. Provided this extrusion of K^+ occurs within a large enough neuronal population, the increase in extracellular K^+ can result in inward K^+ currents that further depolarise the cell membrane [49, 53].

The shifts in E_{GABA} that are associated with intense $GABA_A$R activation are transient, such that once $GABA_A$R activity subsides $[Cl^-]_i$ returns to baseline levels within seconds or minutes [25, 54]. Any factor that affects the rate of Cl^- accumulation during $GABA_A$R activation will affect how rapidly and by how much E_{GABA} shifts. For instance, the volume of the neuronal compartment that receives the GABAergic input is one important parameter. For a given amount of synaptic $GABA_A$R stimulation and its accompanying Cl^- influx, smaller postsynaptic volumes will result in relatively larger increases in $[Cl^-]_i$. As a result, dendritic compartments are more susceptible to Cl^- accumulation (and hence depolarising shifts in E_{GABA}) than the soma [25, 41]. In a theoretical paper, Qian and Sejnowski [55] utilised this reasoning to suggest that $GABA_A$R-mediated inhibition is likely to be ineffective on dendritic spines. Due to their minute volume, even small amounts of Cl^- influx would result in a local increase in $[Cl^-]_i$ that would rapidly depolarise E_{GABA}. Consistent with this idea, it has since been confirmed that most GABAergic synapses are localised to dendritic shafts as opposed to spines [56, 57]. As described above, another important factor that affects Cl^- accumulation during $GABA_A$R activity is the presence, affinity and capacity of carbonic anhydrase. Given the significance of cell volume and carbonic anhydrase activity, it is perhaps not surprising therefore that different cell types might differ in their susceptibility to Cl^- accumulation. For example, Lamsa and Taira [54] found that 10–100 Hz stimulation trains produce depolarising switches in the E_{GABA} of interneurons of the CA3 stratum pyramidale and stratum oriens regions, but were unable to evoke similar shifts in CA3 pyramidal neurons.

In order to evoke the depolarising shifts in E_{GABA} described above, intense $GABA_A$R activation has been elicited either by exogenous application of $GABA_A$R agonists or high-frequency stimulation of GABAergic afferents. Evidence that such short-term changes in E_{GABA} could occur in vivo have come from studies of hyperactive network activity patterns, such as those generated in experimental models of epilepsy. It is believed that the intense activation of $GABA_A$Rs that occurs during seizures can cause rapid Cl^- accumulation [58–62]. Indeed, the resultant erosion of $GABA_A$R-mediated inhibition serves to initiate or exacerbate the hyperexcitability that is characteristic of epileptiform events [63]. Beyond seizure activity, it is currently an open question as to what range of physiologically relevant activity patterns could lead to short-term changes to E_{GABA}, and what the functional impact upon circuit function might be. Nevertheless it is interesting that levels of $[Cl^-]_i$ accumulation would appear to increase linearly with the intensity and number of stimulations, and even relatively weak stimulation can produce small changes in $[Cl^-]_i$ [62, 64].

Another area that has yet to be fully investigated concerns how short-term activity-dependent shifts in E_{GABA} might affect developing neurons. It has already been established that during the first two weeks of postnatal life, rat hippocampal neurons express low levels of intracellular carbonic anhydrase and therefore do not exhibit the HCO_3^- dependent $GABA_A$R depolarisation that mature neurons display following high-frequency synaptic activity [52, 65]. And it seems likely that other properties of immature neurons would contribute to a different susceptibility to activity-driven Cl^- accumulation or depletion. These include the higher resting $[Cl^-]_i$ observed in young neurons, plus different expression patterns of Cl^- transporter proteins [5, 66, 67] and Cl^- permeable channels [45, 68]. One area for future work will be to dissect the role that short-term activity-driven shifts in E_{GABA} play in both the normal and abnormal development of neural circuits.

4. Long-Term Temporal Changes in E_{GABA}

As we saw in the previous section, brief periods of high-intensity synaptic activity can give rise to short-term changes in the ionic driving force for $GABA_A$Rs. There are however, a growing number of examples whereby different forms of neural activity can give rise to more enduring changes in E_{GABA} and the underlying $[Cl^-]_i$ (see Figure 1). Many of these long-term changes in E_{GABA} are linked to hyperexcitability disorders, such as epilepsy [8–10, 15, 16, 69] and neuropathic pain [7, 17, 70, 71] and have also been observed in other cases of neuronal trauma such as neural axotomy [11], ischemia [12, 13], and in spasticity models following spinal cord injury [14]. Yet similar long-lasting changes to E_{GABA} have also been reported in healthy tissue following certain neural activity patterns [27, 28, 72–78]. In order to better understand these shifts in inhibitory plasticity and their roles in both healthy and pathological neural signalling, a number of studies have begun to investigate the underlying mechanisms behind long-term activity-dependent changes to E_{GABA}.

One of the first such investigations focused on the effects of epileptiform activity in hippocampal slices. Here, interictal activity, brought on with low Mg^{2+} conditions, switched the driving force of GPSPs from hyperpolarising to depolarising in CA1 pyramidal cells [10]. This depolarising shift in E_{GABA} corresponded to a significant reduction in

KCC2 mRNA and protein levels, as well as an increased rate of removal of the Cl^- cotransporter from the cell membrane [10]. Similar reductions in KCC2 mRNA and protein could also be observed following *in vivo* kindling [9], and in both cases the activity-led downregulation in KCC2 expression was found to be dependent on BDNF signaling. Scavenging endogenous BDNF with TrkB receptor bodies, or pharmacologically inhibiting TrkB, blocked the activity-induced downregulation of KCC2 and thus suggests that the mechanism involves a BDNF-TrkB signalling interaction [9, 10]. A similar role for BDNF-TrkB signalling has since been reported in the context of positive shifts in E_{GABA} and reductions in KCC2 levels within neuropathic and inflammatory pain models [7, 79, 80], suggesting that endogenous BDNF signalling may be a common mechanism by which KCC2 is downregulated during aberrant neural activity.

Aside from pathological models, changes to E_{GABA} and the resultant inhibitory plasticity have also been investigated in the context of more normal physiological signalling. For example, periods of paired pre- and postsynaptic spiking activity have been found to lead to a small but persistent depolarising shift in the postsynaptic E_{GABA}, of around 3-4 mV in mature rat hippocampal pyramidal neurons [27]. Such long-term depolarising shifts in E_{GABA} have also been observed following sustained periods of postsynaptic spiking at frequencies of 10–20 Hz, without presynaptic activity [28]. In both cases the reduction in GABAergic synaptic inhibition was linked to a sustained decrease in KCC2 transporter activity, which in turn was dependent upon Ca^{2+} signalling via L-type Ca^{2+} channels [27, 28]. Further investigation revealed that the activity-dependent downregulation in KCC2 activity requires protein kinase C (PKC) activity, although other studies have since shown that PKC can promote KCC2 activity by stabilising the cotransporter at the membrane surface [81]. Interestingly, Wang et al. [77] recorded from neurons of the subthalamic nucleus and showed for the first time that GABAergic plasticity could be induced in either direction, either generating hyperpolarising or depolarising shifts in GPSPs depending on the degree of rebound spiking activity. Based on further pharmacological experiments the authors proposed that the level of Ca^{2+} increases may be key to determining the nature of GABA$_A$R plasticity, with large increases being associated with negative shifts in E_{GABA} and small rises in Ca^{2+} leading to positive shifts in E_{GABA} [77].

Developmental stage would appear to be critically important for determining the nature and mechanism underlying long-term changes in the ionic driving force for GABA$_A$Rs. Within mature cells, such activity-driven changes appear to work by targeting KCC2 and reducing the activity and/or expression of this transporter. This raises the question of what happens within younger neurons when E_{GABA} is still depolarising and levels of KCC2 protein are typically low. Are immature neural networks subject to similar activity-dependent long-term $[Cl^-]_i$ alterations and if so, what are the downstream targets for such mechanisms? To date only a small number of studies have addressed this question directly but already an interesting dichotomy between mature and immature GABA$_A$R plasticity regulation is beginning to emerge. For example, as already mentioned, in mature hippocampal slices when E_{GABA} is hyperpolarising, application of seizure models has been linked to a depolarising shift in E_{GABA} values coupled with a downregulation in KCC2 expression [9, 10]. By contrast, in neonatal hippocampal slices, seizure activity induced by kainic acid have been found to result in either a depolarising [8, 82] or a hyperpolarising shift in E_{GABA} [83, 84]. In the latter cases, more negative E_{GABA} values have been linked to an increase in KCC2 expression and activity [84, 85].

Such variations may be partially due to the type of seizure model used, yet similar age-dependent differences can also be found in other examples of activity-driven E_{GABA} changes. For example, a protocol of paired pre- and postsynaptic spiking at 5 Hz, which has been shown previously to elicit depolarising E_{GABA} shifts in the mature rat hippocampus [27], actually produces a long-term hyperpolarising shift when applied to the same neurons earlier in development [72, 78]. Rather than targeting KCC2, the hyperpolarising shift in E_{GABA} in immature neurons occurs via a down-regulation of the NKCC1 transporter, which results in a decrease in $[Cl^-]_i$ [72]. As in mature systems, the direction of such GABA$_A$R shifts in developing neurons can change according to the nature of the stimulus. While paired pre- and postsynaptic spiking at 5 Hz hyperpolarised E_{GABA} at developing synapses, stimulation at higher frequencies (20–50 Hz) produces the opposite effect and results in E_{GABA} values that are more depolarising [78]. This long-term shift in driving force for GABA$_A$Rs induced by high-frequency paired spiking was again mediated through the regulation of NKCC1 activity and required increases in intracellular Ca^{2+}, either via L-type Ca^{2+} channels or from internal Ca^{2+} stores [78]. Thus, while spiking-induced activation of L-type Ca^{2+} channels can result in a similar increase in $[Cl^-]_i$ in both mature and immature hippocampal neurons, the frequency at which it occurs, and the Cl^- cotransporter that is targeted, varies according to developmental stage. A similar phenomenon has been observed following periods of experimentally induced postsynaptic spiking. Prolonged spiking at 20 Hz has been shown to lead to depolarising shifts in E_{GABA} within both the mature [28] and immature hippocampus [86]. Yet while the underlying mechanism has been linked to Ca^{2+} influx and KCC2 downregulation in mature cells [28], in younger neurons the change in E_{GABA} would appear to occur via a different mechanism. Here, postsynaptic spiking is believed to trigger increases in Na^+-K^+-ATPase activity, which alters the balance of Na^+ across the membrane. This shifts the thermodynamic equilibrium of NKCC1 and results in an increase in the rate at which Cl^- is transported into the cell [86]. Thus, just as spatial regulation of E_{GABA} can show age-specific variation, the mechanisms underlying long-term activity-dependent changes in E_{GABA} can also vary according to the developmental stage of the neuron.

What are the functional consequences of such long-term alterations to E_{GABA}? Changes to $[Cl^-]_i$ and the resultant Cl^- driving force for GABA$_A$Rs have been speculated to be involved in long-term potentiation (LTP)—the best

studied form of persistent change in synaptic efficacy. In a recent study, Ormond and Woodin [73] found that paired stimulation protocols designed to induce glutamatergic LTP in mature rat hippocampal slices also produced depolarising shifts in E_{GABA}. The resultant reduction in the strength of inhibitory synaptic input occurred in parallel to "classical" LTP at glutamatergic synapses, with both serving to potentiate synaptic transmission. As with classical LTP, this form of disinhibition-mediated potentiation was found to be dependent upon Ca^{2+} influx via NMDARs [73]. Indeed, other work has shown that activation of NMDARs can lead to a rapid and enduring decrease in KCC2-mediated Cl^- transport [87], while NMDAR signalling during LTP induction leads to a reduction in the total levels of KCC2 [88]. It has yet to be established whether or not this apparent GABAergic plasticity is relatively synapse specific, as has been reported for glutamatergic LTP, or whether GABAergic inputs are affected across larger parts of the dendrite or indeed across the entire cell. Nevertheless, these findings raise the possibility that the expression of NMDAR-mediated LTP might involve a component of GABAergic plasticity.

Amongst neurological disorders, neural trauma and hyperactivity have been shown to lead to long-term changes in the E_{GABA} of the affected neurons. Yet such changes to the ionic driving force for GABA$_A$Rs may in turn work to contribute to, or exacerbate, the abnormal activity patterns associated with these pathological states. In a landmark paper investigating the propagation of epileptic activity between two interconnected and intact hippocampi, Khalilov et al. [8] showed that seizure activity in one hippocampus could propagate to the naive hippocampus and eventually transform it into an epileptic structure capable of generating seizures. Subsequent investigation of the E_{Cl^-} of neurons in this secondary epileptic focus revealed that the cells had undergone an excitatory shift in the driving force of their GABA$_A$R synapses. Stimulating GABA$_A$Rs within the secondary focus resulted in bursts of action potentials in the absence of any glutamatergic signalling, leading the authors to conclude that such excitatory actions of GABA may generate seizures in the newly epileptic tissue [8]. Such shifts in the E_{GABA} do not need to be overtly excitatory in order to alter neural circuit activity. In rat dentate granule cells, induction of status epilepticus via *in vivo* pilocarpine injections can lead to depolarising E_{GABA} and impaired Cl^- extrusion capabilities [15]. The depolarising GPSPs increased the probability of action potential generation when paired with excitatory inputs and compromised the ability of the dentate gyrus to filter inputs from the entorhinal cortex [15].

The long-term and short-term changes to E_{GABA} observed in pathological states, or following pathological activity patterns, can be considered as relatively large changes, often switching the driving force of GABA$_A$Rs from hyperpolarising to depolarising and even excitatory [8, 10, 15, 60–62, 70]. By contrast, changes to $[Cl^-]_i$ following what could be considered more physiologically normal spiking activity typically result in much smaller modifications to the driving force for GABA$_A$Rs, usually within the range of approximately 3–10 millivolts. Given these relatively modest

shifts an important question is to what extent such plasticity might alter subsequent activity in the affected cells. Artificially setting the E_{GABA} of a neuron is one way of exploring how changes to the ionic driving force for GABA$_A$Rs may impact activity. This has been achieved experimentally by either altering $[Cl^-]_i$ via intracellular dialysis of different Cl^- concentrations during whole-cell recordings, or by simulating GABAergic inputs with different E_{GABA} values using the dynamic-clamp recording configuration. In several studies that have adopted these approaches, shifting E_{GABA} to depolarising values led to increased neuronal excitability, resulting in enhanced spiking probability and reduced spike latencies in response to GABAergic inputs, as well as facilitation of voltage-sensitive NMDAR transmission [89–92]. Another approach which has made it possible to explore the functional impact of relatively small changes in $[Cl^-]_i$ and E_{GABA} has been computational modelling. These studies have shown that modest shifts in E_{GABA} can have a significant impact on neural signalling. For example, changing the E_{GABA} in a model of a mature CA1 pyramidal neuron from -75 mV to -70 mV (a similar level of long-term depolarising shift to that observed experimentally) results in an increase in action potential firing frequency by approximately 40% [92]. Likewise, positive shifts in inhibitory reversal potentials by as little as 10 mV can markedly shorten the duration of inhibitory inputs within the soma [33]. Changes to neural output resulting from modest shifts in E_{GABA} can be further exaggerated depending on other factors such as the frequency and location of GABAergic inputs [33, 93]. For instance, in neonatal spinal cord, GPSPs are depolarising but still mediate inhibitory effects via shunting actions. In computational models of these neurons, moving the E_{Cl^-} to more depolarised values reduces the time window over which GPSPs exert functional inhibition of excitatory activity within the soma, particularly when the shift in E_{Cl^-} occurs at distal inhibitory inputs so that shunting effects associated with the GABAergic conductance have less impact [33].

Modest changes to E_{GABA} are likely to be especially significant when the balance between GABA$_A$R inhibition and facilitation is a fine one. For example, in neocortical layer 5 pyramidal neurons E_{GABA} has been calculated to lie at values more depolarising than the resting membrane potential, but below the action potential threshold [94]. Depending on their timing in relation to glutamatergic inputs, somatic GABA$_A$R inputs can either shunt or facilitate excitatory inputs, which can impose a bidirectional modulation on neuronal firing rates [94]. By simulating different timing relationships between GABAergic and glutamatergic inputs in a model neocortical neuron, Morita et al. [95] showed that such bidirectional modulation of firing rates by GABA$_A$Rs was possible when the E_{GABA} lies within a narrow range of values close to the original E_{GABA} value calculated by Gulledge and Stuart [94]. Increasing E_{GABA} by only a few millivolts was enough to severely reduce the relative timing window in which GABA$_A$R inputs could have an inhibitory effect upon neuronal firing rate compared to a facilitating one. Moving E_{GABA} more negative by a few millivolts, such that it was equal to the resting membrane potential of the model cell, was sufficient to render GABA$_A$R inputs

completely inhibitory, regardless of their relative timing to glutamatergic inputs [95]. Similarly, it has been shown that when E_{GABA} falls within a specific voltage range, GPSPs can have a bidirectional effect on spike times in visual cortex—either delaying or advancing the time of spikes relative to oscillatory changes in membrane potential [96]. As precise spike timing has been widely implicated in neural processing [97–99] and synaptic plasticity [97, 98], the alterations in spiking activity brought on by small shifts in E_{GABA} may therefore have important consequences for information coding and brain development.

5. Summary

In summary, the driving force for GABA$_A$Rs should not be considered a fixed parameter underlying fast synaptic inhibition, but rather a dynamic parameter, that exhibits both spatial and activity-dependent modulation. The concept that E_{GABA} changes in the context of neural development and certain neuropathological conditions is well established. However, more recent studies in this area have revealed that neurons have a range of sophisticated mechanisms for regulating the ionic driving force for GABA$_A$Rs. E_{GABA} has been reported to vary between different cellular compartments and may even exhibit synapse-specific variation within a single neuron. In addition, the driving force for GABA$_A$Rs can be changed "on the fly" and is subject to both short- and long-term temporal changes via a range of activity-dependent mechanisms. These processes are further subject to developmental regulation, where changes in activity patterns can target different regulators of $[Cl^-]_i$ and drive E_{GABA} in different directions depending on the age of the neuron. Further dissecting the mechanisms that regulate such a fundamental aspect of GABAergic transmission should improve our understanding of synaptic integration mechanisms in both health and disease.

Acknowledgments

References

[1] Y. Ben-Ari, E. Cherubini, R. Corradetti, and J. L. Gaiarsa, "Giant synaptic potentials in immature rat CA3 hippocampal neurones," *Journal of Physiology*, vol. 416, pp. 303–325, 1989.

[2] R. Tyzio, G. L. Holmes, Y. Ben-Ari, and R. Khazipov, "Timing of the developmental switch in GABA mediated signaling from excitation to inhibition in CA3 rat hippocampus using gramicidin perforated patch and extracellular recordings," *Epilepsia*, vol. 48, supplement 5, pp. 96–105, 2007.

[3] R. Khazipov, I. Khalilov, R. Tyzio, E. Morozova, Y. Ben-Ari, and G. L. Holmes, "Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus," *European Journal of Neuroscience*, vol. 19, no. 3, pp. 590–600, 2004.

[4] K. Ganguly, A. F. Schinder, S. T. Wong, and M. M. Poo, "GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition," *Cell*, vol. 105, no. 4, pp. 521–532, 2001.

[5] J. A. Payne, C. Rivera, J. Voipio, and K. Kaila, "Cation-chloride co-transporters in neuronal communication, development and trauma," *Trends in Neurosciences*, vol. 26, no. 4, pp. 199–206, 2003.

[6] E. Papp, C. Rivera, K. Kaila, and T. F. Freund, "Relationship between neuronal vulnerability and potassium-chloride cotransporter 2 immunoreactivity in hippocampus following transient forebrain ischemia," *Neuroscience*, vol. 154, no. 2, pp. 677–689, 2008.

[7] J. A. Coull, S. Beggs, D. Boudreau et al., "BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain," *Nature*, vol. 438, no. 7070, pp. 1017–1021, 2005.

[8] I. Khalilov, G. L. Holmes, and Y. Ben-Ari, "In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures," *Nature Neuroscience*, vol. 6, no. 10, pp. 1079–1085, 2003.

[9] C. Rivera, H. Li, J. Thomas-Crusells et al., "BDNF-induced TrkB activation down-regulates the K^+-Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion," *Journal of Cell Biology*, vol. 159, no. 5, pp. 747–752, 2002.

[10] C. Rivera, J. Voipio, J. Thomas-Crusells et al., "Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2," *Journal of Neuroscience*, vol. 24, no. 19, pp. 4683–4691, 2004.

[11] J. Nabekura, T. Ueno, A. Okabe et al., "Reduction of KCC2 expression and GABA receptor-mediated excitation after In vivo axonal injury," *Journal of Neuroscience*, vol. 22, no. 11, pp. 4412–4417, 2002.

[12] B. B. Pond, K. Berglund, T. Kuner, G. Feng, G. J. Augustine, and R. D. Schwartz-Bloom, "The chloride transporter Na^+-K^+-Cl^- cotransporter isoform-1 contributes to intracellular chloride increases after in vitro ischemia," *Journal of Neuroscience*, vol. 26, no. 5, pp. 1396–1406, 2006.

[13] N. Jaenisch, O. W. Witte, and C. Frahm, "Downregulation of potassium chloride cotransporter KCC2 after transient focal cerebral ischemia," *Stroke*, vol. 41, no. 3, pp. e151–e159, 2010.

[14] P. Boulenguez, S. Liabeuf, R. Bos et al., "Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury," *Nature Medicine*, vol. 16, no. 3, pp. 302–307, 2010.

[15] H. R. Pathak, F. Weissinger, M. Terunuma et al., "Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy," *Journal of Neuroscience*, vol. 27, no. 51, pp. 14012–14022, 2007.

[16] I. Cohen, V. Navarro, S. Clemenceau, M. Baulac, and R. Miles, "On the origin of interictal activity in human temporal lobe epilepsy in vitro," *Science*, vol. 298, no. 5597, pp. 1418–1421, 2002.

[17] Y. Lu, J. Zheng, L. Xiong, M. Zimmermann, and J. Yang, "Spinal cord injury-induced attenuation of GABAergic inhibition in spinal dorsal horn circuits is associated with downregulation of the chloride transporter KCC2 in rat," *Journal of Physiology*, vol. 586, no. 23, pp. 5701–5715, 2008.

[18] M. Martina, S. Royer, and D. Pare, "Cell-type-specific GABA responses and chloride homeostasis in the cortex and amygdala," *Journal of Neurophysiology*, vol. 86, no. 6, pp. 2887–2895, 2001.

[19] J. Chavas and A. Marty, "Coexistence of excitatory and inhibitory GABA synapses in the cerebellar interneuron network," *Journal of Neuroscience*, vol. 23, no. 6, pp. 2019–2031, 2003.

[20] A. Gulacsi, C. R. Lee, A. Sík et al., "Cell type-specific differences in chloride-regulatory mechanisms and GABA(A) receptor-mediated inhibition in rat substantia nigra," *Journal of Neuroscience*, vol. 23, no. 23, pp. 8237–8246, 2003.

[21] A. Woodruff et al., "Depolarizing effect of neocortical chandelier neurons," *Front Neural Circuits*, vol. 3, p. 15, 2009.

[22] S. Khirug, J. Yamada, R. Afzalov, J. Voipio, L. Khiroug, and K. Kaila, "GABAergic depolarization of the axon initial segment in cortical principal neurons is caused by the Na-K-2Cl cotransporter NKCC1," *Journal of Neuroscience*, vol. 28, no. 18, pp. 4635–4639, 2008.

[23] J. Szabadics, C. Varga, G. Molnár, S. Oláh, P. Barzó, and G. Tamás, "Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits," *Science*, vol. 311, no. 5758, pp. 233–235, 2006.

[24] R. Baldi, C. Varga, and G. Tamas, "Differential distribution of KCC2 along the axo-somato-dendritic axis of hippocampal principal cells," *European Journal of Neuroscience*, vol. 32, no. 8, pp. 1319–1325, 2010.

[25] K. J. Staley and W. R. Proctor, "Modulation of mammalian dendritic GABA(A) receptor function by the kinetics of Cl^- and HCO3- transport," *Journal of Physiology*, vol. 519, part 3, pp. 693–712, 1999.

[26] K. J. Staley, B. L. Soldo, and W. R. Proctor, "Ionic mechanisms of neuronal excitation by inhibitory GABA(A) receptors," *Science*, vol. 269, no. 5226, pp. 977–981, 1995.

[27] M. A. Woodin, K. Ganguly, and M. M. Poo, "Coincident pre- and postsynaptic activity modifies GABAergic synapses by postsynaptic changes in Cl^- transporter activity," *Neuron*, vol. 39, no. 5, pp. 807–820, 2003.

[28] H. Fiumelli, L. Cancedda, and M. M. Poo, "Modulation of GABAergic transmission by activity via postsynaptic Ca^{2+}-dependent regulation of KCC2 function," *Neuron*, vol. 48, no. 5, pp. 773–786, 2005.

[29] A. R. Woodruff, S. A. Anderson, and R. Yuste, "The enigmatic function of chandelier cells," *Front Neurosci*, vol. 4, p. 201, 2010.

[30] L. L. Glickfeld, J. D. Roberts, P. Somogyi, and M. Scanziani, "Interneurons hyperpolarize pyramidal cells along their entire somatodendritic axis," *Nature Neuroscience*, vol. 12, no. 1, pp. 21–23, 2009.

[31] G. Molnar, S. Oláh, G. Komlósi et al., "Complex events initiated by individual spikes in the human cerebral cortex," *PLoS Biology*, vol. 6, no. 9, article e222, 2008.

[32] K. J. Staley and I. Mody, "Shunting of excitatory input to dentate gyrus granule cells by a depolarizing GABA(A) receptor-mediated postsynaptic conductance," *Journal of Neurophysiology*, vol. 68, no. 1, pp. 197–212, 1992.

[33] C. Jean-Xavier, G. Z. Mentis, M. J. O'Donovan, D. Cattaert, and L. Vinay, "Dual personality of GABA/glycine-mediated depolarizations in immature spinal cord," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 27, pp. 11477–11482, 2007.

[34] J. Duebel, S. Haverkamp, W. Schleich et al., "Two-photon imaging reveals somatodendritic chloride gradient in retinal on-type bipolar cells expressing the biosensor clomeleon," *Neuron*, vol. 49, no. 1, pp. 81–94, 2006.

[35] T. Waseem et al., "Genetically encoded Cl^- Sensor as a tool for monitoring of Cl^- dependent processes in small neuronal compartments," *The Journal of Neuroscience Methods*, vol. 193, no. 1, pp. 14–23, 2010.

[36] P. Andersen, R. Dingledine, and L. Gjerstad, "Two different responses of hippocampal pyramidal cells to application of gamma-amino butyric acid," *Journal of Physiology*, vol. 305, pp. 279–296, 1980.

[37] M. Hara, M. Inoue, T. Yasukura, S. Ohnishi, Y. Mikami, and C. Inagaki, "Uneven distribution of intracellular Cl^- in rat hippocampal neurons," *Neuroscience Letters*, vol. 143, no. 1-2, pp. 135–138, 1992.

[38] K. L. Perkins and R. K. Wong, "Ionic basis of the postsynaptic depolarizing GABA response in hippocampal pyramidal cells," *Journal of Neurophysiology*, vol. 76, no. 6, pp. 3886–3894, 1996.

[39] T. Kuner and G. J. Augustine, "A genetically encoded ratiometric indicator for chloride: capturing chloride transients in cultured hippocampal neurons," *Neuron*, vol. 27, no. 3, pp. 447–459, 2000.

[40] H. Romo-Parra, M. Treviño, U. Heinemann, and R. Gutiérrez, "GABA actions in hippocampal area CA3 during postnatal development: differential shift from depolarizing to hyperpolarizing in somatic and dendritic compartments," *Journal of Neurophysiology*, vol. 99, no. 3, pp. 1523–1534, 2008.

[41] P. Jedlicka et al., "Activity-dependent intracellular chloride accumulation and diffusion controls GABA(A) receptor-mediated synaptic transmission," *Hippocampus*, 2010.

[42] C. Földy, S.-H. Lee, R. J. Morgan et al., "Regulation of fast-spiking basket cell synapses by the chloride channel ClC-2," *Nature Neuroscience*, vol. 13, no. 9, pp. 1047–1049, 2010.

[43] K. Staley, "The role of an inwardly rectifying chloride conductance in postsynaptic inhibition," *Journal of Neurophysiology*, vol. 72, no. 1, pp. 273–284, 1994.

[44] R. L. Smith, G. H. Clayton, C. L. Wilcox, K. W. Escudero, and K. J. Staley, "Differential expression of an inwardly rectifying chloride conductance in rat brain neurons: a potential mechanism for cell-specific modulation of postsynaptic inhibition," *Journal of Neuroscience*, vol. 15, no. 5, part 2, pp. 4057–4067, 1995.

[45] I. Rinke, J. Artmann, and V. Stein, "ClC-2 voltage-gated channels constitute part of the background conductance and assist chloride extrusion," *Journal of Neuroscience*, vol. 30, no. 13, pp. 4776–4786, 2010.

[46] P. Blaesse, M. S. Airaksinen, C. Rivera, and K. Kaila, "Cation-chloride cotransporters and neuronal function," *Neuron*, vol. 61, no. 6, pp. 820–838, 2009.

[47] B. E. Alger and R. A. Nicoll, "GABA-mediated biphasic inhibitory responses in hippocampus," *Nature*, vol. 281, no. 5729, pp. 315–317, 1979.

[48] S. M. Thompson and B. H. Gahwiler, "Activity-dependent disinhibition. II. effects of extracellular potassium, furosemide, and membrane potential on ECl- in hippocampal CA3 neurons," *Journal of Neurophysiology*, vol. 61, no. 3, pp. 512–523, 1989.

[49] K. Kaila, K. Lamsa, S. Smirnov, T. Taira, and J. Voipio, "Long-lasting GABA-mediated depolarization evoked by high-frequency stimulation in pyramidal neurons of rat hippocampal slice is attributable to a network-driven, bicarbonate-dependent K^+ transient," *Journal of Neuroscience*, vol. 17, no. 20, pp. 7662–7672, 1997.

[50] K. Kaila, "Ionic basis of GABA(A) receptor channel function in the nervous system," *Progress in Neurobiology*, vol. 42, no. 4, pp. 489–537, 1994.

[51] N. Lambert and L. Grover, "The mechanism of biphasic GABA responses," *Science*, vol. 269, no. 5226, pp. 928–929, 1995.

[52] C. Rivera, J. Voipio, and K. Kaila, "Two developmental switches in GABAergic signalling: the K^+-Cl^- cotransporter KCC2 and carbonic anhydrase CAVII," *Journal of Physiology*, vol. 562, part 1, pp. 27–36, 2005.

[53] T. Viitanen, E. Ruusuvuori, K. Kaila, and J. Voipio, "The K$^+$-Cl$^-$ cotransporter KCC2 promotes GABAergic excitation in the mature rat hippocampus," *Journal of Physiology*, vol. 588, part 9, pp. 1527–1540, 2010.

[54] K. Lamsa and T. Taira, "Use-dependent shift from inhibitory to excitatory GABA receptor action in SP-O interneurons in the rat hippocampal CA3 area," *Journal of Neurophysiology*, vol. 90, no. 3, pp. 1983–1995, 2003.

[55] N. Qian and T. J. Sejnowski, "When is an inhibitory synapse effective?" *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 20, pp. 8145–8149, 1990.

[56] T. F. Freund and G. Buzsaki, "Interneurons of the hippocampus," *Hippocampus*, vol. 6, no. 4, pp. 347–470, 1996.

[57] M. Megias, Z. Emri, T. F. Freund, and A. I. Gulyás, "Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells," *Neuroscience*, vol. 102, no. 3, pp. 527–540, 2001.

[58] V. Lopantsev and M. Avoli, "Participation of GABA(A)-mediated inhibition in ictallike discharges in the rat entorhinal cortex," *Journal of Neurophysiology*, vol. 79, no. 1, pp. 352–360, 1998.

[59] K. Lamsa and K. Kaila, "Ionic mechanisms of spontaneous GABAergic events in rat hippocampal slices exposed to 4-amino-pyridine," *Journal of Neurophysiology*, vol. 78, no. 5, pp. 2582–2591, 1997.

[60] Y. Fujiwara-Tsukamoto, Y. Isomura, A. Nambu, and M. Takada, "Excitatory gaba input directly drives seizure-like rhythmic synchronization in mature hippocampal CA1 pyramidal cells," *Neuroscience*, vol. 119, no. 1, pp. 265–275, 2003.

[61] Y. Fujiwara-Tsukamoto, Y. Isomura, M. Imanishi, T. Fukai, and M. Takada, "Distinct types of ionic modulation of GABA actions in pyramidal cells and interneurons during electrical induction of hippocampal seizure-like network activity," *European Journal of Neuroscience*, vol. 25, no. 9, pp. 2713–2725, 2007.

[62] Y. Isomura, M. Sugimoto, Y. Fujiwara-Tsukamoto, S. Yamamoto-Muraki, J. Yamada, and A. Fukuda, "Synaptically activated Cl$^-$ accumulation responsible for depolarizing GABAergic responses in mature hippocampal neurons," *Journal of Neurophysiology*, vol. 90, no. 4, pp. 2752–2756, 2003.

[63] B. Lasztoczi, G. Nyitrai, L. Héja, and J. Kardos, "Synchronization of GABAergic inputs to CA3 pyramidal cells precedes seizure-like event onset in juvenile rat hippocampal slices," *Journal of Neurophysiology*, vol. 102, no. 4, pp. 2538–2553, 2009.

[64] K. Berglund, W. Schleich, H. Wang et al., "Imaging synaptic inhibition throughout the brain via genetically targeted clomeleon," *Brain Cell Biology*, vol. 36, no. 1–4, pp. 101–118, 2008.

[65] E. Ruusuvuori, H. Li, K. Huttu et al., "Carbonic anhydrase isoform VII acts as a molecular switch in the development of synchronous gamma-frequency firing of hippocampal CA1 pyramidal cells," *Journal of Neuroscience*, vol. 24, no. 11, pp. 2699–2707, 2004.

[66] V. I. Dzhala, D. M. Talos, D. A. Sdrulla et al., "NKCC1 transporter facilitates seizures in the developing brain," *Nature Medicine*, vol. 11, no. 11, pp. 1205–1213, 2005.

[67] C. Rivera, J. Voipio, J. A. Payne et al., "The K$^+$/Cl$^-$ cotransporter KCC2 renders GABA hyperpolarizing during neuronal maturation," *Nature*, vol. 397, no. 6716, pp. 251–255, 1999.

[68] C. M. Becker, H. Betz, and H. Schroder, "Expression of inhibitory glycine receptors in postnatal rat cerebral cortex," *Brain Research*, vol. 606, no. 2, pp. 220–226, 1993.

[69] E. Palma, M. Amici, F. Sobrero et al., "Anomalous levels of Cl$^-$ transporters in the hippocampal subiculum from temporal lobe epilepsy patients make GABA excitatory," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 22, pp. 8465–8468, 2006.

[70] J. A. Coull, D. Boudreau, K. Bachand et al., "Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain," *Nature*, vol. 424, no. 6951, pp. 938–942, 2003.

[71] S. W. Cramer, C. Baggott, J. Cain et al., "The role of cation-dependent chloride transporters in neuropathic pain following spinal cord injury," *Molecular Pain*, vol. 4, article 36, p. 36, 2008.

[72] T. Balena and M. A. Woodin, "Coincident pre- and postsynaptic activity downregulates NKCC1 to hyperpolarize E(Cl) during development," *European Journal of Neuroscience*, vol. 27, no. 9, pp. 2402–2412, 2008.

[73] J. Ormond and M. A. Woodin, "Disinhibition mediates a form of hippocampal long-term potentiation in area CA1," *PLoS One*, vol. 4, no. 9, article e7224, 2009.

[74] M. Ouardouz and B. R. Sastry, "Activity-mediated shift in reversal potential of GABA-ergic synaptic currents in immature neurons," *Developmental Brain Research*, vol. 160, no. 1, pp. 78–84, 2005.

[75] M. Ouardouz, J. Y. Xu, and B. R. Sastry, "Theta bursts set up glutamatergic as well as GABA-ergic plasticity in neonatal rat hippocampal CA1 neurons," *Brain Research*, vol. 1068, no. 1, pp. 65–69, 2006.

[76] J. Y. Xu and B. R. Sastry, "Theta-bursts induce a shift in reversal potentials for GABA-A receptor-mediated postsynaptic currents in rat hippocampal CA1 neurons," *Experimental Neurology*, vol. 204, no. 2, pp. 836–839, 2007.

[77] L. Wang, S. T. Kitai, and Z. Xiang, "Activity-dependent bidirectional modification of inhibitory synaptic transmission in rat subthalamic neurons," *Journal of Neuroscience*, vol. 26, no. 28, pp. 7321–7327, 2006.

[78] C. Xu, M. X. Zhao, M. M. Poo, and X. H. Zhang, "GABA(B) receptor activation mediates frequency-dependent plasticity of developing GABAergic synapses," *Nature Neuroscience*, vol. 11, no. 12, pp. 1410–1418, 2008.

[79] G. Miletic and V. Miletic, "Loose ligation of the sciatic nerve is associated with TrkB receptor-dependent decreases in KCC2 protein levels in the ipsilateral spinal dorsal horn," *Pain*, vol. 137, no. 3, pp. 532–539, 2008.

[80] W. Zhang, L. Y. Liu, and T. L. Xu, "Reduced potassium-chloride co-transporter expression in spinal cord dorsal horn neurons contributes to inflammatory pain hypersensitivity in rats," *Neuroscience*, vol. 152, no. 2, pp. 502–510, 2008.

[81] H. H. Lee, J. A. Walker, J. R. Williams, R. J. Goodier, J. A. Payne, and S. J. Moss, "Direct protein kinase C-dependent phosphorylation regulates the cell surface stability and activity of the potassium chloride cotransporter KCC2," *Journal of Biological Chemistry*, vol. 282, no. 41, pp. 29777–29784, 2007.

[82] R. Nardou, Y. Ben-Ari, and I. Khalilov, "Bumetanide, an NKCC1 antagonist, does not prevent formation of epileptogenic focus but blocks epileptic focus seizures in immature rat hippocampus," *Journal of Neurophysiology*, vol. 101, no. 6, pp. 2878–2888, 2009.

[83] A. S. Galanopoulou, "Dissociated gender-specific effects of recurrent seizures on GABA signaling in CA1 pyramidal neurons: Role of GABA receptors," *Journal of Neuroscience*, vol. 28, no. 7, pp. 1557–1567, 2008.

[84] S. Khirug et al., "A single seizure episode leads to rapid functional activation of KCC2 in the neonatal rat hippocampus," *The Journal of Neuroscience*, vol. 30, no. 36, pp. 12028–12035, 2010.

[85] A. S. Galanopoulou, "Developmental patterns in the regulation of chloride homeostasis and GABA receptor signaling by seizures," *Epilepsia*, vol. 48, supplement 5, pp. 14–18, 2007.

[86] A. C. Brumback and K. J. Staley, "Thermodynamic regulation of NKCC1-mediated Cl$^-$ cotransport underlies plasticity of GABA signaling in neonatal neurons," *Journal of Neuroscience*, vol. 28, no. 6, pp. 1301–1312, 2008.

[87] A. Kitamura, H. Ishibashi, M. Watanabe, Y. Takatsuru, M. Brodwick, and J. Nabekura, "Sustained depolarizing shift of the GABA reversal potential by glutamate receptor activation in hippocampal neurons," *Neuroscience Research*, vol. 62, no. 4, pp. 270–277, 2008.

[88] W. Wang, N. Gong, and T. L. Xu, "Downregulation of KCC2 following LTP contributes to EPSP-spike potentiation in rat hippocampus," *Biochemical and Biophysical Research Communications*, vol. 343, no. 4, pp. 1209–1215, 2006.

[89] Y. F. Wang, X. B. Gao, and A. N. van den Pol, "Membrane properties underlying patterns of GABA-dependent action potentials in developing mouse hypothalamic neurons," *Journal of Neurophysiology*, vol. 86, no. 3, pp. 1252–1265, 2001.

[90] G. Valeeva et al., "Temporal coding at the immature depolarizing GABAergic synapse," *Front Cell Neurosc*, vol. 4, 2010.

[91] C. J. Akerman and H. T. Cline, "Depolarizing GABAergic conductances regulate the balance of excitation to inhibition in the developing retinotectal circuit in vivo," *Journal of Neuroscience*, vol. 26, no. 19, pp. 5117–5130, 2006.

[92] F. Saraga, T. Balena, T. Wolansky, C. T. Dickson, and M. A. Woodin, "Inhibitory synaptic plasticity regulates pyramidal neuron spiking in the rodent hippocampus," *Neuroscience*, vol. 155, no. 1, pp. 64–75, 2008.

[93] S. A. Prescott, T. J. Sejnowski, and Y. de Koninck, "Reduction of anion reversal potential subverts the inhibitory control of firing rate in spinal lamina I neurons: towards a biophysical basis for neuropathic pain," *Molecular Pain*, vol. 2, article 32, 2006.

[94] A. T. Gulledge and G. J. Stuart, "Excitatory actions of GABA in the cortex," *Neuron*, vol. 37, no. 2, pp. 299–309, 2003.

[95] K. Morita, K. Tsumoto, and K. Aihara, "Bidirectional modulation of neuronal responses by depolarizing GABAergic inputs," *Biophysical Journal*, vol. 90, no. 6, pp. 1925–1938, 2006.

[96] K. M. Stiefel, V. Wespatat, B. Gutkin, F. Tennigkeit, and W. Singer, "Phase dependent sign changes of GABAergic synaptic input explored in-silico and in-vitro," *Journal of Computational Neuroscience*, vol. 19, no. 1, pp. 71–85, 2005.

[97] B. A. Richards, O. P. Voss, and C. J. Akerman, "GABAergic circuits control stimulus-instructed receptive field development in the optic tectum," *Nature Neurosci*, vol. 13, no. 9, pp. 1098–1106, 2010.

[98] Y. Mu and M. M. Poo, "Spike timing-dependent LTP/LTD mediates visual experience-dependent plasticity in a developing retinotectal system," *Neuron*, vol. 50, no. 1, pp. 115–125, 2006.