ABSTRACT: In the CNS, prolonged activation of GABA<sub>A</sub> receptors (GABA<sub>ARs</sub>) has been shown to evoke biphasic postsynaptic responses, consisting of an initial hyperpolarization followed by a depolarization. A potential mechanism underlying the depolarization is an acute chloride (Cl<sup>-</sup>) accumulation resulting in a shift of the GABA<sub>A</sub> reversal potential ($E_{\text{GABA}}$). The amount of GABA-evoked Cl<sup>-</sup> accumulation and accompanying depolarization depends on presynaptic and postsynaptic properties of GABAergic transmission, as well as on cellular morphology and regulation of Cl<sup>-</sup> intracellular concentration ([Cl<sup>-</sup>]),. To analyze the influence of these factors on the Cl<sup>-</sup> and voltage behavior, we studied spatiotemporal dynamics of activity-dependent [Cl<sup>-</sup>], changes in multicompartmental models of hippocampal cells based on realistic morphological data. Simulated Cl<sup>-</sup> influx through GABA<sub>ARs</sub> was able to exceed physiological Cl<sup>-</sup> extrusion rates thereby evoking HCO<sub>3</sub><sup>-</sup>-dependent $E_{\text{GABA}}$ shift and depolarizing responses. Depolarizations were observed in spite of GABA<sub>A</sub> receptor desensitization. The amplitude of the depolarization was frequency-dependent and determined by intracellular Cl<sup>-</sup> accumulation. Changes in the dendritic diameter and in the speed of GABA clearance in the synaptic cleft were significant sources of depolarization variability. In morphologically reconstructed granule cells subjected to an intense GABAergic background activity, dendritic inhibition was more affected by accumulation of intracellular Cl<sup>-</sup> than somatic inhibition. Interestingly, $E_{\text{GABA}}$ changes induced by activation of a single dendritic synapse propagated beyond the site of Cl<sup>-</sup> influx and affected neighboring synapses. The simulations suggest that $E_{\text{GABA}}$ may differ even along a single dendrite supporting the idea that it is necessary to assign $E_{\text{GABA}}$ to a given GABAergic input and not to a given neuron. © 2010 Wiley-Liss, Inc.

KEY WORDS: bicarbonate; GABA reversal potential; GABAergic depolarization; KCC2; chloride diffusion; dendritic inhibition

INTRODUCTION

Inhibitory transmission regulates membrane potential dynamics in cortical neurons and their rhythmic activity (e.g., Vida et al., 2006; Rudolph et al., 2007; Atallah and Scanziani, 2009). Furthermore, fast GABAergic signaling is important for stabilizing persistent cortical states (Shu et al., 2003; Mann et al., 2009). This fast inhibition is mainly mediated by GABA<sub>A</sub> receptors (GABA<sub>ARs</sub>). Depending on the GABA<sub>A</sub> reversal potential ($E_{\text{GABA}}$), GABA-elicited currents induce hyperpolarizing, shunting, or depolarizing postsynaptic potentials. Hyperpolarizing inhibition acts linearly (subtraction), whereas shunting inhibition has divisive effects for membrane voltages (Ulrich, 2003), modulates neuronal gain (Chance et al., 2002; Mitchell and Silver, 2003; Prescott and De Koninck, 2003), and promotes coherent oscillations (Jeong and Gutkin, 2005; Stiefel et al., 2005; Vida et al., 2006).

In the developing neural tissue, GABA<sub>A</sub>R activation is depolarizing due to high intracellular Cl<sup>-</sup> concentration ([Cl<sup>-</sup>]) (Cherubini et al., 1991; Rivera et al., 1999). In adult neurons, repetitive activation of GABA<sub>ARs</sub> evokes biphasic postsynaptic responses: an initial hyperpolarization followed by a delayed depolarization. These are referred to in the literature as GDPSPs or GABA<sub>AR</sub>-mediated depolarizing postsynaptic potentials (Kaila et al., 1997; Herrero et al., 2002). Such biphasic GABA responses are seen in several brain areas (Staley and Proctor, 1999 and references therein). Experiments showed the GDPSP to depend on the HCO<sub>3</sub> reversibility of GABA<sub>ARs</sub> (Kaila et al., 1993; Bonnet and Bingmann, 1995; Dallwig et al., 1999; Sun et al., 2001; Herrero et al., 2002; Perez Velazquez, 2003).

More generally, $E_{\text{GABA}}$ is determined by equilibrium potentials of Cl<sup>-</sup> and HCO<sub>3</sub> ($E_{\text{Cl}}, E_{\text{HCO}_3}$). According to the Cl<sup>-</sup> accumulation hypothesis, intense GABA<sub>A</sub>R activation substantially increases [Cl<sup>-</sup>], so that $E_{\text{Cl}}$ shifts toward resting membrane potential ($E_{\text{rest}}$) (Kaila et al., 1989; Staley et al., 1995; Backus et al., 1998; Frech et al., 1999;
Staley and Proctor, 1999). During such stimulation, the HCO$_3^-$ gradient remains largely constant. As a consequence, $E_{\text{GABA}}$ may rise above $E_{\text{rest}}$, thus leading to GABAergic depolarizations. Kuner and Augustine (2000) demonstrated that GABA$_A$ input activation brings about local increase of [Cl$^-$]i spreading into nearby regions of the cell and shifting $E_{\text{GABA}}$. Thus, in addition to global changes, local alterations of [Cl$^-$]i occur under physiological as well as pathological conditions (c.f. Isomura et al., 2003; Vreugdenhil et al., 2005).

The amount of Cl$^-$ accumulation and accompanying depolarization following tetanic activation of GABAergic afferents depends on presynaptic and postsynaptic properties of GABAergic transmission, on regulation of neuronal [Cl$^-$]i and on particular cellular morphology. Our goal here is to address the following questions: How do Cl$^-$ accumulation and the subsequent GDPS depend on the frequency and synchronicity of GABA$_A$R activation, the Cl$^-$ extrusion rate and the volume of postsynaptic compartments? What is the impact of GABA release changes on the GABAergic depolarization? How do the spatiotemporal changes of [Cl$^-$]i result from the intracellular Cl$^-$ diffusion influence the GDPS? Does repetitive activation of a single synapse affect $E_{\text{GABA}}$ at neighboring synapses? To clarify these issues, we developed a biophysically realistic model of GABAergic neurotransmission. We use this model to study the interplay of key factors modulating the spatiotemporal dynamics of dendritic Cl$^-$ and membrane voltage. We further employ the Cl$^-$ diffusion model to predict activity-dependent Cl$^-$ accumulation in morphologically reconstructed hippocampal neurons subjected to a simulated in vivo-like bombardment of GABAergic synaptic conductances.

**METHODS**

**Membrane potential dynamics**

We used an equivalent circuit representation of neuronal compartment incorporating a GABA$_A$R permeability, a leak conductance responsible for the resting membrane potential, and a Cl$^-$ extrusion mechanism. In each compartment of a multicompartmental neuron model, membrane potential ($V$) was generated by activity-dependent ($E_{\text{Cl}}$) and activity-independent ($E_{\text{rest}}, E_{\text{HCO}_3}$) electrochemical potentials.

In the master equation, the sum of GABAergic and leak current was equal to the capacitative current as follows:

$$-C\cdot \frac{dV}{dt} = I_{\text{GABA}} + I_{\text{leak}} = I_{\text{GABA}} + g_{\text{rest}} \cdot (V - E_{\text{rest}})$$

Two parallel Cl$^-$ and HCO$_3^-$ ionic pathways were used to describe the GABAergic current as follows:

$$I_{\text{GABA}} = I_{\text{Cl}} + I_{\text{HCO}_3}$$

**GABA$_A$ Ionic Currents**

The Goldman-Hodgkin-Katz (GHK) constant field equation was used for the GABA$_A$ Cl$^-$ and for the HCO$_3^-$ current as well as for GABA$_A$ reversal potential (Kaila et al., 1989). To calculate synaptic currents flowing through a population of GABA$_A$Rs present in the considered compartment, we multiplied the single channel Cl$^-$ and HCO$_3^-$ current by the total number of receptors ($R_{\text{number}}$) and by the time-varying fraction of open receptors (Open) as follows:

$$I_{\text{Cl}} = P_{\text{Cl}} \left( \frac{VF^2}{RT} \right) \left( [\text{Cl}^-]_i - [\text{Cl}^-]_o \exp\left( \frac{VF}{RT} \right) \right) R_{\text{number}} \cdot \text{Open}$$

$$I_{\text{HCO}_3} = P_{\text{HCO}_3} \left( \frac{VF^2}{RT} \right) \left( [\text{HCO}_3^-]_i - [\text{HCO}_3^-]_o \exp\left( \frac{VF}{RT} \right) \right) R_{\text{number}} \cdot \text{Open}$$

$$E_{\text{GABA}} = -RT \frac{F}{R} \ln \left( \frac{[\text{Cl}^-]_o + P_{\text{rel}} [\text{HCO}_3^-]_o}{[\text{Cl}^-]_i + P_{\text{rel}} [\text{HCO}_3^-]_i} \right)$$

where $P_{\text{rel}}$ represents $P_{\text{HCO}_3}/P_{\text{Cl}}$ and $R$, $T$, $F$ have their usual meanings.

Differentiation ($d/dV$) of the GHK equation written for a monovalent anion yields the GABA-induced conductance for anion “a” per unit area of membrane (Kaila et al., 1989):

$$g_a =$$

$$P_a \frac{F^2}{RT} \left[ a[i][a]o + [a][i]z + ([a][i] - [a][o]) \left( \frac{VF}{RT} \right) z + [a][o] z^2 \right]$$

$$1 - z^2$$

where $z = \exp \left( \frac{VF}{RT} \right)$ and “a” is Cl$^-$ or HCO$_3^-$.

We adjusted the number of synaptic receptors ($R_{\text{number}}$) to get a synaptic GABAergic conductance of 1.5 nS. In simulations testing the effects of GABA$_A$ conductance changes, GABA$_A$ synapses were simulated as a postsynaptic parallel Cl$^-$ and HCO$_3^-$ conductance with exponential rise and exponential decay as follows:

$$I_{\text{GABA}} = I_{\text{Cl}} + I_{\text{HCO}_3} = (1 - P) \cdot g_{\text{GABA}} \cdot (V - E_{\text{Cl}})$$

$$+ P \cdot g_{\text{GABA}} \cdot (V - E_{\text{HCO}_3})$$

where $P$ is a fractional ionic conductance that was used to split the GABA$_A$ conductance into Cl$^-$ and HCO$_3^-$ conductance. $g_{\text{GABA}}$ was determined by two state kinetic scheme described by rise time ($\tau_{\text{a1}}$) and decay time constant ($\tau_{\text{a2}}$): $g_{\text{GABA}} = B - A$, $dA/dt = -A/\tau_{\text{a1}}$, $dB/dt = -B/\tau_{\text{a2}}$.

$E_{\text{Cl}}$ and $E_{\text{HCO}_3}$ were calculated from Nernst equation.

**GABA$_A$R Kinetic Model**

To incorporate GABA-induced gating of postsynaptic receptors, the Markov model of GABA$_A$R established by Jones and Westbrook (1995) was used (Supporting Information Fig. 4). The NMODL translator (Hines and Carnevale, 2000) converted the gating scheme into a family of differential equations and solved
them numerically assuming that at $t = 0$ ms no bound, open, or desensitized receptors were present. The model features two GABA binding steps (Bound1, Bound2) each providing access to open (Open1, Open2) and desensitized (D_{bound}, D_{bound}) states. Occupancies of open states (Open1, Open2) yield together the total fraction of open receptors needed for solving the GHK equations:

$$\text{Open} = \text{Open}_1 + \text{Open}_2$$

GABA-Induced $[\text{Cl}^-]_i$ Change

The contribution of GABA$_A$ chloride currents to the $\text{Cl}^-$ concentration inside the cellular compartment was calculated as follows:

$$\frac{d[\text{Cl}^-]_i}{dt} = \frac{1}{F} \cdot \frac{I_{\text{Cl}}}{\text{volume}} + \frac{[\text{Cl}^-]_{\text{rest}} - [\text{Cl}^-]_i}{\tau_{\text{Cl}}}$$

The equation represents GABA-mediated $\text{Cl}^-$ accumulation with exponential recovery (decay time constant $\tau_{\text{Cl}}$) to resting level $[\text{Cl}^-]_{\text{rest}}$. The decay approximates an outward $\text{Cl}^-$ transport mechanism with first order kinetics (c.f. Wagner et al., 2001). In some simulations (Fig. 1, Supporting Information Figs. 1 and 2), the $\text{Cl}^-$ pump velocity ($v$) was computed according to Lineweaver-Burke equation (Staley and Proctor, 1999):

$$\frac{1}{v} = K_D / ([\text{Cl}^-]_{\text{pump}} + 1/v_{\text{max}})$$

where $K_D$ is the neuronal $\text{Cl}^-$ concentration at which the extrusion rate is half maximal and $v_{\text{max}}$ is the maximum rate of $\text{Cl}^-$ transport. $K_D$ and $v_{\text{max}}$ were taken from the data by Staley and Proctor (1999). The contribution of GABA$_A$ chloride currents to the $\text{Cl}^-$ concentration inside the cellular compartment was calculated as follows:

$$\frac{d[\text{Cl}^-]_i}{dt} = \frac{1}{F} \cdot \frac{I_{\text{Cl}}}{\text{volume}} + \text{leak} - v_{\text{max}} \cdot \frac{[\text{Cl}^-]_i}{K_d + [\text{Cl}^-]_i}$$

where volume is the volume of the structure into which the current flows and leak is the $\text{Cl}^-$ leak that was included to achieve steady-state resting level of $[\text{Cl}^-]_i$ (8 mM).

Cl$^-$ Diffusion

Longitudinal $\text{Cl}^-$ diffusion was modeled as the exchange of $\text{Cl}^-$ between adjacent compartments. For radial diffusion, the volume was discretized into a series of concentric shells around a cylindrical core (De Schutter and Smolen, 1998). Diffusion coefficient was 2 $\mu$m$^2$/ms (Kuner and Augustine, 2000; Brumberg and Staley, 2008). Thus, we used standard compartmental diffusion modeling (De Schutter and Smolen, 1998) instead of modeling based on the electro-diffusion equation (Qian and Sejnowski, 1989, 1990). Our rationale was that for dendrites with their relatively large electrostatic size, the diffusion model is sufficient for our simulations. We based our reasoning on a number of points. First, Qian and Sejnowski (1989) pointed out that electrodiffusion proved significant corrections for highly electrotonically restricted structures such as spines or thin processes but not for relatively large dendrites. Second, it has been argued that the original electro-diffusion models used by Qian and Sejnowski (1989) may be of limited applicability to model ionic diffusion in dendrites as they were derived from simplified assumptions on charge carriers (De Schutter and Smolen, 1998). At the same time, an updated version of that model taking into account the details of charge carriers (Lopreore et al., 2008) presents technical complexity beyond the scope of our article (extensive required simulation times for presumably minor corrections). We thus decided that for purposes of our study including electro-diffusive terms would not significantly influence our results (see also De Schutter, 2010).

Time Course of GABA in the Synaptic Cleft

The GABA pulse was simulated as an exponentially decaying GABA transient:

$$[\text{GABA}] = A \cdot \exp(-t/\tau_{\text{GABA}})$$

where $A$ is the peak concentration and $\tau_{\text{GABA}}$ is the time constant of GABA clearance ($A = 2$ mM, $\tau_{\text{GABA}} = 0.1$ ms; Barberis et al., 2004).

Noise in Stimulus Spike Train

Interspike intervals were randomized by including fractional noise (0—no noise, 1—fully noisy). Fractional noise is a parameter in a NEURON’s built-in mechanism called NetStim using a Poisson distribution of the intervals between events.

Morphology of Reconstructed Neurons

We inserted GABA$_A$ synapses into a dendrite (Supporting Information Fig. 3A) of calbindin-containing CA1 interneuron (Gulyas et al., 1999; www.koki.hu/~gulyas/ca1cells). The diameter of the dendrite varied (0.92–0.3–0.26 $\mu$m) along its length (618.37 $\mu$m). In simulations of activity-dependent $\text{Cl}^-$ accumulation in granule cells of the dentate gyrus, GABA$_A$ synapses were placed on soma (25%) and dendritic (75%) branches (Halasy and Somogyi, 1993). The morphologically realistic passive granule cell models (Schmidt-Hieber et al., 2007) were downloaded from http://senselab.med.yale.edu/ModelDB/ShowModel.asp?model=95960.

Numerical Integration

The model was implemented in the simulation environment NEURON (www.neuron.yale.edu). The source code for GABAergic synaptic and ionic mechanisms was written in the model description language NMODL (Hines and Carnevale, 2000) and is available on request. Parameters used in our simulations were as follows: temperature $= 35^\circ\text{C}$, $g_{\text{leak}} = 0.2$ mS/cm$^2$, $E_{\text{rest}} = -60$ mV, $C = 1$ uF/cm$^2$, $R_a = 150$ ohm-cm, $[\text{Cl}^-]_o = 8$ mM, $[\text{Cl}^-]_i = 133.5$ mM, $[\text{HCO}_3^-]_o = 16$ mM, $[\text{HCO}_3^-]_i = 26$ mM, $P_{\text{rel}} = 0.25$, $P_{\text{Cl}} = 8 \times 10^{-14}$ cm$^3$/s, $\tau_{\text{GABA}} = 0.1$ ms, $t_{\text{Cl}} = 3$ s, $[\text{GABA}] = 2$ mM, diffusion coefficient $= 2$ $\mu$m$^2$/ms. The rates of GABA$_A$R kinetic model were identical to those of Barberis et al. (2004). Some of these parameters were varied as explained in the relevant figures. In

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simulations of background GABAergic activity in granule cells, following parameters were used: basal $[\text{Cl}^-]$ = 5 mM corresponding to $E_{\text{GABA}}$ of $-73.8$ mV; somatic synapses: rise time 0.25 ms, decay time 5.5 ms, 1.6 nS conductance; dendritic synapses: 0.5 ms rise time, 6 ms decay time, 0.5 nS conductance (Santhakumar et al., 2005); $V_{\text{rest}}$: $-70$ mV (passive properties were taken from Schmidt-Hieber et al., 2007).

Due to the low resting potential ($-80$ mV), IPSPs are usually slightly depolarizing in dentate granule cells. To test whether $\text{Cl}^-$ accumulation is able to evoke hyper-to-depolarization switch, we set the resting potential of granule cells to $-70$ mV.

Rationale for this amendment: We describe the $-70$ mV resting potential of granule cells also in the legend for the Figure 7 (see Fig. 7) but it would be better if this information was also in the Methods section.

**FIGURE 1.** Dependence of GABA-induced voltage responses and corresponding $[\text{Cl}^-]$ changes on the relative $\text{HCO}_3^-/\text{Cl}^-$ permeability ($P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$) in a single compartment dendritic model. GABAergic synapses (20) were inserted into a dendritic segment (volume = 75 $\mu$m$^3$; Bracci et al., 2001) and activated. In A–C, the black, brighter black and gray traces represent voltage/[Cl$^-$] changes at $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ = 0.3, 0.2, and 0, respectively. The duration of stimulation was 3 s. Resting membrane potential level is indicated by the dashed line. D: The relation between stimulation frequencies, $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ and GABA-induced changes of membrane potential. GDPSP: maximal GABA$_A$R-mediated depolarizing potential. The range of relative $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ values (0.18–0.44) experimentally determined (Bormann et al., 1987; Fatima-Shad and Barry, 1993) is indicated by the gray area between the vertical lines. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
RESULTS

First, we wanted to test whether the activation of GABA<sub>A</sub>Rs leads to significant Cl<sup>-</sup> influx and accounts for corresponding electrophysiological responses. To do so, we created a biophysical model of GABAergic synapse containing GABA<sub>A</sub>Rs (see Methods) based on the kinetic GABA<sub>A</sub>R model of Jones and Westbrook (1995). We inserted 20 GABAergic synapses into a single compartment model of dendritic segment with a defined volume (75 μm<sup>3</sup>, cf. Bracci et al., 2001). We then determined the dependence of the voltage–time relation at different stimulation frequencies on the relative HCO<sub>3</sub><sup>-</sup> permeability (Fig. 1).

As expected, the GABA-induced depolarization (GDPSP) increased with increasing relative ratio of HCO<sub>3</sub><sup>-</sup> to Cl<sup>-</sup> permeability (P<sub>HCO<sub>3</sub></sub>/P<sub>Cl</sub>). Using the single compartment model, we also investigated the dependence of GDPSP on relevant physical parameters: the Cl<sup>-</sup> extrusion rate, the number of activated synapses, and the dendritic diameter (see Supporting Information Figs. 1 and 2). The [Cl<sup>-</sup>]<sub>i</sub> necessary to drive E<sub>GABA</sub> to a more positive value than V<sub>rest</sub> was 10.6 mM (Supporting Information Fig. 1). The GDPSP amplitude increased with decreasing maximum Cl<sup>-</sup> pump velocity (Supporting Information Fig. 1G), increasing number of simultaneously active GABAergic inputs (Supporting Information Fig. 2A), and with shrinking diameter of postsynaptic dendritic compartment (Supporting Information Fig. 2B). These results were in agreement with simplified simulations of GABA<sub>A</sub> depolarizing responses (Staley and Proctor, 1999; Bracci et al., 2001).

Although useful for basic estimates of changes and interactions of important variables affecting GABA-induced responses, the single compartment model of the dendrite provides only limited amount of information (see also Staley and Proctor,
1999; Bracci et al., 2001). Specifically, such a single compartment model by design neglects both longitudinal and radial Cl\textsuperscript{−} diffusion within neurons with complex geometries. Therefore, to study spatial Cl\textsuperscript{−} concentration changes under morphologically realistic conditions, we inserted the model of GABA\textsubscript{A}Rs including the Cl\textsuperscript{−} diffusion into a dendritic compartment of a reconstructed neuron. We first chose a calbindin-containing CA1 interneuron (Gulyas et al., 1999) since it allowed us to study spatiotemporal Cl\textsuperscript{−} dynamics within a long unbranched dendrite.

To study the impact of synapse location on GDPSPs, we placed a GABAergic synapse at three different positions: at the distal end (diameter \( \approx 0.26 \) μm), the proximal end (diameter \( \approx 0.92 \) μm), and in the middle (diameter \( \approx 0.3 \) μm) of the dendrite (length \( \approx 618.37 \) μm). 10 Hz (regular or noisy) stimulation train was applied and resulting voltage and [Cl\textsuperscript{−}] changes were computed at corresponding locations (Fig. 2). Interestingly, 10 Hz activation of a single synapse at the distal dendritic end was sufficient to induce significant local [Cl\textsuperscript{−}] change and a switch to locally depolarizing responses (Fig. 2A, D). In contrast, proximally located synapses remained hyperpolarizing.

Given the above results, we reasoned that simultaneous activation of GABAergic synapses may lead to a spatial and temporal summation of Cl\textsuperscript{−} and voltage transients and affect dendritic \( E_{GABA} \). Therefore, we next examined the electrochemical consequences of a larger number of stimulated GABAergic synaptic inputs present on the dendritic surface (Fig. 3). Synchronous 10 Hz activation (20 pulses) of 13 synapses (located in the dendrite in equidistant positions, Supporting Information Fig. 3) evoked Cl\textsuperscript{−} accumulation was more pronounced in distal dendritic segments than in proximal ones. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**FIGURE 3.** Intracellular Cl\textsuperscript{−} accumulation and diffusion and GABA-induced responses following tetanic activation of dendritic inputs in a multicompartmental neuronal model. Upper graph: Local voltage changes brought about by synchronous repetitive (10 Hz, 20 pulses) activation of 13 GABA\textsubscript{A} synapses. Voltage values were recorded at the distal end, the proximal end, and in the middle of the dendrite. Lower graph: GABA\textsubscript{A} conductance decreases with time because of GABA\textsubscript{A}R desensitization (see Supporting Information Figs. 3, 4). Nevertheless, the desensitization does not prevent continuous accumulation of Cl\textsuperscript{−} within distal dendritic and middle segments as indicated by the monotonic increase of \([\text{Cl}^−]/g_{GABA}\) ratio. Shape plot: Spatial pattern of intracellular Cl\textsuperscript{−} concentration [Cl\textsuperscript{−}], at the end of the tetanic stimulation (synchronous 10 Hz activation of 13 GABA\textsubscript{A} synapses). The color code indicates [Cl\textsuperscript{−}]; change. [Cl\textsuperscript{−}] increased significantly in the stimulated dendrite. Note that the Cl\textsuperscript{−} diffusion to nearby “silent” dendrites did not markedly change their [Cl\textsuperscript{−}]. Cl\textsuperscript{−} accumulation was more pronounced in distal dendritic segments than in proximal ones. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
accumulation varying along the dendrite and leading to a hyperpolarizing/depolarizing switch in distal and in middle dendritic segments (Fig. 3). Thus, Cl\(^{-}\) accumulation and diffusion due to cooperative action of multiple synapses converted also synaptic responses in the middle of the dendrite to a depolarization (c.f. Figs. 2B and 3). By contrast, the proximal segments did not display depolarizing voltage changes (Fig. 3). This demonstrates the region-dependence and input-specificity of \(E_{\text{GABA}}\) within a given neuron. Repetitive activation of GABA\(_{\text{A}}\)Rs leads to their desensitization (Jones and Westbrook, 1995) and a gradual decrease in GABA\(_{\text{A}}\) channel conductance (Supporting Information Fig. 3B).

However, even in the presence of desensitization, ongoing accumulation of Cl\(^{-}\) was observed in distal and middle dendritic segments as shown by the monotonic increase of \([\text{Cl}^{-}]_{i}/[\text{GABA}]_{o}\) ratio (Fig. 3). Hence, activity-dependent depolarization switch occurred in spite of GABA\(_{\text{A}}\)R desensitization. Nevertheless, under different conditions (e.g., at weaker GABA\(_{\text{A}}\) synapses with lower initial conductance and larger postsynaptic volume), Cl\(^{-}\) accumulation might be counteracted more effectively by the desensitization (c.f. Fig. 4C). In summary, tetanic stimulation of dendritic GABAergic afferents is able to evoke significant Cl\(^{-}\) accumulation and locally depolarizing \(E_{\text{GABA}}\) shifts in distal dendritic branches.

FIGURE 4. Factors affecting dendritic GDPSP amplitudes in the multicompartmental neuronal model. A–F: Synaptic density, dendritic size (diameter), GABA\(_{\text{A}}\)R conductance amplitude, conductance decay time, stimulation noise and synaptic GABA transient, respectively, determine the amplitude of GDPSPs following tetanic stimulation (10 or 40 Hz, B–F: 19 synapses). E: Each data point represents an average of five runs obtained with different random number generator seeds for individual synapses. Fractional noise randomizes the intervals between spikes (0: no noise; 1: noisy). F: First four columns represent GDPSP amplitudes in case of exponentially decaying synaptic GABA pulses with various values of \(\tau_{\text{GABA}}\) (0.1, 0.15, 0.2, 0.25 ms). The GDPSP in the fifth column was mediated by repetitive 1 ms square pulses of GABA, activating dendritic GABAergic synapses.
To investigate the relationship between the GDPSP amplitudes and the synaptic density, we varied the number of active dendritic synapses while recording voltage changes in the middle of the stimulated dendrite. Density greater than 1.5 synapses per 100 μm was sufficient to induce GABA-mediated depolarization at stimulation frequency of 10 Hz (Fig. 4A). We would like to note that in the dendritic tree of CA1 calbindin-containing interneurons there are ~18–50 synapses per 100 μm length of dendrite (Gulyás et al., 1999). Hence, we would expect such neurons to show the activity-dependent hyper-to-depolarization switch of their GABAergic synaptic potentials.

To determine the influence of dendritic size, synaptic strength and kinetics of synaptic responses on the amplitude of GDPSPs, dendritic diameter, GABA_A conductance, and conductance decay time were systematically modified (Figs. 4B–D). The simulations revealed a strongly nonlinear relationship between dendritic diameter and GDPSP amplitudes, with small dendritic diameters leading to large synaptically activated intracellular Cl^- accumulation and depolarization (Fig. 4B). In contrast, GDPSPs were found to be almost linearly proportional to GABA_A conductance amplitudes and decay times (Figs. 4C,D). Next, we wanted to assess the role of synchronization of synaptic inputs in inducing GDPSPs (Fig. 4C). A decrease of synaptic activation synchrony (due to noise in spike trains) reduced but did not abolish GDPSP amplitudes (Fig. 4E). Finally, we varied the time course of GABA concentration in the synaptic cleft and determined its modulatory effect on Cl^- accumulation and resulting depolarization. The GDPSP amplitude was sensitive to changes in decay time constant τ\textsubscript{GABA} (Fig. 4F).

Because of the slow Cl^- transport (Staley and Proctor, 1999; Wagner et al., 2001), the increased [Cl^-], outlasts GABA-mediated depolarization thus providing a higher [Cl^-], starting level for succeeding synaptic activity. This creates an opportunity for subsequent stimuli to evoke delayed GABA-dependent depolarizations. Therefore, to test this possibility, we studied the effect of single pulse stimulation at different time points after conditioning tetanic stimulation (Fig. 5). Indeed, when using a physiological extrusion rate (τ\textsubscript{Cl} = 3 s) (Staley and Proctor, 1999; Wagner et al., 2001), the GABA-induced Cl^- accumulation persisted for several seconds on a level sufficient to induce delayed GDPSPs.

Cortical neurons in vivo are subject to an intense excitatory and inhibitory synaptic bombardment due to high-frequency network activity (Steriade, 2001; Destexhe and Contreras, 2006). Therefore, we set out to determine how the intense GABAergic background activity in hippocampal neurons (Alger and Nicoll, 1980) affects their [Cl^-], and E\textsubscript{GABA}. To address this question, we monitored Cl^- dynamics in models of morphologically reconstructed granule cells (Schmidt-Hieber et al., 2007; see Methods) in which synaptic background activity was arising from the random release of dendritic and somatic GABA_A synapses. In these simulations, 75% of all GABAergic synapses were located on granule cell dendrites (synaptic density: 0.5/μm; Megias et al., 2001) and 25% on granule cell somata, in agreement with electron microscopic studies (Halász and Somogyi, 1993). Conductances and kinetics of dendritic and somatic GABA_A synapses were based on electrophysiological data (Santhakumar et al., 2005; see Methods). Stochastic (Poisson) low frequency (0.1 Hz) activation of somatic and dendritic inhibitory synapses induced only minimal changes in somatic ([Cl^-] = 0.04 ± 0.01 mM) and dendritic ([Cl^-] = 0.08 ± 0.01 mM; n = 8 granule cells; Fig. 6). Dendritic increase of [Cl^-] was significantly higher than the somatic increase (P = 0.04), but did not lead to the depolarizing GABA switch (not shown). In contrast, stochastic (Poisson) high frequency background synaptic activity (10 Hz per synapse) evoked considerable rise of somatic ([Cl^-] = 2.8 ± 0.3 mM) and dendritic ([Cl^-] = 3.9 ± 0.3 mM; n = 8 granule cells; Fig. 6) leading to depolarizing dendritic and somatic potentials (not shown). Again, dendritic [Cl^-] changes were significantly greater than those observed in soma (P = 0.03). In summary, we predict that in dentate granule cells, intense synaptic GABAergic background activity may lead to substantial changes in Cl^- concentration potentially evoking depolarizing E\textsubscript{GABA} shifts. In addition, our simulations confirm that dendritic compartments are more prone to [Cl^-], changes as compared with somata of hippocampal neurons.

Activation of synaptic GABA_ARs induces focal increase in Cl^- spreading by diffusion to adjacent dendritic areas. Therefore, we wanted to test how repetitive activation of a single dendritic synapse affects neighboring synapses in the dendritic tree of granule cells. We determined E\textsubscript{GABA} as a function of distance from synaptic input located at distal or central dendritic site in eight reconstructed cells (Figs. 7A,B). While stochastic 100 Hz, 40 Hz, and 10 Hz stimulation induced significant Cl^- accumulation associated with a spatial E\textsubscript{GABA} change, stimulation at 1 Hz frequency...
evoked only minimal changes. In the model granule cells (with a resting potential of \(-70\) mV; Santhakumar et al., 2005; see Methods), a change in \(E_{\text{GABA}}\) of more than \(3.8\) mV caused GABAergic synaptic input to switch from hyperpolarization to depolarization. Interestingly, depending on its location and frequency, activity at a single synapse was able to switch \(E_{\text{GABA}}\) at neighboring synaptic sites. The depolarization switch induced by 40 and 100 Hz stimulation of distal GABA\(_A\) input spread within 36 ± 6 \(\mu\)m and 72 ± 5 \(\mu\)m beyond the site of Cl\(^-\) influx, respectively (Fig. 7A). Activation of GABA\(_A\) input in the center of a dendrite at 100 Hz frequency also induced the depolarizing switch spreading 33 ± 13 \(\mu\)m and 7 ± 2 \(\mu\)m distally (toward the “sealed” end) and proximally (to soma), respectively (Fig. 7B). Furthermore, our simulations showed that the spatial propagation of \(E_{\text{GABA}}\) depends strongly on the strength of GABAergic synapses and dendritic diameter (Figs. 7C,D).

FIGURE 6. Cl\(^-\) accumulation and \(E_{\text{GABA}}\) shift in granule cells subjected to a stochastic activity of dendritic and somatic GABA\(_A\) synapses. A: Shape plot of granule cell morphology. The color code indicates \(E_{\text{GABA}}\) change following stochastic activation of dendritic and somatic GABA\(_A\) synapses. The main shape plot and the inset show changes of \(E_{\text{GABA}}\) in the same granule cell following background GABAergic activity with a mean frequency of 10 Hz and 0.1 Hz, respectively. Note that whereas 10 Hz stimulation induced significant Cl\(^-\) accumulation and a shift of \(E_{\text{GABA}}\) (most prominent in distal dendritic segments), 0.1 Hz stimulation produced only minimal changes. B: Quantification of \(E_{\text{GABA}}\) and [Cl\(^-\)] change in soma and distal dendrites of eight reconstructed granule cells subjected to 0.1 Hz and 10 Hz background activity at GABAergic synapses. Simulation parameters: density of dendritic synapses: 0.5/\(\mu\)m (Megias et al., 2001); relative number of dendritic versus somatic GABA\(_A\) synapses: 75 versus 25% (Halasy and Somogyi 1993). Morphology and passive properties were taken from Schmidt-Hieber et al. (2007). Conductance values and kinetics of dendritic and somatic GABA\(_A\) synapses were taken from Santhakumar et al. (2005) (see Methods). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

In this study, we analyzed spatiotemporal Cl\(^-\) and voltage dynamics in neuronal dendrites and soma using computational modeling approach. Our simulations indicate that GABA\(_A\)-mediated Cl\(^-\) accumulation is sufficient to generate depolarizations in small-volume neuronal compartments receiving intense GABAergic input. The amplitude of GABAergic depolarizations was frequency-dependent and followed the intracellular Cl\(^-\) accumulation. Dendritic Cl\(^-\) influx through GABA\(_A\)Rs following their tectonic activation was able to exceed physiological Cl\(^-\) extrusion rates thereby increasing the [Cl\(^-\)]

and shifting \(E_{\text{GABA}}\). These findings are in agreement with previous experimental studies (Dallwig et al., 1999; Staley and Proctor, 1999; Bracci et al., 2001; Isomura et al., 2003). Our computational results predict distal dendritic GABAergic transmission to be more influenced by prolonged stimulation than proximal dendritic GABAergic transmission. Furthermore, in model granule cells subjected to an intense GABAergic background activity, dendritic inhibition was more affected by accumulation of intracellular Cl\(^-\) than somatic inhibition. The simulations suggest that \(E_{\text{GABA}}\) may differ even along a single dendrite supporting the idea that it is necessary to assign \(E_{\text{GABA}}\) to a given GABAergic input and not to a given neuron (Blaesse et al., 2009).

Cl\(^-\) Accumulation and GABAergic Depolarization

The general concept that GABA-induced chloride flux can substantially alter [Cl\(^-\)], has been proposed and supported by several studies (Huguenard and Alger, 1986; Akaie et al., 1987; Kaila et al., 1989; Thompson and Gähwiler, 1989; Ling and Benardo, 1995; Kuner and Augustine, 2000; De Fazio and Hablitz, 2001; Wagner et al., 2001; Isomura et al., 2003; Berglund et al., 2008). As an additional mechanism, network driven K\(^+\) accumulation is thought to enhance the depolarizing response by both direct membrane depolarization and a reduction of Cl\(^-\) extrusion (McCarren and Alger, 1985; Kaila et al., 1997; Bazhenov et al., 2008; see note added in proof; see also Perkins and Wong, 1996; Perkins, 1999). Our simulations suggest that under appropriate conditions, the GDPSP may be evoked by tectonic stimulation even if GABAergic activity is not accompanied by extracellular K\(^+\) accumulation. Thus, substantial CI\(^-\) concentration changes in small dendrites are sufficient to shift \(E_{\text{GABA}}\) to depolarizing values. Importantly, we show that Cl\(^-\) and \(E_{\text{GABA}}\) changes can propagate beyond the site of
synaptic $\text{Cl}^-$ influx (Kuner and Augustine, 2000) and that activity at a single synapse can affect $E_{\text{GABA}}$ of adjacent synapses located within tens of $\mu$m away from the active synapse (see also Doyon et al., 2008).

**Impact of $\text{Cl}^-$ Extrusion Mechanisms**

By varying the velocity of $\text{Cl}^-$ pump, we found higher $\text{Cl}^-$ extrusion rates to decrease GDPSPs. However, when using a physiological $\text{Cl}^-$ extrusion rate (Staley and Proctor, 1999; Wagner et al., 2001), in most conditions the GABA-induced $\text{Cl}^-$ accumulation reached a level sufficient to induce GDPSPs. Interestingly, electrophysiological experiments have shown that it is possible to fit the recovery of $[\text{Cl}^-]_i$ by a single exponent (Staley and Proctor, 1999; Wagner et al., 2001). This suggests that a single transporter (described by single exponential process) plays a crucial role in the recovery from $\text{Cl}^-$ accumulation (Staley and Proctor, 1999; Wagner et al., 2001). In most adult CNS neurons, $\text{K}^+\text{Cl}^-$ cotransporter 2 (KCC2) has been identified as the main chloride exporter (Gamba, 2005; Blaesse et al., 2009; as opposed to developing neurons where $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransporter 1 (NKCC1) plays a dominant role, Gamba, 2005; but see also Khirug et al., 2008). Nevertheless, in addition to GABA-mediated $\text{Cl}^-$ accumulation and KCC2-mediated $\text{Cl}^-$ extrusion, other $\text{Cl}^-$ influx/efflux pathways such as $\text{Cl}^--\text{HCO}_3^-$ exchangers, ATP-driven $\text{Cl}^-$ pumps and voltage-sensitive $\text{Cl}^-$ channels may contribute to $[\text{Cl}^-]_i$ changes (Isomura et al., 2003; Gamba, 2005; Rinke et al., 2010). Thus, further studies are necessary to analyze the contribution of these additional mechanisms to GABA-induced intracellular $\text{Cl}^-$ dynamics in mature neurons. Moreover, in future work, it would be interesting to analyze $\text{Cl}^-$ homeostasis in detailed models of mature neurons.

FIGURE 7. Spatial spread of $E_{\text{GABA}}$ shift in granule cells triggered by repetitive activation of a single dendritic GABA$_A$ input. A: Frequency dependence of $E_{\text{GABA}}$ shift in eight reconstructed granule cells following stochastic activation of a single GABA$_A$ synapse located at the distal end of the dendrite. B: Frequency dependence of $E_{\text{GABA}}$ changes following stochastic activation of a single GABA$_A$ synapse located in the center of the dendrite. See text for more details. C and D: Spatial $E_{\text{GABA}}$ changes triggered by activation of a distal dendritic synapse depend strongly on its conductance and dendritic diameter. Simulation parameters: A, B, C, and D: $g_{\text{GABA}}$ rise time 0.5 ms, decay time 6 ms (Santhakumar et al., 2005); resting potential: $-70$ mV; initial $E_{\text{GABA}} = -73.83$ mV; stimulation duration: 5 s; A, B, and C: $g_{\text{GABA}} = 0.5$ nS (Santhakumar et al., 2005).
immature neuronal cells where Cl\textsuperscript{−} extrusion/intrusion rates and morphological properties are different.

**Modulation of Synaptic GABA Release**

As tetanic stimulation can modulate GABA release (Ghijisen et al., 2007), presynaptic short-term plasticity mechanisms might be at work influencing the frequency dependence of GDPSs (Manuel and Davies, 1998; Patenaude et al., 2003). Indeed, the magnitude of GDPSs is promoted by blocking GABA\textsubscript{A} receptors (Cobb et al., 1999). GABA\textsubscript{B} receptors antagonists enhance the duration of GABA release making the depolarizing GABA response excitatory and proconvulsive (Kantrowitz et al., 2005).

This implies that presynaptic GABA\textsubscript{B} autoreceptors mediate activity-dependent depression of GDPSs thereby preventing the development of pathological depolarizing GABA responses. Consistent with this presynaptic mechanism, in our simulations, Cl\textsuperscript{−} accumulation and depolarization were highly sensitive to changes of GABA time course in the synaptic cleft. Changes in the speed of GABA clearance were an important source of GDPS amplitude variability. This can be explained by the fact that peak GABA concentration (2 mM) was subsaturating (Mozrymas et al., 2003; Barberis et al., 2004), thus leaving space for variability due to changes in GABA decay.

**Time Course of Cl\textsuperscript{−} Accumulation**

Although Cl\textsuperscript{−} accumulation can account for high-frequency induced depolarizing GABAergic potentials (Isomura et al., 2003), some phenomena seen under experimental conditions do not seem to be explicable by Cl\textsuperscript{−} accumulation alone. Lamsa and Taïra (2003) have observed that single pulse stimuli elicited depolarizing PSPs in hippocampal interneurons until 45 s after 40 Hz tetanus. In neurons, the decay of the [Cl\textsuperscript{−}]\textsubscript{i} can be considerably slow, lasting several seconds (Staley and Proctor, 1999; Kuner and Augustine, 2000; Wagner et al., 2001; Marandi et al., 2002; Jin et al., 2005). However, the long duration of readiness for depolarizing responses after conditioning stimulus train would require even slower decay of the [Cl\textsuperscript{−}]\textsubscript{i} (tens of seconds). Extracellular K\textsuperscript{+} accumulation due to intense network activity may be an extra mechanism for slowing down or reversing the activity of K\textsuperscript{+}-Cl\textsuperscript{−} cotransporters (Jarolimek et al., 1999) and thus maintaining internal Cl\textsuperscript{−} elevation for longer time periods (Fujitawa-Tsukamoto et al., 2007; see also note added in proof). Another possibility is Ca\textsuperscript{2+}-dependent downregulation of K\textsuperscript{+}-Cl\textsuperscript{−} cotransporter function (Woodin et al., 2003; Fiumelli et al., 2005; Lee et al., 2007) which would enhance the Cl\textsuperscript{−} accumulation and prolong the increase of [Cl\textsuperscript{−}]\textsubscript{i}.

Intriguingly, a recent study has reported that intracellular Cl\textsuperscript{−} ions directly modulate GABA\textsubscript{A}R kinetics thus conferring an additional level of complexity to the time course of Cl\textsuperscript{−} accumulation and GABA-mediated synaptic responses (Houston et al., 2009).

**Background Activity and Compartment-Specific Changes of Cl\textsuperscript{−} and \(E_{GABA}\)**

Hippocampal neurons typically receive a tonic bombardment of inhibitory synaptic currents (Alger and Nicoll, 1980; Otis et al., 1991). Therefore, we studied how background activity at GABAergic synapses impacts intracellular Cl\textsuperscript{−} and \(E_{GABA}\) in anatomically realistic models of granule cells (Schmidt-Hieber et al., 2007). Intense stochastic activation of dendritic and somatic GABA\textsubscript{A} synapses evoked significant changes in Cl\textsuperscript{−} concentration and \(E_{GABA}\). Of note, our prediction that high-frequency background activity may influence [Cl\textsuperscript{−}]\textsubscript{i} can be tested by monitoring Cl\textsuperscript{−} and \(E_{GABA}\) changes following experimental manipulation of spontaneous network activity (e.g., using TTX and KCl).

Our simulations further showed that dendrites were subject to larger [Cl\textsuperscript{−}] changes when compared with granule cell bodies. In line with these computational results, in hippocampal principal neurons, dendritic inhibition has been shown to be more affected by accumulation of intracellular Cl\textsuperscript{−} than somatic inhibition (Alger and Nicoll, 1979; Andersen et al., 1980; Staley and Proctor, 1999).

All in all we would suggest that a significant somato-dendritic GABA-reversal gradient would appear in an activity-dependent manner in neurons subjected to physiologically relevant rates of GABAergic inputs. Hence, the GABA synapses in the dendrites would have a lower inhibitory impact on the cell. We may speculate that if it was important for the GABA efficacy to be stable regardless of the network activity, such dendritic GABA-reversal collapse would need to be compensated. Interestingly, a recent study has revealed an axo-somatic-dendritic gradient of steady state \(E_{GABA}\) and Cl\textsuperscript{−} likely reflecting distinct expression of Cl\textsuperscript{−} transporters within respective cellular domains of cortical neurons (Khur et al., 2008; but see Glickfeld et al., 2009; see also Bajet el al., 2006; Gavrikov et al., 2006). This evidence points toward compartment-specific mechanisms of \(E_{GABA}\) regulation that possibly may counteract an excessive activity-dependent collapse of \(E_{GABA}\) in dendrites.

**Functional Consequences of GABAergic Depolarization**

In several brain areas, GABA\textsubscript{A} receptor-mediated inhibition is functionally relevant for the generation of synchronous network activity (Nakanishi and Kukita, 2000; Nusser et al., 2001; Lamsa and Taïra, 2003; Atallah and Scanziani, 2009). The complex role of depolarizing \(E_{GABA}\) in network synchronization and excitability has recently been investigated in a num-
ber of studies. The value of $E_{\text{GABA}}$ has been found to dramatically affect action potential generation and firing rate modulation (Gulledge and Stuart, 2003; Morita et al., 2006; Prescott et al., 2006; Saraga et al., 2008). Most importantly, $E_{\text{GABA}}$ interacts with such factors as the speed of the synapse, the synaptic delay, and the dynamics of spike generation to determine the stability and synchrony of neuronal oscillations (Jeong and Gutkin, 2005; Stiefel et al., 2005; Morita et al., 2006; Vida et al., 2006; see also Jedlicka and Backus, 2006).

In conclusion, our simulations show that neurons, when exposed to in vivo-like conditions, should change their $[\text{Cl}^-]$, hence modifying the $E_{\text{GABA}}$ in an activity- and spatially dependent manner. This implies possible functional segregation of perisomatic and distal-dendritic fast inhibitory synaptic transmission.

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Note added in proof

Viitanen et al. (2010) have recently uncovered a positive feedback loop between GABA-induced intraneuronal chloride accumulation and potassium transients. Intraneuronal accumulation of chloride activates extrusion of chloride and potassium by the KCC2, thereby giving rise to depolarizing potassium transients.

REFERENCES

Akaike N, Inomata N, Tokutomi N. 1987. Contribution of chloride shifts to the fade of gamma-aminobutyric acid-gated currents in frog dorsal root ganglion cells. J Physiol 391:219–234.

Alger BE, Nicoll RA. 1979. GABA-mediated biphasic inhibitory responses in hippocampus. Nature 281:315–317.

Alger BE, Nicoll RA. 1980. Spontaneous inhibitory post-synaptic potentials in hippocampus: Mechanism for tonic inhibition. Brain Res 200:195–200.

Andersen P, Dingledine R, Gjerstad L, Langmoen IA, Laursen AM. 1990. Two different responses of hippocampal pyramidal cells to application of gamma-amino butyric acid. J Physiol 305:279–296.

Atallah BV, Scanziani M. 2009. Instantaneous modulation of gamma oscillation frequency by balancing excitation with inhibition. Neuron 62:566–577.

Bach KH, Deitmer JW, Friauf E. 1998. Glycine-activated currents are changed by coincident membrane depolarization in developing rat auditory brainstem neurons. J Physiol 507(Part 3):783–794.

Barberis A, Petriti EM, Cherubini E. 2004. Presynaptic source of quantal size variability at GABAergic synapses in rat hippocampal neurons in culture. Eur J Neurosci 20:1803–1810.

Bazhenov M, Timofeev I, Froehlich F, Sejnowski TJ. 2008. Dynamic modulation of excitation and inhibition during stimulation at gamma and beta frequencies in the CA1 hippocampal region. J Neurophysiol 85:2412–2422.

Bregestovski P, Waseem T, Mukhtarov M. 2009. Genetically encoded optical sensors for monitoring of intracellular chloride and chloride-selective channel activity. Front Mol Neurosci 2:15.

Bracci E, Vreugdenhil M, Hack SP, Jefferys JGR. 2001. Dynamic modulation of excitation and inhibition during stimulation at gamma and beta frequencies in the CA1 hippocampal region. J Neurophysiol 85:2412–2422.

Bormann J, Hamill OP, Sakmann B. 1987. Mechanism of anion permeation through channels gated by glycine and gamma-aminobutyric acid in mouse cultured spinal neurones. J Physiol 385:243–286.

Brusch E, Vreugdenhil M, Hack SP, Jefferys JGR. 2001. Dynamic modulation of excitation and inhibition during stimulation at gamma and beta frequencies in the CA1 hippocampal region. J Neurophysiol 85:2412–2422.

Bonnet U, Bingmann D. 1995. GABA-A-responses of CA3 neurones: Contribution of bicarbonate and of Cl(-)-extrusion mechanisms. Neureport 6:700–704.

Borodinsky L, Raiteri D, Scanziani M. 2009. Instantaneous modulation of gamma oscillation frequency by balancing excitation with inhibition. Neuroreport 20:195–200.

Bracci E, Vreugdenhil M, Hack SP, Jefferys JGR. 2001. Dynamic modulation of excitation and inhibition during stimulation at gamma and beta frequencies in the CA1 hippocampal region. J Neurophysiol 85:2412–2422.
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Glickfeld LL, Roberts JD, Somogyi P, Scanziani M. 2009. Interneurons hyperpolarize pyramidal cells along their entire somatodendritic axis. Nat Neurosci 12:21–23.

Gulledge AT, Stuart GJ. 2003. Excitatory actions of GABA in the cortex. Neuron 37:299–309.

Gulyás AI, Megias M, Emri Z, Freund TF. 1999. Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. J Neurosci 19:10082–10097.

Halasy K, Somogyi P. 1993. Distribution of GABAergic synapses and their targets in the dentate gyrus of rat: A quantitative immunoelectron microscopic analysis. J Hirnforsch 34:299–308.

Herrero Al, Del Olmo N, González-Escalada JR, Solis JM. 2002. Two new actions of topitamate: Inhibition of depolarizing GABA(A)-mediated responses and activation of a potassium conductance. Neuropharmacology 42:210–220.

Hines ML, Carnevale NT. 2000. Expanding NEURON’s repertoire of mechanisms with NMOLD. Neural Comput 12:995–1007.

Houston CM, Bright DR, Sivilotti LG, Beato M, Smart TG. 2009. Intracellular chloride ions regulate the time course of GABA-mediated inhibitory synaptic transmission. J Neurophysiol 92:10416–10423.

Huguenard JR, Alger BE. 1986. Whole-cell voltage-clamp study of the fading of GABA-activated currents in acutely dissociated hippocampal neurons. J Neurophysiol 56:1–18.

Hull C, von Gersdorff H. 2004. Fast endocytosis is inhibited by GABA-mediated chloride influx at a presynaptic terminal. Neuron 44:469–482.

Isomura Y, Sugimoto M, Fujiwara-Tsukamoto Y, Yamamoto-Muraki S, Yamada J, Fukuda A. 2003. Synaptically activated Cl– accumulation responsible for depolarizing GABAergic responses in mature hippocampal neurons. J Neurophysiol 90:2752–2756.

Jarolimek W, Lewen A, Miss格尔 U. 1999. A furosemide-sensitive K+–Cl– cotransporter counteracts intracellular Cl– accumulation and depletion in cultured rat midbrain neurons. J Neurosci 19:4695–4704.

Jedlicka P, Backus KH. 2006. Inhibitory transmission, activity-dependent ionic changes and neuronal network oscillations. Physiol Rev 55:139–149.

Jeong HY, Gutkin B. 2005. Study on the role of GABAergic synapses in synchronization. Neurocomputing 65/66:859–868.

Jones MV, Westbrook GL. 1995. Desensitized states prolong GABA(A)-mediated channel responses to brief agonist pulses. Neuron 15:181–191.

Kaila K, Pasternack M, Saarikoski J, Voipio J. 1989. Influence of GABA-gated bicarbonate conductance on potential, current and intracellular chloride in crayfish muscle fibres. J Physiol 416:161–181.

Kaila K, Voipio J, Paalasmaa P, Pasternack M, Deisz RA. 1993. The role of bicarbonate in GABA(A) receptor-activated IPSPs of rat neocortical neurons. J Physiol 464:273–289.

Kaila K, Lamsa K, Smirnov S, Taira T, Voipio J, Kaila K. 2000. 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. J Neurosci 17:7662–7672.

Kantrowitz JT, Francis NN, Saleh A, Perkins KL. 2005. Synaptic depolarizing GABA response in adults is excitatory and proconvulsive when GABA(B) receptors are blocked. J Neurophysiol 93:2656–2667.

Khurug S, Yamada J, Affalov R, Voipio J, Khiroug L, Kaila K. 2008. GABAergic depolarization of the axon initial segment in cortical principal neurons is caused by the Na-K-2Cl co-transporter NKCC1. J Neurosci 28:4635–4639.

Kuner T, Augustine GJ. 2000. A genetically encoded ratiometric indicator for chloride: Capturing chloride transients in cultured hippocampal neurons. Neuron 27:447–459.

Lamas K, Taira T. 2003. Use-dependent shift from inhibitory to excitatory GABA(A) receptor action in SP-O interneurons in the rat hippocampal CA3 area. J Neurophysiol 90:1983–1995.

Lee HH, Walker JA, Williams JR, Goodyer 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 282:29777–29784.

Ling DS, Benardo LS. 1995. Activity-dependent depression of monosynaptic fast IPSCs in hippocampus: Contributions from reductions in chloride driving force and conductance. Brain Res 670:142–146.

Lopreore CL, Bartol TM, Coggan JS, Keller DX, Sosinsky GE, Ellis MH, Sejnowski TJ. 2008. Computational modeling of three-dimensional electrodiffusion in biological systems: Application to the node of Ranvier. Biophys J 95:2624–2635.

Mann EO, Kohl MM, Paulsen O. 2009. Distinct roles of GABA(A) and GABA(B) receptors in balancing and terminating persistent cortical activity. J Neurosci 29:7513–7518.

Manuel NA, Davies CH. 1998. Pharmacological modulation of GABA(A) receptor-mediated postsynaptic potentials in the CA1 region of the rat hippocampus. Br J Pharmacol 125:1529–1542.

Marandi N, Konnerth A, Garaschuk O. 2002. Two-photon chloride imaging in neurons of brain slices. Pflugers Arch 445:357–365.

Mccarren M, Alger BE. 1985. Use-dependent depression of IPSPs in rat hippocampal pyramidal cells in vitro. J Neurophysiol 53:557–571.

Megias M, Emri Z, Freund TF, Gulyas AI. 2001. Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. Neuroscience 102:527–540.

Mitchell SJ, Silver RA. 2003. Shunting inhibition modifies neuronal gain during synaptic excitation. Neuron 38:433–445.

Morita K, Tsumoto K, Aihara K. 2006. Bidirectional modulation of neuronal responses by depolarizing GABAergic inputs. Biophys J 90:1925–1938.

Mozzynska JW, Zarmowska ED, Pytel M, Mercik K. 2003. Modulation of GABA(A) receptors by hydrogen ions reveals synaptic GABA transient and a crucial role of the desensitization process. J Neurosci 23:7981–7992.

Nakaniishi K, Kukita F. 2000. Intracellular [Cl–] modulates synchronous electrical activity in rat neocortical neurons in culture by way of GABAergic inputs. Brain Res 863:192–204.

Nusser Z, Kay LM, Laurent G, Homanics GE, Mody I. 2001. Disruption of GABA(A) receptors on GABAergic interneurons leads to increased oscillatory power in the olfactory bulb network. J Neurophysiol 86:2823–2833.

Otis TS, Staley KJ, Mody I. 1991. Perpetual inhibitory activity in mammalian brain slices generated by spontaneous GABA release. Brain Res 545:142–150.

Pateanu C, Chapman CA, Bertrand S, Congar P, Lacaille JC. 2003. GABA(B)- and mGluR-dependent long-term potentiation of rat hippocampal GABA synaptic transmission. J Physiol 553(Part 1):155–167.

Perez Velasquez JL. 2003. Bicarbonate-dependent depolarizing potentials in pyramidal cells and interneurons during epileptiform activity. Eur J Neurosci 18:1337–1342.

Perkins KL, Wong RK. 1996. Ionic basis of the postsynaptic depolarizing GABA response in hippocampal pyramidal cells. J Neurophysiol 76:3886–3894.

Perkins KL. 1999. Cl– accumulation does not account for the depolarizing phase of the synaptic GABA response in hippocampal pyramidal cells. J Neurophysiol 82:768–777.

Prescott SA, De Koninck Y. 2003. Gain control of firing rate by shunting inhibition: Roles of synaptic noise and dendritic saturation. Proc Natl Acad Sci USA 100:2076–2081.

Prescott SA, Sejnowski TJ, De Koninck Y. 2006. Reduction of anion reversal potential subverts the inhibitory control of firing rate in spinal lamina I neurons: Towards a biophysical basis for neuropathic pain. Mol Pain 2:32.

Hippocampus
Rinke I, Artmann J, Stein V. 2010. ClC-2 voltage-gated channels constitute part of the background conductance and assist chloride extrusion. J Neurosci 30:4776–4786.

Qian N, Sejnowski TJ. 1989. An electro-diffusion model for computing membrane potentials and ionic concentrations in branching dendrites, spines and axons. Biol Cybern 62:1–15.

Qian N, Sejnowski TJ. 1990. When is an inhibitory synapse effective? Proc Natl Acad Sci USA 87:8145–8149.

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. 1999. The K⁺/Cl⁻ co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251–255.

Rudolph M, Pospischil M, Timofeev I, Destexhe A. 2007. Inhibition determines membrane potential dynamics and controls action potential generation in awake and sleeping cat cortex. J Neurosci 27:5280–5290.

Santhakumar V, Aradi I, Soltesz I. 2005. Role of mossy fiber sprouting and mossy cell loss in hyperexcitability: A network model of the dentate gyrus incorporating cell types and axonal topography. J Neurophysiol 93:437–453.

Saraga F, Balena T, Wolansky T, Dickson CT, Woodin MA. 2008. Inhibitory synaptic plasticity regulates pyramidal neuron spiking in the rodent hippocampus. Neuroscience 155:64–75.

Schmidt-Hieber C, Jonas P, Bischofberger J. 2007. Subthreshold dendritic signal processing and coincidence detection in dentate gyrus granule cells. J Neurosci 27:8430–8441.

Shu Y, Hasenstaub A, McCormick DA. 2003. Turning on and off recurrent balanced cortical activity. Nature 423:288–293.

Staley KJ, Soldo BL, Proctor WR. 1995. Ionic mechanisms of neuronal excitation by inhibitory GABAA receptors. Science 269:977–981.

Staley KJ, Proctor WR. 1999. Modulation of mammalian dendritic GABA(A) receptor function by the kinetics of Cl⁻ and HCO₃⁻ transport. J Physiol 519(3):693–712.

Steriade M. 2001. Impact of network activities on neuronal properties in corticothalamic systems. J Neurophysiol 86:1–39.

Stiefel KM, Wespatat V, Gutkin B, Tennygkeit F, Singer W. 2005. Phase dependent sign changes of GABAergic synaptic input explored in-silico and in-vitro. J Comput Neurosci 19:71–85.

Sun MK, Zhao WQ, Nelson TJ, Alkon DL. 2001. Theta rhythm of hippocampal CA1 neuron activity: Gating by GABAergic synaptic depolarization. J Neurophysiol 85:269–279.

Thompson SM, Gahwiler BH. 1989. Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus in vitro. J Neurophysiol 61:501–511.

Ulrich D. 2003. Differential arithmetic of shunting inhibition for voltage and spike rate in neocortical pyramidal cells. Eur J Neurosci 18:2159–2165.

Vida I, Bartos M, Jonas P. 2006. Shunting inhibition improves robustness of gamma oscillations in hippocampal interneuron networks by homogenizing firing rates. Neuron 49:107–117.

Vitarien T, Ruusuvuori E, Kaila K, Voipio J. 2010. The K⁺–Cl⁻ cotransporter KCC2 promotes GABAergic excitation in the mature rat hippocampus. J Physiol doi: 10.1113/jphysiol.2009.181826.

Vreugdenhil M, Bracci E, Jefferys JGR. 2005. Layer-specific pyramidal cell oscillations evoked by tetanic stimulation in the rat hippocampal area CA1 in vitro and in vivo. J Physiol 562:149–164.

Wagner S, Sagiv N, Yarom Y. 2001. GABA-induced current and circadian regulation of chloride in neurones of the rat suprachiasmatic nucleus. J Physiol 537:853–869.

Woodin MA, Ganguly K, Poo M. 2003. Coincident pre- and postsynaptic activity modifies GABAergic synapses by postsynaptic changes in Cl⁻ transporter activity. Neuron 39:807–820.