Activity-Dependent Intracellular Chloride Accumulation and Diffusion Controls GABA_A Receptor-Mediated Synaptic Transmission

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ABSTRACT: In the CNS, prolonged activation of GABA_A receptors (GABA_ARs) 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[−]) accumulation resulting in a shift of the GABA_A reversal potential (E_{GABA}). The amount of GABA-evoked Cl[−] accumulation and accompanying depolarization depends on presynaptic and postsynaptic properties of GABAergic transmission, as well as on cellular morphology and regulation of Cl[−] intracellular concentration ([Cl[−]]_i). To analyze the influence of these factors on the Cl[−] and voltage behavior, we studied spatiotemporal dynamics of activity-dependent [Cl[−]]_i changes in multicompartimental models of hippocampal cells based on realistic morphological data. Simulated Cl[−] influx through GABA_ARs was able to exceed physiological Cl[−] extrusion rates thereby evoking HCO₃[−]-dependent E_{GABA} shift and depolarizing responses. Depolarizations were observed in spite of GABA_A receptor desensitization. The amplitude of the depolarization was frequency-dependent and determined by intracellular Cl[−] 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[−] than somatic inhibition. Interestingly, E_{GABA} changes induced by activation of a single dendritic synapse propagated beyond the site of Cl[−] influx and affected neighboring synapses. The simulations suggest that E_{GABA} may differ even along a single dendrite supporting the idea that it is necessary to assign E_{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_A receptors (GABA_ARs). Depending on the GABA_A reversal potential (E_{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_AR activation is depolarizing due to high intracellular Cl[−] concentration ([Cl[−]]_i) (Cherubini et al., 1991; Rivera et al., 1999). In adult neurons, repetitive activation of GABA_ARs evokes biphasic postsynaptic responses: an initial hyperpolarization followed by a delayed depolarization. These are referred to in the literature as GDPSPs or GABA_AR-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 HCO₃[−] permeability of GABA_ARs (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_{GABA} is determined by equilibrium potentials of Cl[−] and HCO₃[−] (E_{Cl} , E_{HCO_3}). According to the Cl[−] accumulation hypothesis, intense GABA_AR activation substantially increases [Cl[−]]_i so that E_{Cl} shifts toward resting membrane potential (E_{rest}) (Kaila et al., 1989; Staley et al., 1995; Backus et al., 1998; Frech et al., 1999;

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Additional Supporting Information may be found in the online version of this article.

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Staley and Proctor, 1999). During such stimulation, the HCO_3^- gradient remains largely constant. As a consequence, E_{GABA} may rise above E_{rest} , thus leading to GABAergic depolarizations. Kuner and Augustine (2000) demonstrated that GABA_A input activation brings about local increase of $[\text{Cl}^-]_i$ spreading into nearby regions of the cell and shifting E_{GABA} . Thus, in addition to global changes, local alterations of $[\text{Cl}^-]_i$ 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 $[\text{Cl}^-]_i$ and on particular cellular morphology. Our goal here is to address the following questions: How do Cl^- accumulation and the subsequent GDPSP depend on the frequency and synchronicity of GABA_AR 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 $[\text{Cl}^-]_i$ resulting from the intracellular Cl^- diffusion influence the GDPSP? Does repetitive activation of a single synapse affect E_{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

The Computational Model

Membrane potential dynamics

We used an equivalent circuit representation of neuronal compartment incorporating a GABA_AR 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_{Cl}) and activity-independent (E_{rest} , E_{HCO_3}) electrochemical potentials.

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

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

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_ARs present in the considered compartment, we multiplied the single channel Cl^- and HCO_3^- current by the total number of receptors (R_{number}) and by the time-varying fraction of open receptors (Open) as follows:

$$I_{\text{Cl}} = P_{\text{Cl}} \left(\frac{VF^2}{RT} \right) \left(\frac{[\text{Cl}^-]_i - [\text{Cl}^-]_o \exp(VF/RT)}{1 - \exp(VF/RT)} \right) R_{\text{number}} \text{Open}$$

$$I_{\text{HCO}_3} = P_{\text{HCO}_3} \left(\frac{VF^2}{RT} \right) \left(\frac{[\text{HCO}_3^-]_i - [\text{HCO}_3^-]_o \exp(VF/RT)}{1 - \exp(VF/RT)} \right) R_{\text{number}} \text{Open}$$

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

where P_{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):

$$g_a = P_a \frac{F^2}{RT} \frac{[a]_i([a]_o + [a]_i)z + ([a]_i - [a]_o)(VF/RT)z + [a]_o z^2}{(1 - z)^2}$$

where $z = \exp(VF/RT)$ and "a" is Cl^- or HCO_3^- .

We adjusted the number of synaptic receptors (R_{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_{GABA} was determined by two state kinetic scheme described by rise time (τ_{u1}) and decay time constant (τ_{u2}): $g_{\text{GABA}} = B - A$, $dA/dt = -A/\tau_{\text{u1}}$, $dB/dt = -B/\tau_{\text{u2}}$.

E_{Cl} and E_{HCO_3} were calculated from Nernst equation.

GABA_AR Kinetic Model

To incorporate GABA-induced gating of postsynaptic receptors, the Markov model of GABA_AR 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_{slow} , D_{fast}) 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 Cl^- concentration inside the cellular compartment was calculated as follows:

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

The equation represents GABA-mediated Cl^- accumulation with exponential recovery (decay time constant τ_{Cl}) to resting level $[\text{Cl}^-]_i^{\text{rest}}$. The decay approximates an outward 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 Cl^- pump velocity (v) was computed according to Lineweaver-Burke equation (Staley and Proctor, 1999):

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

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

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

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

Cl^- Diffusion

Longitudinal Cl^- diffusion was modeled as the exchange of 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 $2 \mu\text{m}^2/\text{ms}$ (Kuner and Augustine, 2000; Brumback 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 electrotonic 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 τ_{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 μm) along its length (618.37 μm). In simulations of activity-dependent 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°C , $g_{\text{leak}} = 0.2$ mS/ cm^2 , $E_{\text{rest}} = -60$ mV, $C = 1$ uF/ cm^2 , $R_a = 150$ ohm-cm, $[\text{Cl}^-]_i = 8$ mM, $[\text{Cl}^-]_o = 133.5$ mM, $[\text{HCO}_3^-]_i = 16$ mM, $[\text{HCO}_3^-]_o = 26$ 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 = $2 \mu\text{m}^2/\text{ms}$. The rates of GABA_AR kinetic model were identical to those of Barberis et al. (2004). Some of these parameters were varied as explained in the relevant figures. In

FIGURE 1. Dependence of GABA-induced voltage responses and corresponding $[Cl^-]_i$ changes on the relative HCO_3^-/Cl^- permeability ($P_{HCO_3^-}/P_{Cl^-}$) 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/ $[Cl^-]_i$ changes at $P_{HCO_3^-}/P_{Cl^-} = 0.3, 0.2$, and 0 , respectively. The duration of stimulation was 3 s. Resting membrane potential level is indi-

cated by the dashed line. D: The relation between stimulation frequencies, $P_{HCO_3^-}/P_{Cl^-}$ and GABA-induced changes of membrane potential. GDPSP: maximal GABA_AR-mediated depolarizing potential. The range of relative $P_{HCO_3^-}/P_{Cl^-}$ 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 www.interscience.wiley.com.]

simulations of background GABAergic activity in granule cells, following parameters were used: basal $[Cl^-]_i = 5$ mM corresponding to E_{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_{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 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 2. Voltage and $[\text{Cl}^-]_i$ changes following repetitive activation of a single dendritic GABA_A synapse in a multicompartmental neuronal model. A GABAergic synapse was placed at the distal end (A), the proximal end (B), and in the middle (C) of a dendrite in a morphologically reconstructed hippocampal interneuron (Gulyas et al., 1999; see Supporting Information Fig. 3B). A–C: regular repetitive (10 Hz, 20 pulses) synaptic activation, D: noisy repetitive (mean frequency 10 Hz) synaptic activation at the distal dendritic end.

Voltage and $[\text{Cl}^-]_i$ changes were recorded at the synaptic location. The time constant for Cl^- extrusion (τ_{Cl}) was 3 s (Staley and Proctor, 1999; Wagner et al., 2001). Dendritic diameter varied (distal end: 0.26; middle: 0.3; proximal end: 0.92 μm) along the length (618.37 μm). Note that, at the distal dendritic end, both regular and stochastic activation of the GABA_A synapse lead to significant Cl^- accumulation and depolarizing switch.

RESULTS

First, we wanted to test whether the activation of GABA_ARs leads to significant Cl^- influx and accounts for corresponding electrophysiological responses. To do so, we created a biophysical model of GABAergic synapse containing GABA_ARs (see Methods) based on the kinetic GABA_AR 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^3 , cf. Bracci et al., 2001). We then determined the dependence of the voltage–time relation at different stimulation frequencies on the relative HCO_3^- permeability (Fig. 1). As expected, the GABA-induced depolarization (GDPSP) increased with increasing relative ratio of HCO_3^- to Cl^- permeability ($P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$). Using the single compartment model, we also investigated the dependence of GDPSP on relevant bio-

physical parameters: the Cl^- extrusion rate, the number of activated synapses, and the dendritic diameter (see Supporting Information Figs. 1 and 2). The $[\text{Cl}^-]_i$ necessary to drive E_{GABA} to a more positive value than V_{rest} was 10.6 mM (Supporting Information Fig. 1). The GDPSP amplitude increased with decreasing maximum Cl^- 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_A 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,

FIGURE 3. Intracellular Cl^- 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_A synapses. Voltage values were recorded at the distal end, the proximal end, and in the middle of the dendrite. Lower graph: GABA_A conductance decreases with time because of GABA_A R desensitization (see Supporting Information Figs. 3, 4). Nevertheless, the desensitization does not prevent continuous accumulation of Cl^- within distal dendritic

and middle segments as indicated by the monotonic increase of $[\text{Cl}^-]_i/g_{\text{GABA}}$ ratio. Shape plot: Spatial pattern of intracellular Cl^- concentration $[\text{Cl}^-]_i$ at the end of the tetanic stimulation (synchronous 10 Hz activation of 13 GABA_A synapses). The color code indicates $[\text{Cl}^-]_i$ change. $[\text{Cl}^-]_i$ increased significantly in the stimulated dendrite. Note that the Cl^- diffusion to nearby “silent” dendrites did not markedly change their $[\text{Cl}^-]_i$. Cl^- 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 www.interscience.wiley.com.]

1999; Bracci et al., 2001). Specifically, such a single compartment model by design neglects both longitudinal and radial Cl^- diffusion within neurons with complex geometries. Therefore, to study spatial Cl^- concentration changes under morphologically realistic conditions, we inserted the model of GABA_A Rs including the Cl^- 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^- dynamics within a long unbranched dendrite.

To study the impact of synapse location on GDPSPs, we placed a GABA_A ergic synapse at three different positions: at the distal end (diameter = 0.26 μm), the proximal end (diameter = 0.92 μm), and in the middle (diameter = 0.3 μm) of the dendrite (length = 618.37 μm). 10 Hz (regular or noisy)

stimulation train was applied and resulting voltage and $[\text{Cl}^-]_i$ 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 $[\text{Cl}^-]_i$ 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 GABA_A ergic synapses may lead to a spatial and temporal summation of Cl^- and voltage transients and affect dendritic E_{GABA} . Therefore, we next examined the electrochemical consequences of a larger number of stimulated GABA_A ergic 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^-

FIGURE 4. Factors affecting dendritic GDPSP amplitudes in the multicompartmental neuronal model. A–F: Synaptic density, dendritic size (diameter), GABA_AR 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 τ_{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.

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_{GABA} within a given neuron. Repetitive activation of GABA_ARs leads to their desensitization (Jones and Westbrook, 1995) and a gradual decrease in GABA_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 $[\text{Cl}^-]_i/g_{\text{GABA}}$ ratio (Fig. 3). Hence, activity-dependent depolarization switch occurred in spite of GABA_AR desensitization. Nevertheless, under different conditions (e.g., at weaker GABA_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_{GABA} shifts in distal dendritic branches.

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 (Gulyas 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_{GABA} (Fig. 4F).

Because of the slow Cl^- transport (Staley and Proctor, 1999; Wagner et al., 2001), the increased $[\text{Cl}^-]_i$ outlasts GABA-mediated depolarization thus providing a higher $[\text{Cl}^-]_i$ 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 ($\tau_{\text{Cl}} = 3$ s) (Staley and Proctor, 1999; Wagner et al., 2001), the GABA-induced Cl^- accumulation persisted 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 $[\text{Cl}^-]_i$ and E_{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 (Halasy and Somogyi, 1993). Conductances and kinetics of dendritic and somatic GABA_A synapses were based on electrophysiologi-

FIGURE 5. GABA-evoked Cl^- concentration changes outlast conditioning tetanic stimulation train leading to delayed GABAergic depolarizations. Superimposed voltage (upper graph) and $[\text{Cl}^-]_i$ (lower graph) traces evoked by single pulse stimuli (asterisks, 1 middle synapse activated) applied at different time points following 20 pulses of 10 Hz stimulation of 19 evenly spaced synapses. Voltage and $[\text{Cl}^-]_i$ were recorded in the middle of the stimulated dendrite. Note that single pulse stimuli following the conditioning stimulation train induced three GDPSPs with amplitudes decaying with increasing stimulus delays.

cal 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 (0.04 ± 0.01 mM) and dendritic $[\text{Cl}^-]_i$ (0.08 ± 0.01 mM; $n = 8$ granule cells; Fig. 6). Dendritic increase of $[\text{Cl}^-]_i$ 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 (2.8 ± 0.3 mM) and dendritic $[\text{Cl}^-]_i$ (3.9 ± 0.3 mM; $n = 8$ granule cells; Fig. 6) leading to depolarizing dendritic and somatic potentials (not shown). Again, dendritic $[\text{Cl}^-]_i$ 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_{GABA} shifts. In addition, our simulations confirm that dendritic compartments are more prone to $[\text{Cl}^-]_i$ 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_{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_{GABA} change, stimulation at 1 Hz frequency

FIGURE 6. Cl^- accumulation and E_{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_{GABA} change following stochastic activation of dendritic and somatic GABA_A synapses. The main shape plot and the inset show changes of E_{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_{GABA} (most prominent in distal dendritic segments), 0.1 Hz stimulation produced only minimal changes. **B:** Quantification of E_{GABA} and $[Cl^-]_i$ changes 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/ μm (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 www.interscience.wiley.com.]

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_{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_{GABA} at neighboring synaptic sites. The depolarization switch induced by 40 and 100 Hz stimulation of distal GABA_A input spread within $36 \pm 6 \mu m$ and $72 \pm 5 \mu m$ beyond the site of Cl^- influx, respec-

tively (Fig. 7A). Activation of GABA_A input in the center of a dendrite at 100 Hz frequency also induced the depolarizing switch spreading $33 \pm 13 \mu m$ and $7 \pm 2 \mu m$ distally (toward the “sealed” end) and proximally (to soma), respectively (Fig. 7B). Furthermore, our simulations showed that the spatial propagation of E_{GABA} depends strongly on the strength of GABAergic synapses and dendritic diameter (Figs. 7C,D).

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_ARs following their tetanic activation was able to exceed physiological Cl^- extrusion rates thereby increasing the $[Cl^-]_i$ and shifting E_{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_{GABA} may differ even along a single dendrite supporting the idea that it is necessary to assign E_{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 $[Cl^-]_i$ has been proposed and supported by several studies (Huguenard and Alger, 1986; Akaike 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 tetanic stimulation even if GABAergic activity is not accompanied by extracellular K^+ accumulation. Thus, substantial Cl^- concentration changes in small dendrites are sufficient to shift E_{GABA} to depolarizing values. Importantly, we show that Cl^- and E_{GABA} changes can propagate beyond the site of

FIGURE 7. Spatial spread of E_{GABA} shift in granule cells triggered by repetitive activation of a single dendritic GABA_A input. **A:** Frequency dependence of E_{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_{GABA} changes following stochastic activation of a single GABA_A synapse located in the center of the den-

drite. See text for more details. **C and D:** Spatial E_{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_{GABA} rise time 0.5 ms, decay time 6 ms (Santhakumar et al., 2005); resting potential: -70 mV; initial $E_{GABA} = -73.83$ mV; stimulation duration: 5 s; **A, B,** and **C:** $g_{GABA} = 0.5$ nS (Santhakumar et al., 2005).

synaptic Cl^- influx (Kuner and Augustine, 2000) and that activity at a single synapse can affect E_{GABA} of adjacent synapses located within tens of μm away from the active synapse (see also Doyon et al., 2008).

Impact of Cl^- Extrusion Mechanisms

By varying the velocity of Cl^- pump, we found higher Cl^- extrusion rates to decrease GDPSPs. However, when using a physiological Cl^- extrusion rate (Staley and Proctor, 1999; Wagner et al., 2001), in most conditions the GABA-induced Cl^- accumulation reached a level sufficient to induce GDPSPs. Interestingly, electrophysiological experiments have shown that it is possible to fit the recovery of $[Cl^-]_i$ 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 Cl^- accumulation (Staley and Proctor, 1999; Wagner et al., 2001). In most adult CNS neurons, $K^+ Cl^-$ cotransporter 2 (KCC2) has been identified as the main chloride exporter (Gamba, 2005; Blaesse et al., 2009; as opposed to developing neurons where $Na^+ K^+ 2Cl^-$ cotransporter 1 (NKCC1) plays a dominant role, Gamba, 2005; but see also Khirug et al., 2008). Nevertheless, in addition to GABA-mediated Cl^- accumulation and KCC2-mediated Cl^- extrusion, other Cl^- influx/efflux pathways such as Cl^- - HCO_3^- exchangers, ATP-driven Cl^- pumps and voltage-sensitive Cl^- channels may contribute to $[Cl^-]_i$ 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 Cl^- dynamics in mature neurons. Moreover, in future work, it would be interesting to analyze Cl^- homeostasis in detailed models of

immature neuronal cells where Cl^- extrusion/intrusion rates and morphological properties are different.

Modulation of Synaptic GABA Release

As tetanic stimulation can modulate GABA release (Ghijsen et al., 2007), presynaptic short-term plasticity mechanisms might be at work influencing the frequency dependence of GDPSPs (Manuel and Davies, 1998; Patenaude et al., 2003). Indeed, the magnitude of GDPSPs is promoted by blocking GABA_B receptors (Cobb et al., 1999). GABA_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_B autoreceptors mediate activity-dependent depression of GDPSPs thereby preventing the development of pathological depolarizing GABA responses. Consistent with this presynaptic mechanism, in our simulations, Cl^- 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 GDPSP amplitude variability. This can be explained by the fact that peak GABA concentration (2 mM) was subsaturating (Mozrzymas et al., 2003; Barberis et al., 2004), thus leaving space for variability due to changes in GABA decay.

Time Course of Cl^- Accumulation

Although Cl^- 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^- accumulation alone. Lamsa and Taira (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 $[\text{Cl}^-]_i$ 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 $[\text{Cl}^-]_i$ (tens of seconds). Extracellular K^+ accumulation due to intense network activity may be an extra mechanism for slowing down or reversing the activity of K^+ - Cl^- cotransporters (Jarolimek et al., 1999) and thus maintaining internal Cl^- elevation for longer time periods (Fujiwara-Tsukamoto et al., 2007; see also note added in proof). Another possibility is Ca^{2+} -dependent downregulation of K^+ - Cl^- cotransporter function (Woodin et al., 2003; Fiumelli et al., 2005; Lee et al., 2007) which would enhance the Cl^- accumulation and prolong the increase of $[\text{Cl}^-]_i$. Intriguingly, a recent study has reported that intracellular Cl^- ions directly modulate GABA_A kinetics thus conferring an additional level of complexity to the time course of Cl^- accumulation and GABA-mediated synaptic responses (Houston et al., 2009).

Background Activity and Compartment-Specific Changes of Cl^- 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^- and E_{GABA} in anatomically realistic models of granule cells (Schmidt-Hieber et al., 2007). Intense stochastic activation of dendritic and somatic GABA_A synapses evoked significant changes in Cl^- concentration and E_{GABA} . Of note, our prediction that high-frequency background activity may influence $[\text{Cl}^-]_i$ can be tested by monitoring Cl^- 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 $[\text{Cl}^-]_i$ 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^- 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-somato-dendritic gradient of steady state E_{GABA} and Cl^- likely reflecting distinct expression of Cl^- transporters within respective cellular domains of cortical neurons (Khurug et al., 2008; but see Glickfeld et al., 2009; see also Duebel et al., 2006; Gavrikov et al., 2006). This evidence points toward compartment-specific mechanisms of E_{GABA} regulation that possibly may counter-act an excessive activity-dependent collapse of E_{GABA} in dendrites.

Intriguingly, GABA_A receptors are able to produce transient microdomains of high Cl^- (Hull and von Gersdorff, 2004). Thus, besides global Cl^- concentration changes, local increases in Cl^- near the plasma membrane play a physiologically relevant role. Therefore, quantitative knowledge of local Cl^- concentration dynamics is necessary to understand not only membrane potential changes but also biochemical phenomena, as Cl^- plays a "second messenger" role, modulating biochemical processes close to the cell surface (Hull and von Gersdorff, 2004). Spatial and temporal discrimination of fluorescence methods for measuring Cl^- concentration is not yet sufficient to estimate microdomain Cl^- concentration changes, particularly in hardly accessible cellular compartments like dendrites (Kuner and Augustine, 2000; Marandi et al., 2002; Isomura et al., 2003; Berglund et al., 2008; Bregestovski et al., 2009). Thus, computational modeling that we present here is a valuable complementary method for the assessment of small scale Cl^- dynamics.

Functional Consequences of GABAergic Depolarization

In several brain areas, GABA_A receptor-mediated inhibition is functionally relevant for the generation of synchronous network activity (Nakanishi and Kukita, 2000; Nusser et al., 2001; Lamsa and Taira, 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_{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_{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 $[\text{Cl}^-]_i$; hence modifying the E_{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.

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