Development of dendritic tonic GABAergic inhibition regulates excitability and plasticity in CA1 pyramidal neurons

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Backpropagating action potentials (BAPs) play a critical role in spike timing-dependent synaptic plasticity (STDP) and convey information from the soma to the dendrites and spines (Campanac and Debanne 2008). BAPs are modulated by a wide range of voltage-gated ion channels, notably A-type potassium channels and h-channels (Andrasfalvy et al. 2008; Magee 1998). However, the regulation of BAPs by ligand-gated channels, such as GABA receptors, is less well studied, although GABAergic inhibition is a potent regulator of neuronal excitability (Leung and Peloquin 2006; Tsubokawa and Ross 1996) and could, therefore, affect BAPs and consequently synaptic plasticity (Andrasfalvy et al. 2008; Gasparini et al. 2007). In adult hippocampal CA1 pyramidal neurons, postsynaptic bursts of action potentials (APs) rather than single APs are necessary for STDP (Buchanan and Mellor 2007; Meredith et al. 2003; Pike et al. 1999). This higher threshold for plasticity has been hypothesized to reflect developmental differences in AP propagation into dendrites (Buchanan and Mellor 2007).

Inhibition plays a key role in neuronal signal integration and information processing (Brickley and Mody 2012; Lovett-Barron et al. 2012). In CA1 hippocampus, GABAergic inhibition is critical for hippocampal-dependent behaviors, network oscillations and synaptic plasticity (Mann and Paulsen 2007; Mody 2005; Moser et al. 2008). During postnatal development, GABAergic inhibition is upregulated and does not mature until the second postnatal month (Banks et al. 2002; Cohen et al. 2000). However, the impact of these developmental changes on BAPs and dendritic signal processing remains poorly understood.

GABAergic inhibition can act both phasically and tonically across different compartments of a CA1 pyramidal neuron. Phasic inhibition occurs at the synapse via α1–3-, β- and γ-subunits at a millisecond level (Brickley and Mody 2012). In
contrast, tonic inhibition, mediated via α5- and δ-subunits at perisynaptic or extrasynaptic receptors, acts over seconds to minutes (Belelli et al. 2009; Brickley and Mody 2012). Whilst the effect of precisely timed phasic inhibition upon CA1 dendrites is demonstrated (Kwag and Paulsen 2009; Royer et al. 2012; Tsubokawa and Ross 1996), the effect of tonic inhibition on dendritic excitability has not been investigated. Tonic GABA(A) receptors are activated by the release of extracellular GABA by interneurons and astrocytes (Brickley and Mody 2012; Heja et al. 2012). Both cell types have spatially restricted target regions (Heja et al. 2012; Klausberger 2009). Therefore, distinct dendritic regions could be differently regulated by tonic inhibition.

Here, using a combination of somatic and dendritic electrophysiology with two-photon calcium imaging, we addressed how tonic GABAergic inhibition regulates dendritic BAPs at different preadolescent (PreAd) and adolescent (Ad) postnatal ages. We investigated the consequence for synaptic plasticity in CA1 pyramidal neurons and modeled the impact of different dendritic distributions of tonic inhibition on BAPs. Together, our results show that tonic GABAergic inhibition is mediated by α5-containing GABA(A) receptors and regulates dendritic excitability in Ad but not younger, PreAd CA1 pyramidal neurons. This effect was localized to the distal dendrites and could be explained by a distal expression pattern of dendritic α5-receptor-mediated tonic inhibition.

MATERIALS AND METHODS

All animal use was approved by the Animal Welfare Committee of the VU University Amsterdam, according to Dutch and European law.

Hippocampal slice preparation. Acute horizontal hippocampal slices were obtained from 2- to 3-wk (PreAd) and 4- to 6-wk (Ad) male Wistar rats, with 2- to 3-wk (PreAd) and 4- to 7-wk old (Ad) rats used for synaptic plasticity experiments. After decapitation, the brains were rapidly dissected in 4°C slicing solution (110 mM choline chloride, 11.6 mM Na-ascorbate, 7 mM MgCl₂, 3.1 mM Na-pyruvate, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 0.5 mM CaCl₂, 26 mM NaHCO₃, 10 mM glucose) (Bureau et al. 2006). Three hundred-micrometer slices were cut using a LEICA VT1000S vibratome. The slices were transferred to a slice container filled with recording artificial cerebrospinal fluid (aCSF) (125 mM NaCl, 3 mM KCl, 1.2 mM NaH₂PO₄, 0.5 mM CaCl₂, 26 mM NaHCO₃, 10 mM glucose) (Bureau et al. 2006). Timing-dependent long-term potentiation (tLTP) was induced by pairing a CA1 pyramidal neuron and stimulating an excitatory postsynaptic potential (EPSP) with an extracellular stimulation electrode placed in the Schaffer collateral pathway (50 μs, 10–300 μA, 0.1 Hz). After reaching a stable baseline of 12- to 15-min duration, presynaptic stimulation via the stimulation electrode was paired with a single somatic AP (5 ms pulse) at the soma via a current injection with a delay of 5 ms, repeated 30 times, a protocol shown to induce tLTP in PreAd animals (Meredith et al. 2003). Somatic efficacy was assessed by the slope and amplitude of the induced EPSP 20 min after the pairing protocol over a 3-min period. Circa 20–25 min later, within the same cell, a second tLTP pairing was applied (5 ms pre-post delay) but with a postsynaptic AP burst (20-ms duration, 2–3 APs) instead of a single AP.

Two-photon calcium imaging. To assess the modulation of dendritic excitability by tonic and phasic GABA(A) inhibition during development, backpropagation of single APs and bursts of spikes was measured using two-photon calcium imaging in the presence of GABA(A) blockers. To measure dendritic excitability during normal spontaneous glutamatergic and GABAergic network activity, CA1 pyramidal neurons were patched with intracellular solution containing physiological chloride concentration (148 mM K-gluconate, 1 mM KCl, 10 mM HEPES, 4 mM Mg-ATP, 4 mM K₂ phosphocreatine, 0.4 mM GTP and 0.20% biocytin). During normal spontaneous calcium changes within the dendrites. After break-in, the dyes were allowed to diffuse into the dendritic tree for 20 min after which recordings started.

To dissect the influence of tonic and phasic currents, BAP-induced calcium transients were measured in the presence of different GABA(A) receptor-modulating drugs described above.

Electrophysiology. To assess tonic and spontaneous phasic currents at the soma and in the dendrites, CA1 pyramidal cells were measured in voltage clamp mode with a high chloride intracellular containing K-glucuronate (70 mM), KCl (70 mM), HEPES (10 mM), Mg-ATP (4 mM), K₂ phosphocreatine (4 mM), GTP (0.4 mM) and biocytin (0.2%) with a pH of 7.3. In addition CPG (4 μM), DL-2-Amino-5-phosphonopentanoic acid (100 μM), and 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM) were added to the recording aCSF to isolate GABA(A) receptor-mediated currents. To assess tonic currents, the holding current before and after gabazine application (10 μM, 20-min wash-in period) was determined. Cells that showed a 20% or greater increase in series resistance during the recording were excluded from the analysis.

For plasticity experiments, CA1 pyramidal neurons were recorded in current clamp mode with intracellular pipette solution containing: K-glucuronate (110 mM), HEPES (40 mM), NaCl (4 mM), Mg-ATP (4 mM), GTP (0.3 mM) with a pH of 7.2–7.3. Timing-dependent long-term potentiation (tLTP) was induced by pairing a CA1 pyramidal neuron and stimulating an excitatory postsynaptic potential (EPSP) with an extracellular stimulation electrode placed in the Schaffer collateral pathway (50 μs, 10–300 μA, 0.1 Hz). After reaching a stable baseline of 12- to 15-min duration, presynaptic stimulation via the stimulation electrode was paired with a single somatic AP (5 ms pulse) at the soma via a current injection with a delay of 5 ms, repeated 30 times, a protocol shown to induce tLTP in PreAd animals (Meredith et al. 2003). Somatic efficacy was assessed by the slope and amplitude of the induced EPSP 20 min after the pairing protocol over a 3-min period. Circa 20–25 min later, within the same cell, a second tLTP pairing was applied (5 ms pre-post delay) but with a postsynaptic AP burst (20-ms duration, 2–3 APs) instead of a single AP.

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Model simulations. To assess the effect of the developmental increase in dendrite complexity, six reconstructed neurons per age group were imported into the NEURON simulation environment (Hines and Carnevale 1997). The model consists of passive apical and basal dendrites, with membrane capacitance \( C_m = 1 \text{ \mu F/cm}^2 \), axial resistance \( R_s = 123 \text{ \Omega cm} \) and passive conductance \( g_m = 5 \times 10^{-5} \text{ S/cm}^2 \), as well as Hodgkin-Huxley type sodium and potassium channels at the soma, with maximal conductances \( g_{Na} = 0.07 \text{ S/cm}^2 \) and \( g_K = 0.014 \text{ S/cm}^2 \). To assess the input resistance and capacitance, a hyperpolarizing step (300 pA) was applied at the soma. AP backpropagation into the dendrites was assessed by inserting a 20-ms depolarizing pulse at the soma (0.18–0.35 nA, depending on morphology), similar to the experiment.

To assess the effect of tonic currents in the Ad neurons, we adopted a well-established mature CA1 model that contains both active and passive properties (Poirazi et al. 2003; Sterratt et al. 2012). We distributed tonic inhibitory conductances (Pavlov et al. 2009) in the passive properties (Poirazi et al. 2003; Sterratt et al. 2012). We modeled tonic conductances at all. Maximum conductance (\( g_{max} \)) depended on path morphology, similar to the experiment. A depolarizing pulse at the soma (0.18–0.35 nA, depending on morphology) was set to 0.015 S/cm². To assess the input resistance and capacitance, a hyperpolarizing step (300 pA) was applied at the soma. AP backpropagation into the dendrites was assessed by inserting a 20-ms depolarizing pulse at the soma (0.18–0.35 nA, depending on morphology), similar to the experiment.

RESULTS

Electron microscopy. PreAd (P14) and Ad (P28) male Wistar rats (\( n = 4 \) for both age groups) were deeply anesthetized by isoflurane (2%), followed by an intraperitoneal injection of urethane (1.79 g/kg). Brains were fixed by transcardial perfusion with 0.9% NaCl, followed by PFA fixative (4% PFA, 2.5% glutaraldehyde dissolved in 0.1 M phosphate-buffered saline, pH 7.4). Horizontal hippocampal slices of 50 μm were postfixed in 1% OsO₄ and stained with 1% ruthenium. After embedding in Epon, ultrathin sections (100–200 nm) were collected on 400-mesh copper grids, followed by staining with ruthenium (1%) and lead citrate. Digital images of symmetric (GABAergic) and asymmetric (glutamatergic) synapses were taken at ×100,000 magnification using a Jeol (Peabody, MA) 1010 electron microscope.

Only synapses which showed clear post- and presynaptic properties were selected for analysis. For each synapse, the postsynaptic density (PSD) length and cluster size, number of docked vesicles and number of undocked vesicles were determined. Cluster size is defined as the area within the presynaptic terminal that contains synaptic vesicles, both docked and undocked. A vesicle was classified as docked when there was no separation detectable between the presynaptic membrane and the vesicle membrane. The observer was unaware of the age group while counting.

Statistical methods. When comparing multiple datasets, an ANOVA followed by post hoc test with a Bonferroni correction was performed. For a comparison between two datasets, a t-test was performed. Differences were regarded as significant if the \( P \) value was below 0.05 \(( ^*P < 0.05, ^{**}P < 0.01, ^{***}P < 0.001 \text{ in } \text{Figs. 1–9})\).

RESULTS

Induction rules for STDP in the hippocampus differ significantly with increasing postnatal age (Buchanan and Mellor 2007; Meredith et al. 2003). Comparing rodent hippocampal slices taken from a period of preadolescence (P14–19) with adolescence (P28–42), we measured the level of tLTP between Schaffer collateral synapses on to CA1 pyramidal neurons (Fig. 1A). Using two different induction paradigms, we observed a significantly higher level of tLTP induced by a pairing protocol with single postsynaptic APs [single-spike (SS) pairing] in PreAd compared with Ad neurons (SS pairing, Fig. 1B, PreAd: 101 ± 26%, \( n = 11 \) vs Ad: 32 ± 14%, \( n = 11 \), independent samples t-test \( P = 0.030 \)). Following additional pairing with postsynaptic bursts of APs (BS pairing), synaptic strength significantly increased in Ad neurons (Ad: 99 ± 18% to a level that was similar to PreAd neurons (BS pairing, Fig. 1A, PreAd: 127 ± 44%, independent samples t-test \( P = 0.54 \)).

Given the difference in postsynaptic AP requirements for plasticity, we tested whether backpropagation of single APs and bursts of spikes differed in PreAd and Ad dendrites using two-photon calcium imaging. Backpropagation was determined as a change in the fluorescence of the calcium-sensitive dye, Fluo-4, as measured with two-photon calcium imaging at different distances from the soma (Fig. 1B, see MATERIALS AND METHODS). There were significant differences in the level of backpropagation for single APs and bursts across age groups [ANOVA \( F(3,38) = 10.4, P < 0.001 \)]. Single BAPs were detected at significantly greater distances along dendrites from PreAd compared with Ad neurons [PreAd: 408 ± 56 μm (\( n = 7 \)), Ad: 221 ± 27 μm (\( n = 16 \)), post hoc t-test, \( P < 0.01 \), Fig. 1C]. There was no significant change in propagation distance of single APs vs. bursts of spikes in younger neurons [BS values PreAd: 488 ± 46 μm (\( n = 8 \)) \( P = 1 \), nonsignificant, Fig. 1C], but in Ad dendrites, there was a trend for bursts of spikes to propagate significantly further than single APs [SS: 221 ± 27 μm (\( n = 16 \)), BS: 358 ± 34 μm (\( n = 8 \)), \( P = 0.066 \)] to a distance similar to that seen with bursts in PreAd dendrites \( (P = 0.21, \text{Fig. 1C}) \). Thus dendritic excitability in response to backpropagation of single APs and bursts is significantly altered between PreAd and Ad developmental stages.

During hippocampal development, CA1 pyramidal neurons undergo prominent changes in both their neuronal morphology and regulation by GABAergic inhibition. To determine whether either of these mechanisms could underlie the change in tLTP induction rules observed, we first characterized the maturation of dendritic and both excitatory and inhibitory synaptic morphology during the same developmental period (Fig. 2). For both age groups, dendritic morphology of six CA1 pyramidal cells was reconstructed. Total dendritic length of both apical and basal dendrites significantly increased during development [Fig. 2, A and B, apical PreAd 5.7 ± 0.4 mm (\( n = 6 \)), Ad 7.7 ± 0.2 mm (\( n = 6 \)), \( P = 0.0017 \); basal PreAd 1.7 ± 0.2 (\( n = 6 \)), Ad 3.1 ± 0.2 mm (\( n = 6 \)), \( P = 0.0011 \)]. Sholl analysis revealed an increased number of basal and thin oblique dendrites at 200–275 μm in older neurons compared with PreAd neurons (Fig. 2, C and D).

At the ultrastructural level, GABAergic pre- and postsynaptic properties were quantified at the electron microscopy level in two dendritic regions, stratum radiatum (SR) and stratum lacunosum moleculare (SLM) (Fig. 2, E and G). Glutamatergic synapses have asymmetric profiles with a thick strongly labeled PSD, while GABAergic synapses have a symmetric profile with a thin PSD (Fig. 2, E and G) (Lund et al. 2001; Marty et al. 2002; Megias et al. 2001). The number of docked vesicles in GABAergic synapses showed a prominent increase with age in both SR and SLM [Fig. 2H, middle, SR PreAd 2.77 ± 0.11 (\( n = 108 \)), Ad 3.97 ± 0.14 (\( n = 120 \)), \( P < 0.001 \); SLM PreAd 2.54 ± 0.10 (\( n = 114 \)), Ad 4.54 ± 0.19 (\( n = 114 \)), \( P < 0.001 \)]. In the Ad group, this upregulation in number of docked vesicles was significantly greater in SLM than in SR [Fig. 2H, middle, SR 3.97 ± 0.14 vs. SLM 4.54 ± 0.19, \( P < 0.001 \)].
Neuronal morphology can prominently alter dendritic excitability and AP backpropagation (Golding et al. 2001; van Elburg and van Ooyen 2010; Vetter et al. 2001; Yang et al. 2012). In addition, dendrite diameter has significant effects on input resistance and voltage (Migliore et al. 2005). To isolate the effect of morphological changes, we assessed passive neuronal properties and dendritic backpropagation in a simplified passive NEURON model, in which morphology parameters were varied based upon the reconstructed morphologies of CA1 pyramidal neurons (Fig. 2). All models contained the same active Hodgkin-Huxley-like channels in the soma, passive leak channels in dendrites and were stimulated with hyperpolarizing and depolarizing steps at the soma, similar to the experiments with real neurons. There was a clear developmental decrease in input resistance [Fig. 3B, top, PreAd 144 ± 11 (n = 6), Ad 108 ± 11 MΩ (n = 6), P = 0.040], but no significant effect on membrane time constants [Fig. 3B, bottom, PreAd 20.1 ± 0.2 (n = 6), Ad 20.1 ± 0.1 ms (n = 6), P = 0.97]. This pattern replicated the experimental data [Fig. 3A, input resistance PreAd 182 ± 12 (n = 6), Ad 126 ± 15 MΩ (n = 6), P = 0.014; τ PreAd 31.0 ± 3.6 (n = 6), Ad 27.5 ± 2.7 ms (n = 6), P = 0.47], showing that the increased morphological complexity observed during development resulted in a lower input resistance. However, backpropagation into distal dendritic regions, indicated by peak voltage of BAP, was not affected by morphological differences [Fig. 3, C and D, exponential decay PreAd 329 ± 67 µm (n = 6), Ad 331 ± 30 µm (n = 6), P = 0.98]. Thus developmental changes in morphology can explain maturation...
of neuronal passive properties but do not significantly alter distance-dependent backpropagation into distal dendrites.

Dendritic BAP is necessary for induction of STDP (Feldman 2012; Kampa et al. 2007). An increase in GABAergic inhibition onto dendrites, as observed during the Ad stage (Fig. 2; Banks et al. 2002; Cohen et al. 2000), is predicted to decrease excitability of neurons and to modulate dendritic BAP. To investigate the functional consequences of increased GABAergic inhibition upon STDP, tLTP induction paradigms were tested in Ad neurons in the presence of the GABA(A) receptor antagonist, gabazine (Fig. 4).

The contributions of tonic and phasic inhibition were separated using differing concentrations of gabazine (Stell and Mody 2002). Blocking phasic but leaving tonic inhibition intact, Ad neurons still required BS for tLTP, similar to control aCSF conditions [Fig. 4, A and C, 200 nM gabazine: single AP (SS) pairing: 124 ± 19% (n = 5), P = 0.28 from baseline, burst (BS) pairing: 180 ± 7% EPSP slope potentiation (n = 5), P < 0.001]. However, blocking both tonic and phasic inhibition with a higher gabazine concentration lowered the threshold for tLTP to that of PreAd neurons [Fig. 4, B and C, 10 μM gabazine: single AP (SS) pairing: 132 ± 14%, n = 11, burst (BS) pairing: 199 ± 18% EPSP slope potentiation, n = 11, P < 0.001].
To directly determine whether the GABAergic regulation of plasticity was underpinned by changes in dendritic excitability, we measured AP backpropagation along apical dendrites and blocked all GABA(A) receptor-mediated inhibition (gabazine, 10 μM). In PreAd CA1 pyramidal neurons, there was no change in backpropagation of APs for either BAP(burst) or BAP(ss) into apical dendrites when GABA(A) receptors were blocked (Fig. 5A). The amplitude of the BAP-induced calcium transients with GABA(A) receptor blockers decreased linearly with distance, similar to the distance dependency observed in control conditions (aCSF) [Fig. 5A, linear regression slope BAP(ss): control (n = 6 cells) -1.6 ± 0.3 (SD) %ΔF/R·mm⁻¹, gabazine (10 μM, n = 5 cells) -1.6 ± 0.3 (SD) %ΔF/R·mm⁻¹; BAP(burst): control (n = 6 cells) -3.9 ± 0.5 (SD) %ΔF/R·mm⁻¹, gabazine (10 μM, n = 5 cells) -2.9 ± 0.6 (SD) %ΔF/R·mm⁻¹; two-way ANOVA BAP(ss) P = 0.54, BAP(burst) P = 0.41].

In contrast, blockade of all GABA(A) receptor activity significantly modulated backpropagation along dendrites of Ad neurons. Backpropagation of AP bursts (Fig. 5B) was significantly enhanced in distal dendritic regions relative to control (250–450 μm from soma, P < 0.001, two-way ANOVA followed by Bonferroni post hoc tests). BAP(ss) showed the same trend [distance 50% amplitude BAP(burst): control 262 ± 65 μm (n = 10), gabazine (10 μM) 493 ± 81 μm (n = 6), P < 0.001; BAP(ss): control 229 ± 27 μm (n = 9), gabazine (10 μM) 292 ± 54 μm (n = 2), P = 0.35]. Intriguingly, propagation into the proximal dendritic regions was not affected by blocking GABAergic inhibition, indicating a distance-dependent increase in dendritic inhibition. The kinetics, both rise and decay, of BAP-induced calcium transients were not distance dependent in either age group [Fig. 5, C and D, regression analysis (ANOVA) decay PreAd P = 0.54, Ad P = 0.57; rise PreAd P = 0.76, Ad P = 0.088]. However, the decay of fluorescent signals was significantly slower in PreAd compared with Ad dendrites [Fig. 5E, PreAd 0.74 ± 0.15 s (n = 9), Ad 0.45 ± 0.05 s (n = 17), P = 0.029]. Furthermore, blockade of GABA(A) receptors resulted in a significantly slower decay of calcium transients in Ad dendrites, to a level not significantly different from transients in PreAd dendrites [Fig. 5E, Ad control 0.45 ± 0.05 s (n = 17), Ad gabazine 0.78 ± 0.18 s (n = 8), P = 0.028]. This modulation of calcium fluorescence decay kinetics by GABA(A) inhibition was not seen in younger dendrites [Fig. 5E, PreAd control 0.74 ± 0.15 s (n = 9), PreAd gabazine 0.69 ± 0.10 s (n = 9), P = 0.76]. Thus upregulation in GABAergic inhibition onto CA1 pyramidal neuron dendrites during late postnatal development significantly attenuated backpropagation in more distal but not proximal regions of Ad dendrites only.

GABAergic inhibition manifests itself in two ways: fast phasic (milliseconds) and slow tonic (seconds) inhibition, mediated by different receptor subunits and involved in different dendritic functions (Brickley and Mody 2012). In the hippocampus, tonic GABAergic currents are mediated by the δ and α5 GABA(A) receptor subunits (Caraiscos et al. 2004; Scimemi et al. 2005). In addition, we have shown that STDP induction rules are dependent on tonic GABAergic inhibition (Fig. 4). We therefore investigated which subunit was responsible for the GABAergic modulation of BAP in Ad neurons. Specific blockade of α5-mediated tonic GABA(A) currents with the antagonist L-655,708 (100 nM) had no effect on spontaneous phasic GABAergic inhibition (data not shown). However, it caused strong modulation of BAP-induced calcium transients in distal dendrites, similar to effects of gabazine at 10 μM [Fig. 6, A and B, distance 50% amplitude control 262 ± 65 μm (n = 10), L-655,708 417 ± 14 μm (n = 6), gabazine (10 μM) 493 ± 81 μm (n = 8), P < 0.001 compared with aCSF control conditions]. This modulation by α5-mediated GABA(A) receptors was seen for both BAP(ss) and BAP(burst) [distance 50% amplitude BAP(ss) control 229 ± 27 μm (n = 9), L-655,708 336 ± 21 μm (n = 4), P = 0.034, BAP(burst) control 262 ± 65 μm (n = 10), L-655,708 417 ± 14 μm (n = 6), P < 0.001].

Tonic GABA(A) currents mediated by the δ-subunit were specifically enhanced using THIP (10 μM) (Olmos-Serrano et al. 2010). THIP had no effect on amplitudes of the calcium transients [Fig. 6, A and B, distance 50% amplitude control 262 ± 65 μm (n = 10), THIP 264 ± 77 μm (n = 10), P = 0.95],
indicating that δ-subunits did not underlie the distance-dependent modulation of BAP. However, it significantly reduced the decay of the BAP-induced calcium transient [Fig. 6C, τ decay control $0.45 \pm 0.05$ s ($n = 17$), THIP $0.30 \pm 0.03$ s ($n = 11$), $P = 0.044$], suggesting a role for the GABA(A) δ-subunit in BAP kinetics, but not BAP amplitude or spread. Therefore, the developmental increase of GABA(A) receptor-mediated inhibition onto the distal dendrites of CA1 pyramidal neurons is mainly conferred by tonic α5-subunit-containing GABA(A) receptors that significantly attenuate dendritic excitability.

To directly show that α5-containing GABA(A) receptors mediate tonic currents in the distal dendrites, we measured local dendritic holding currents with dendritic patch-clamp recordings following blockade of GABA(A) α5-subunits (Figs. 5, 6, and 7). Application of L-655,708 significantly altered tonic dendritic currents ($8.9 \pm 2.2$ pA relative to baseline, aCSF: −27.6 ± 6.5 pA, L-655,708: −18.7 ± 6.3 pA, $P = 0.016$ paired t-test, $n = 5$, Fig. 7, B and C). Subsequent infusion of 10 μM of gabazine to block all GABA(A) receptors did not further change the holding current ($9.4 \pm 4.0$ pA relative to 8.9 ± 2.2 pA, $P = 0.91$, $n = 4$, Fig. 7, B and C), demonstrating that Ad CA1 pyramidal dendrites showed prominent dendritic tonic currents which are mediated by α5-containing GABA(A) receptors.

Calcium transients in the dendrite, as measured by two-photon calcium imaging, result from activation of voltage-dependent calcium channels (Ross 2012). To test if α5-subunit-mediated effects were due to changes in AP backpropagation of voltage or due to GABAergic effects upon calcium stores or channels, we made dendritic patch-clamp recordings in regions where the most prominent inhibitory modulation was observed (Fig. 5). We measured dendritic AP amplitudes, induced by a depolarizing step, before and after the application of the α5-subunit-specific antagonist, L-655,708 (Fig. 8A). Peak dendritic voltage showed a significant increase after blocking α5 GABA(A) receptor subunits [Fig. 8B, BAP amplitude from baseline membrane potential ($V_m$), pre 71.6 ± 2.5, post 74.0 ± 2.6 mV ($n = 10$), $P = 0.039$], in line with increased dendritic transients recorded in the calcium imaging experiments. No significant increase was observed in aCSF controls (Fig. 8C, $P = 0.36$). In contrast to dendrites, no significant change in AP amplitude was observed at the soma following blockade of α5-specific inhibition (Table 1). These data suggest that, similar to GABAergic regulation of dendritic calcium transients, the voltage signal of the BAP is also modulated by α5-subunit-mediated tonic inhibition in a dendrite-specific manner.

To further investigate the spatial extent of tonic GABAergic modulation both at the soma and in the dendrites, we tested the effects of GABA-receptor modulating drugs on somatic function. Both in PreAd and Ad neurons, there was no effect of α5-receptor subunit blockade on passive ($V_m$, membrane τ, input resistance), or active properties (AP peak, AP halfwidth, AP threshold) measured at the soma (Table 1). These somatic recordings do not reliably detect distal dendritic tonic currents due to strong attenuation within the dendrites and limited spatial reach of somatic voltage clamp (Williams and Mitchell 2008). Together with the observation that proximal dendrites were not affected by blocking GABA(A)-mediated inhibition (Figs. 5C and 6A) and distal dendrites show prominent α5-GABA(A) subunit-mediated tonic current (Fig. 7), this indicates that the prominent effect of α5-subunit-mediated tonic inhibition is specifically targeted toward distal dendritic regions.

Both interneurons and astrocytes, the potential sources of tonic inhibition, target specific regions of the dendrites (Heja et al. 2012; Klausberger 2009), creating different spatial cover-
age. In addition, ion channels, such as HCN channels, show a spatially regulated distribution along CA1 pyramidal dendrites (Hoffman et al. 1997; Magee 1998; Nestor and Hoffman 2012b). To test whether spatial distribution of tonic GABA inhibition in dendrites could explain our observations, we implemented a well-established active model of a mature CA1 pyramidal neuron on to a reconstructed Ad neuron (Poirazi et al. 2003; Sterratt et al. 2012). Tonic GABA(A) conductances (Pavlav et al. 2009) were inserted in the apical dendrite with differing spatial gradients of expression: distributed uniformly along the somato-dendritic axis, in a distally decreasing gradient or restricted to a localized distal expression of dendritic tonic inhibition. Model simulations demonstrate that these findings are consistent with a distally increasing or a localized distal expression of dendritic tonic inhibition.

We show that effects of tonic GABA(A) α5-receptor-mediated inhibition on excitability are exerted in distal dendrites but not at the soma of Ad neurons. α5-Receptor-mediated currents regulate tonic inhibition locally and attenuate the amplitude of somatically generated BAPs. From model simulations, we find that the attenuation of dendritic BAP can be accounted for by distance-dependent tonic inhibition, similar to increasing distance gradients reported for voltage-gated ion channels, h channels and A-type potassium channels (Hoffman et al. 1997; Magee et al. 1998; Nestor and Hoffman 2012a). Our model findings of dendrite-specific localization of tonic currents are consistent with immunohistology: α5-containing GABA receptors are predominantly expressed in the dendritic layers of CA1 hippocampus during this Ad period of development (Hutcheon et al. 2004; Ramos et al. 2004). In the distal SR, the majority of GABAergic inputs to CA1 pyramidal neurons are made on to dendritic shafts (Megias et al. 2001), the same region where we also observed the strongest modulation of calcium transients by BAP. These GABAergic inputs made directly on dendrites are proposed to mediate tonic inhibitory currents (Brickley and Mody 2012). Here, we now show at a functional level that α5-mediated tonic receptors on distal dendrites effectively regulate dendritic excitability.

During pyramidal cell development, the total length of apical and basal dendrites increases, while at the same time firing pattern shifts from regular to burst firing (Degenetias et al. 2002; Franceschetti et al. 1998; Zhang 2004). This change in firing pattern may be directly attributable to increased dendritic size (van Elburg and van Ooyen 2010). However, as we have shown here, these developmental changes in morphol-
Fig. 6. α5-Mediated tonic GABA(A) receptors are responsible for the BAP modulation in Ad neurons. A: distance-dependent BAP(burst) in Ad (P28 – 46) neurons in control conditions (dark blue), 200 nM GBZ (green), 10 μM GBZ (red), L-655,708 (light blue) and THIP (purple). Multiple distances from the soma were recorded for each cell. Amplitudes are shown relative to the first proximal dendritic recording at 100 μm. Dashed line indicates 50% relative amplitude. Binned averages are shown. Inset: absolute amplitudes (ΔF/Δt) plotted against distance from soma for all conditions. B: quantification of the BAP modulation by GABA(A) receptor subunit-mediated inhibition using the distance at which the calcium transient amplitude was 50% of the first proximal recording. Significant differences in distance of 50% amplitude occur between L-655,708 and GBZ (10 μM) conditions compared with control. Individual values and mean ± SE distribution are shown. C: significant alteration in decay time constant of dendritic fluorescence signal in presence of THIP or GBZ (10 μM) compared with control. *P < 0.05, **P < 0.01.

Modulation of dendritic excitability by ligand-gated GABAergic ion channels complements the modulation of dendritic APs via voltage-gated ion channels, including A-type potassium and Ih channels (Andrasfalvy et al. 2008; Magee et al. 1998). The expression levels of some of these channels also alter during Ad maturation (Bender et al. 2001; Guan et al. 2011; Maletic-Savatic et al. 1995; Vasilyev and Barish 2002). Intriguingly, the L-type voltage-gated calcium channel, Cav 1.3, only reaches mature levels in the second postnatal month in rodents (Glazewski et al. 1993; Kramer et al. 2012). It is tempting to speculate that this developmental increase in excitability by calcium channels might counteract the increased inhibition mediated via α5-containing GABA receptors. In our study, we were able to show that the increased inhibition via α5-containing GABA receptors in Ad neurons could explain the altered synaptic plasticity thresholds during development. Levels of excitation and inhibition are kept in a regulatory balance during development and adulthood, a phenomenon called homeostatic scaling (Liu 2004; Mody 2005). Tonic inhibition plays an important role in maintaining the excitation/inhibition balance, as evidenced by the strong upregulation of tonic GABA(A) receptor-mediated currents following the knock-out of excitatory h- or A-type potassium currents (Andrasfalvy et al. 2008; Chen et al. 2010). Development of excitation/inhibition balance as well as the dendritic expression levels of these channels need to be subject to further investigation.

A dendrite-specific localization of tonic inhibition is in line with recent proposals that dendritic compartments act as independent functional units within a neuron where different regions have different functions (Branco et al. 2010). Both excitatory and inhibitory projections onto pyramidal cells are targeted to specific dendritic compartments, depending on presynaptic cell type and brain region, in this way creating different functional regions within the cell (Klausberger 2009; Royer et al. 2012; van Strien et al. 2009). In the hippocampus in vivo, dendritically targeted inhibition changes both frequency and firing mode of pyramidal neurons, depending on their place field selectivity (Royer et al. 2012). In contrast, in the same study somatic inhibition was found to affect only the phase of spiking. Here, we show that tonic currents are specifically directed onto dendritic compartments, controlling both excitability and plasticity in distal dendrites, without affecting somatic function. Given this restricted dendritic compartmentalization, we hypothesize that activating tonic inhibition will enhance the effect of perisomatic inputs upon the soma relative to distal inputs by reducing dendritic excitability.

Tonic inhibitory currents have significant implications for dendritic computation (Fernandez and White 2010; Pavlov et al. 2009). Due to the larger dendritic membrane area covered by tonic extrasynaptic contacts compared with phasic synaptic contacts, activation of tonic receptors causes a larger charge transfer than activation of synaptic receptors alone (Brunig et al. 2002; Olsen and Sieghart 2009). Therefore, tonic receptors, located on dendritic shafts, are optimal candidates to directly modulate dendritic excitability and thereby dendritic computation (Fernandez and White 2010; Pavlov et al. 2009). Our data show that GABA(A) receptor-mediated tonic neurotransmission modulated dendritic excitability (Figs. 5 and 6). This effect occurs locally in dendrites, is developmentally regulated and only observed in Ad neurons. One direct functional consequence of increased tonic GABAergic inhibition is that Ad neurons require a burst of APs rather than SSs for synaptic plasticity (Fig. 1). Tonic GABA(A)-mediated inhibition regulated the threshold for induction of LTP, in agreement with a
decreased threshold for theta-frequency LTP induction in α5 GABA(A) knockout mice (Martin et al. 2010).

In this study, we have focused on the role of dendritic excitability modulation during development. We have shown that GABAergic modulation could explain a difference in plasticity induction requirements. However, this does not exclude additional developmental changes occurring simultaneously at the site of origin, the synapse. Changes in calcium influx at the spine via postsynaptic voltage-gated calcium channel expression (Jones et al. 1997) or developmental regulation of synaptic protein kinases underlying hippocampal plasticity (Luchkina et al. 2014; Yasuda et al. 2003) could also contribute to changes in induction thresholds during postnatal maturation.

In the hippocampus, tonic inhibitory currents are mediated by the α5 and δ GABA(A) receptor subunits (Brickley and Mody 2012), which are both located at peri- and extrasynaptic sites. In our study, blocking of both phasic and tonic inhibition resulted in the same modulation of BAP as blocking tonic inhibition alone (Figs. 5 and 6). The distance-dependent modulation of BAP was fully accounted for by α5-mediated tonic currents (Fig. 6). Intriguingly, dendritic α5-mediated regulation was observed for both calcium transients and voltage deflections, suggesting a direct action on voltage propagation. In addition to regulation by the α5-subunit, we found a small change in calcium fluorescence kinetics in Ad dendrites, in agreement with dendrite-specific δ-subunit expression in the mature hippocampus (Sperk et al. 1997), but this did not affect the range of BAP, which was solely regulated by α5-subunit mediated GABAergic inhibition. During puberty, an upregulation in α4βδ-containing GABA(A) receptors occurs in CA1 hippocampus of female mice, which also impairs LTP induction and, furthermore, impairs spatial learning (Shen et al. 2010). However, in agreement with our findings in CA1 pyramidal neurons from Ad mice, the majority of tonic inhibition measured at the soma in young adult rodents is mediated by α5, with only a small residual component mediated either via the δ-subunit or receptors containing only α- or β-subunits (Glykys et al. 2008). The specific role of the δ-subunit in the kinetics of the BAP requires further investigation and would benefit from the development of a specific antagonist in addition to the currently available agonist THIP.

In rodents, GABAergic function in the hippocampus continues to mature beyond weaning up to the second postnatal month (Banks et al. 2002; Cohen et al. 2000; Danglot et al. 2006). We verified this protracted development of GABAergic inhibition at the electron-microscopic level by the significant development of the synapse and synaptic vesicles at symmetric synapses during late postnatal development (Fig. 1). Consequently, we found a developmental increase in dendritic tonic GABA(A) receptor-mediated modulation of BAP (Figs. 5 and 6). This enhancement of tonic inhibition is consistent with a
### Table 1. Active and passive properties of young (postnatal days 14–19) and mature (postnatal days 28–46) CA1 pyramidal neurons based on spike profile before and after the application of the GABA(A) targeted drugs gabazine (10 μM and 200 nM), THIP, and L-655,708, or artificial cerebrospinal fluid

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<th>Mature CA1 pyramidal neurons</th>
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<td>Pre</td>
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<td>$V_{m}$, mV</td>
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<tr>
<td>8</td>
<td>$-64.1 \pm 1.5$</td>
<td>$-58.1 \pm 1.1$</td>
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<td>Gabazine (10 μM)</td>
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<td>$-65.9 \pm 0.8$</td>
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Values are means ± SE; $n$, no. of neurons. $V_{m}$, membrane potential; $R_{m}$, input resistance; $\tau$, time constant; AP, action potential; Pre, before application; Post, after application. Tonic inhibition does not alter somatic passive and active properties. Active and passive properties of preadolescent (postnatal days 14–19) and adolescent (postnatal days 28–46) CA1 pyramidal neurons based on spike profile before (pre) and after (post) the application of the GABA(A) targeted drugs gabazine (10 μM and 200 nM), THIP, and L-655,708 or artificial cerebrospinal fluid are shown. Statistical analysis is based on repeated-measures mixed ANOVA. Significant difference: * $P < 0.05$, ** $P < 0.01$, or not significant (ns).

Fig. 9. Model predicts localized increasing tonic GABA(A) receptor distribution. A, morphology of a reconstructed Ad neuron incorporated in a 3D NEURON model containing a realistic set of active and passive properties of mature CA1 pyramidal neurons (Sterratt et al. 2012). Scale bar represents 100 μm. B, example traces of voltage deflections at the locations indicated in A, in the presence of uniform tonic currents. C, tonic GABA(A) conductance, as measured by Pavlov et al. (2009), was distributed in the apical dendrites of the model according to Poisson et al. (2009) was distributed in the apical dendrites of the model according to Poisson et al. (2009), with the apical dendrite receiving the strongest tonic currents. D, want to show that the tonic currents have local impact on somatic AP properties. E, modulation of the different tonic currents in A on soma and dendrites as measured by Pavlov et al. (2009). F, modulation of the different tonic currents in A on soma and dendrites as measured by Pavlov et al. (2009).
tonic receptors, the latter via spillover (Glykys and Mody 2007), or whether interneurons specialize in one of the two types of inhibition is as yet unclear. In addition, astrocytes can also release GABA into the extracellular space (Brickley and Mody 2012; Farrant and Nusser 2005; Heja et al. 2012) and could thereby regulate dendritic excitability of the hippocampus. Our data show that effects of tonic GABA(A) receptor-mediated inhibition are not uniform along apical dendrites, suggesting localized GABA release from distal dendrite-targeting interneurons or astrocytes.

In summary, we have shown that dendritically targeted α5-mediated tonic inhibition regulates dendritic excitability and STDP in a developmental manner. Our mechanistic data show that dendrite and soma are differently regulated by tonic α5-mediated inhibition, which has important implications for dendritic computation and hippocampal function.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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