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Hippocampal Network Hyperactivity After Selective Reduction of Tonic Inhibition in $GABA_A$ Receptor α5 Subunit–Deficient Mice

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Glykys, Joseph and Istvan Mody. Hippocampal network hyperactivity following selective reduction of tonic inhibition in $GABA_A$ receptor α5 subunit–deficient mice. *J Neurophysiol* 95: 2796–2807, 2006. First published February 1, 2006; doi:10.1152/jn.01122.2005. Functionally, γ-aminobutyric acid receptor (GABAR)–mediated inhibition can be classified as phasic (synaptic) and tonic (extrasynaptic). The GABARs underlying tonic inhibition assemble from subunits different from those responsible for phasic inhibition. We wanted to assess the excitability of hippocampal pyramidal cell (PC) networks following a selective impairment of tonic inhibition. This is difficult to accomplish by pharmacological means. Because the GABAR α5 subunits mostly mediate the tonic inhibition in CA1 and CA3 PCs, we quantified changes in tonic inhibition and examined network excitability in slices of adult *gabra5*$^{-/-}$ mice. In *gabra5*$^{-/-}$ CA1 and CA3 PCs tonic inhibitory currents were 60 and 53%, respectively, of those recorded in wild type (WT), with no alterations in phasic inhibition. The amount of tonic inhibition recorded in slices was significantly affected by the method of slice storage (interface or submerged chamber). Field recordings in *gabra5*$^{-/-}$ CA3 pyramidal layer showed an increased network excitability that was decreased by the GABAR agonist muscimol at a concentration that restored the tonic inhibition of *gabra5*$^{-/-}$ PCs to the WT level without altering phasic inhibition. Through a battery of pharmacological experiments, we have identified δ subunit–containing GABARs as the mediators of the residual tonic inhibition in *gabra5*$^{-/-}$ PCs. Our study is consistent with an important role of tonic inhibition in the control of hippocampal network excitability and highlights selective enhancers of tonic inhibition as promising therapeutic approaches for diseases involving network hyperexcitability.

INTRODUCTION

γ-Aminobutyric acid receptors (GABARs) are ligand-gated Cl^- channels mediating most of chemical inhibitory activity in the brain. These receptors are pentameric assemblies of seven different subunit classes: α (1–6), β (1–3), γ (1–3), δ, θ, ϵ, and ρ (1–3) (Hevers and Lüddens 1998; Sieghart and Sperck 2002). Functionally, GABARs can be classified as mediating phasic or tonic inhibition (Farrant and Nusser 2005; Mody 2001; Semyanov et al. 2004). Phasic inhibition is mediated by GABARs localized at synapses, whereas tonic inhibition arises from activation of extrasynaptic receptors by low concentrations of ambient GABA (Farrant and Nusser 2005; Mody 2001; Semyanov et al. 2004). These two types of inhibition have been identified in cerebellar granule cells (Brickley et al. 1996; Rossi et al. 2003), dentate gyrus granule cells (Nusser and Mody 2002; Stell and Mody 2002), CA1 pyramidal cells (PCs), and interneurons (Bai et al. 2001; Caraiscos et al. 2004; Semyanov et al. 2003). Extrasynaptic GABARs outnumber those found at synapses (Nusser et al. 1995) and have been suggested to sense GABA spillover (Banks and Pearce 2000; Wei et al. 2003). The different properties of extrasynaptic and synaptic receptors are thought to result from either different subunit composition (Nusser and Mody 2002; Stell and Mody 2002) or from a differential regulation by second messengers (Banks and Pearce 2000).

Phasic inhibition can be selectively enhanced (Nusser and Mody 2002) or decreased (Stell and Mody 2002) by pharmacological means without altering tonic inhibition. In contrast, pharmacological tools are available for specifically enhancing tonic inhibition (Stell et al. 2003; Wallner et al. 2003; Wei et al. 2004), but not for its selective decrease without affecting spontaneous inhibitory currents (Semyanov et al. 2003). Nevertheless, we wanted to study the effects of a selective reduction in tonic inhibition on the excitability of CA1 and CA3 PC networks, the latter having a particularly low threshold for seizure generation (Colom and Saggau 1994; Nagao et al. 1996). Therefore we carried out quantitative measurements of tonic and phasic inhibitions and of network excitability in adult *gabra5*$^{-/-}$ mice known to have a deficit in tonic inhibition (Caraiscos et al. 2004).

The GABAR α5 subunit has a restricted distribution in the rodent brain. It is primarily found in dendritic areas (strata radiatum, oriens/lacunosum moleculare) of hippocampal CA1 and CA3 regions (Fritschy and Möhler 1995; Pirker et al. 2000; Sur et al. 1999; Wisden et al. 1992). In addition, this subunit is thought to contribute almost exclusively to the extrasynaptic GABAR pool of PCs (Brünig et al. 2002; Caraiscos et al. 2004). Inhibition mediated by α5 subunit–containing receptors has been shown to be involved in learning and memory (Collinson et al. 2002), trace fear conditioning (Crestani et al. 2002), generation of gamma oscillations (Towers et al. 2004), as well as in epilepsy (Houser and Esclapez 2003; Rice et al. 1996; Scimemi et al. 2005). In young (P18–P23) *gabra5*$^{-/-}$ mice, CA1 PCs appear to be devoid of tonic GABA currents (Caraiscos et al. 2004). Such a selective involvement of GABAR subunits in tonic inhibition has been shown only for δ subunit–containing receptors (Stell et al. 2003) that have a relatively late development (Laurie et al. 1992).

We further investigated the selective decrease of tonic inhibition in adult *gabra5*$^{-/-}$ mouse hippocampal slices and found a reduction, not a total loss, of tonic inhibition in both CA1 and

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CA3 PCs, without any alteration in phasic inhibition. This selective decrease in tonic inhibition led to epileptiform hyperexcitability in the CA3 pyramidal layer, which could be rescued by restoring tonic inhibition to levels observed in wild-type (WT) PCs. Pharmacological studies using specific modulators of tonic inhibition are consistent with a homeostatic upregulation of δ subunits in *gabra5*$^{-/-}$ mice responsible for the residual tonic current observed in *gabra5*$^{-/-}$ PCs.

METHODS

Slice preparation

This study mainly used adult male (1–4 mo old) C57/Bl6, *gabra5*$^{-/-}$, and *gabra5*$^{+/+}$ littermates, but young (18 days old) *gabra5*$^{-/-}$ animals were also studied. The *gabra5*$^{-/-}$ and WT littermates were raised from heterozygous breeding pairs kindly provided by Merck, Sharp & Dohme (Harlow, UK) and were bred at the UCLA Division of Laboratory Animal Medicine. Mice were anesthetized with halothane according to a protocol approved by the UCLA Chancellor's Animal Research Committee. The brain was removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 D-glucose with pH 7.3–7.4 when bubbled with 95% O_2-5% CO_2. Brain slices, 350 μm thick, were cut with a Leica VT1000S Vibratome (Leica Microsystems, Wetzlar, Germany) in aCSF containing 3 mM kynurenic acid (Sigma, St. Louis, MO). Coronal slices were prepared for CA1 PC whole cell recordings, whereas CA3 PC recordings were obtained in horizontal slices. The CA3 and CA1 regions were surgically disconnected for field recording studies to prevent CA3 from driving the activity in CA1. Slices were stored at 32°C for ≥1 h either in a submerged [tetrahydrodeoxycorticosterone (THDOC) experiments] or an interface chamber (for the rest of experiments) before being transferred to the recording chamber.

Whole cell recordings

CA1 and CA3 PCs were visually identified (Olympus, Melville, NY; BX51WI, IR-DIC videomicroscopy; 40 × water immersion objective) and recorded with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Slices were continuously perfused (about 2.5 ml/min) at 32–34°C with 95% O_2-5% CO_2 bubbled aCSF containing kynurenic acid (3 mM) and other drugs as indicated. Microelectrodes (1–5 MΩ when filled) contained the following internal solution (in mM): 140 CsCl, 1 $MgCl_2$, 10 HEPES, 0.1 EGTA, 4 NaCl, 2 MgATP, and 5 QX-314, pH ≈ 7.25, about 285 mOsm. Whole cell voltage-clamp recordings were performed at −70 mV holding potential. Series resistance and whole cell capacitance were estimated from fast transients evoked by a 5-mV voltage command step using lag values of 7–10 μs and then compensated to 70–80%. Recordings were discontinued if series resistance increased by >25% through an experiment, or the compensated resistance surpassed 20 MΩ at any time during the experiment.

Extracellular field recordings

Simultaneous recordings were made in CA1 and CA3 pyramidal layers in slices held at 32–34°C in a 95% O_2-5% CO_2 aerated interface chamber and perfused with aCSF saturated with 95% O_2-5% CO_2 (flow rate ≈ 2.5 ml/min). Signal amplification was achieved by a custom-made 100X headstage connected to a DC amplifier (Model 440; Brownlee Precision, San Jose, CA). Recording pipettes were filled with aCSF and had resistances of about 5 MΩ.

Drugs

GABA (Sigma) at a concentration of 5 μM was added to the aCSF where indicated and slices were preincubated in this solution for 10 min before starting whole cell recordings. We first determined whether the 5 μM GABA is the real concentration activating the GABA receptors responsible for tonic inhibition when perfused onto

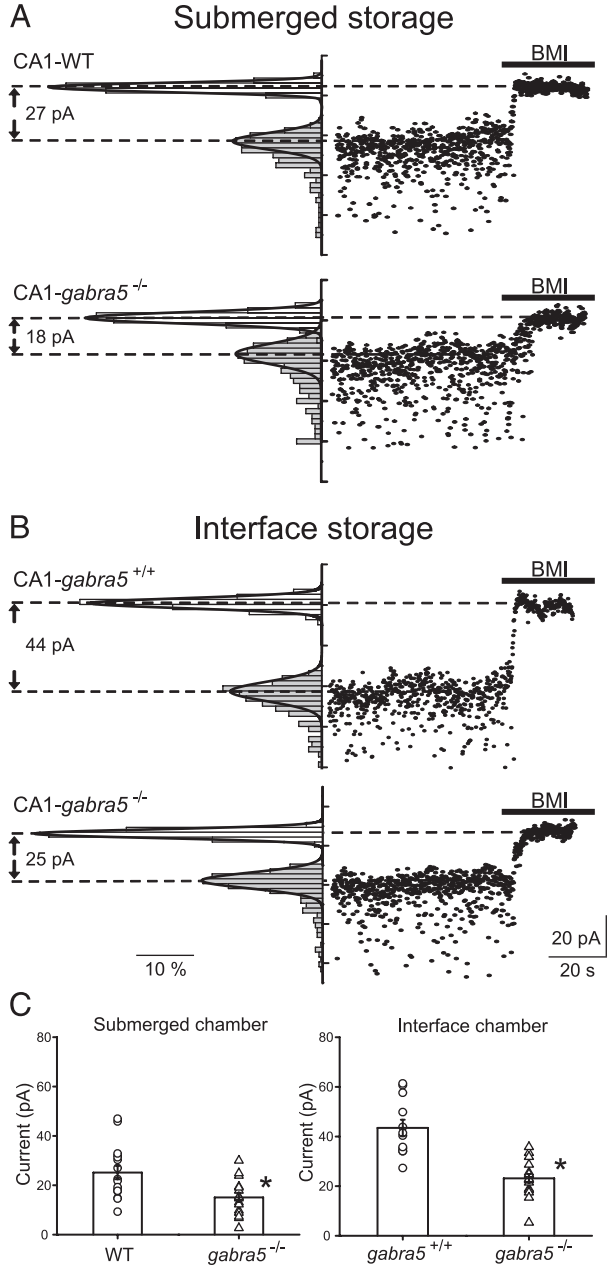

FIG. 1. Residual γ-aminobutyric acid receptor (GABAR)–mediated tonic currents in *gabra5*$^{-/-}$ CA1 pyramidal cells (PCs) and the effect of slice storage condition. *A*: submerged chamber. *B*: interface chamber. Tonic currents in wild-type (WT), *gabra5*$^{+/+}$, and *gabra5*$^{-/-}$ CA1 PCs. *Right*: baseline current measured at 100-ms intervals under control conditions and after the addition of 20 μl bicuculline methiodide (BMI, 10 mM) into the perfusing solution (horizontal bar; $V_h = -70$ mV). *Left*: Gaussian fits to baseline current histograms under control conditions and in the presence of BMI (binwidth of 2.5 pA). Dotted lines indicate the mean baseline current pre- and postapplication of BMI. Note the presence of a residual tonic current in both conditions. *C*: tonic currents histogram from all WT, *gabra5*$^{+/+}$, and *gabra5*$^{-/-}$ CA1 PCs recorded. Circle and triangle represent single-cell tonic current. Error bar indicates SE; (*) indicates statistical significance ($P < 0.01$, unpaired *t*-test).

brain slices. In CA1 PCs of C57/Bl6 mice perfused with aCSF containing 5 μM GABA the tonic current increased to 195% of control, from 22.5 ± 4.6 to 43.9 ± 3.1 pA ($n = 5$; $P = 0.006$; paired *t*-test), on addition of the GAT-1 GABA uptake blocker NO-711 (10 μM). This finding indicates that active GABA transport/degradation mechanisms remove a large part of the externally added GABA. Thus the final concentration of ambient GABA in our brain slices is considerably <5 μM, probably close to the physiological GABA levels measured in the extracellular space by microdialysis in the hippocampus (range 0.2–2.5 μM obtained from pooled values of Ding et al. 1998; Lerma et al. 1986; Tossman et al. 1986). Unless indicated otherwise, tonic currents were recorded in aCSF with 5 μM GABA added to standardize the ambient GABA levels around the recorded neurons, which might otherwise vary with the depth of the neuron in the slice, the level of local spontaneous GABA release, and uptake or other unknown factors.

Stock solutions of THDOC (Sigma) were prepared in 100% ethanol and added to the aCSF where indicated (final concentration: 100 nM THDOC, 0.002% ethanol). Diazepam (DZP) was dissolved in 100% dimethylsulfoxide (DMSO) and added where indicated (final concentration: 0.5 μM, 0.05% DMSO). Muscimol (Fluka, St. Louis, MO) and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride (THIP, Tocris Cookson, Ellisville, MO) were added to the aCSF where indicated. Bicuculline methiodide (BMI, 20 μl of 10 mM stock solution; Sigma) was injected directly into the recording chamber (final concentration ≈ 200 μM) to reveal tonic currents. $CdCl_2$ (50 μM) was dissolved in aCSF to record miniature inhibitory postsynaptic currents (mIPSCs).

Data acquisition and analysis

All recordings were low-pass filtered at 3 kHz and digitized on-line at 10 kHz using a PCI-MIO 16E-4 data-acquisition board (National Instruments, Austin, TX). Spontaneous field activity was detected using a custom-written LabView-based software.

1) Measurement of tonic currents: Values of the mean current measured during 5-ms epochs collected every 100 ms were used to construct a histogram (binwidth 2.5 pA). The histogram had a skewed distribution toward larger negative values because some of the 5-ms epochs landed on segments of inward currents during spontaneous IPSCs (sIPSCs). To ensure that currents generated by IPSCs were not included in our measurement of tonic currents, we fitted a Gaussian only using the unskewed portion of the distribution. The mean of this Gaussian fit was used as the value for the baseline tonic current (see Fig. 1). This analysis was done by using a custom-made macro in IGOR Pro V5.0 (Wavemetrics, Lake Oswego, OR). In a given cell, we

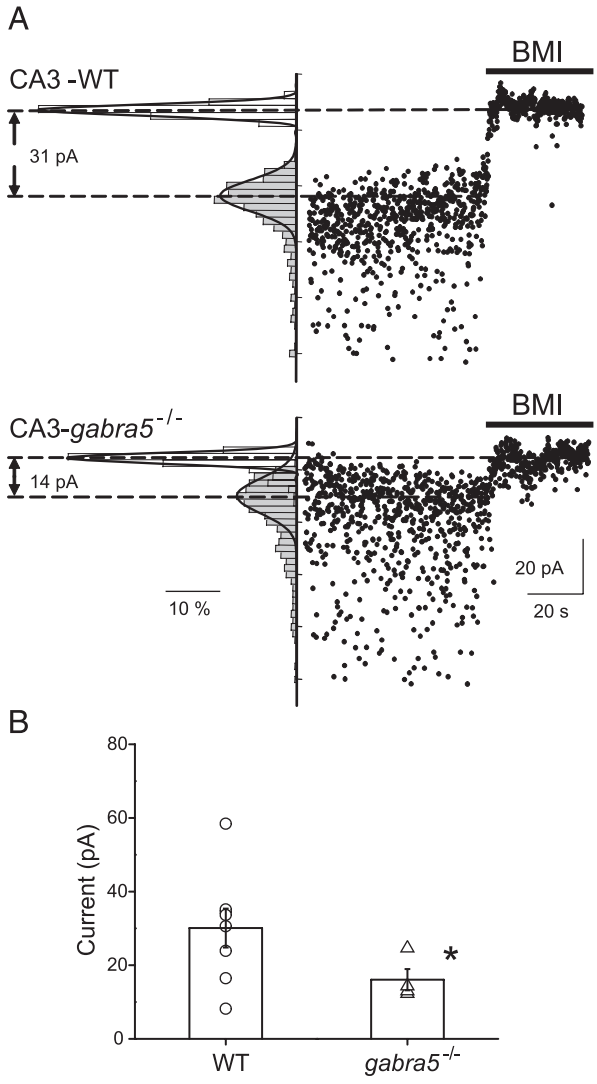

FIG. 2. Residual GABAR-mediated tonic currents in a *gabra5*$^{-/-}$ CA3 PC. *A*: method of measurement tonic currents was the same as described in the legend of Fig. 1. Note the presence of a residual tonic current of 14 pA in the *gabra5*$^{-/-}$ CA3 PC. *B*: tonic currents histogram from all WT and *gabra5*$^{-/-}$ CA3 PCs recorded. Circle and triangle represent single-cell tonic current. Error bar indicates SE; (*) indicates statistical significance ($P < 0.05$, unpaired *t*-test).

TABLE 1. *Tonic and sIPSC characteristics from wild-type (WT), gabra5*$^{+/+}$*, and gabra5*$^{-/-}$ *hippocampal pyramidal cells*

	sIPSC–CA1 (Interface)		sIPSC–CA1 (Submerged)		mIPSC–CA1		sIPSC–CA3 (submerged)	
	gabra5$^{+/+}$	*gabra5*$^{-/-}$	WT	*gabra5*$^{-/-}$	C57/B16	*gabra5*$^{-/-}$	C57/B16	*gabra5*$^{-/-}$
Tonic inhibition								
Tonic current, pA	43.5 ± 3.30	23.1 ± 1.75*	25.1 ± 2.71	15.1 ± 1.82*	N/A	N/A	30.1 ± 5.28	16.1 ± 2.89*
N	12	17	16	16	N/A	N/A	8	4
Phasic inhibition								
Frequency, Hz	26.7 ± 1.79	26.7 ± 2.77	23.7 ± 2.10	25.5 ± 2.19	18.5 ± 1.76	16.2 ± 2.82	30.3 ± 4.54	34.8 ± 3.70
Peak amplitude, pA	49.1 ± 2.76	43.0 ± 2.52	46.3 ± 2.73	49.2 ± 1.88	30.7 ± 1.86	31.0 ± 2.51	46.8 ± 3.42	49.6 ± 3.53
Rise time 10–90, ms	0.70 ± 0.02	0.68 ± 0.02	0.67 ± 0.02	0.64 ± 0.03	0.54 ± 0.02	0.55 ± 0.01	0.73 ± 0.02	0.72 ± 0.03
τ_{decay}, ms	6.59 ± 0.10	6.29 ± 0.14	6.25 ± 0.11	6.23 ± 0.12	5.67 ± 0.16	5.32 ± 0.49	6.25 ± 0.16	6.63 ± 0.12
n	12		12		6	4	7	6

Values are means ± SE. CA1 and CA3 pyramidal cell (PC) sIPSCs, and CA1 PC mIPSCs recorded from WT (C57/B16 + *gabra5*$^{+/+}$), *gabra5*$^{+/+}$, and *gabra5*$^{-/-}$ mice in the presence of 3 mM kynurenic acid and 5 μM GABA ($V_h = -70$ mV). CA1 PC sIPSCs were recorded in slices stored in a modified interface or a submerged incubating chamber. Twelve randomly selected cells were compared in each group by a two-way ANOVA showing no statistical difference between genotype, incubating chamber, or an interaction between both variables. $CdCl_2$ (50 μM) was used to isolate mIPSCs. There were no statistical differences in CA3 sIPSCs and CA1 mIPSCs between WT and *gabra5*$^{-/-}$ mice in any of the matching groups ($P > 0.05$, unpaired *t*-test). *Signifies statistical difference between genotypes ($P < 0.05$, unpaired *t*-test). N/A, nonapplicable.

obtained the magnitude of the tonic current by subtracting the baseline current recorded after the addition of saturating BMI concentrations to the aCSF from that recorded before addition of BMI. The input resistance was calculated during the currents produced by the seal tests.

2) Detection and measurement of sIPSCs: All sIPSCs were detected in 30-s recording segments. Event frequency, 10–90 rise time (RT10–90), and weighted decay time constant (τ_{decay}) values were measured. $IPSC_{slow}$ was defined as any sIPSC with an RT10–90 >1.5 ms. The ratio of $IPSC_{slow}$ over total number of events ($R_{slow/total}$) was determined by dividing the frequency of $IPSC_{slow}$ by the frequency of total sIPSCs. Average data are shown as mean ± SE. Statistical significance was assessed by unpaired *t*-test assuming unequal variances, and by two-way and one-way ANOVAs with a Tukey test for multiple comparisons. To perform ANOVA tests between a pharmacological experimental group and their respective controls, we took a random sample of cells from the respective *gabra5*$^{+/+}$ or *gabra5*$^{-/-}$ control group (columns 1 or 2 of Table 1), equivalent in number to the cells in the pharmacological experiment. The level of significance was set at $P < 0.05$.

3) Analysis of extracellular field recordings: Recordings were sampled at 10 kHz and low-pass filtered at 3 kHz. Spontaneous field events were defined as at least a 0.025-mV deflection in baseline voltage. Interevent intervals and number of spikes per burst were analyzed in spontaneous field recordings. A power spectrum was used to demonstrate fast ripple activity. Statistical differences were determined by χ^2 test and by Wilcoxon matched-pairs signed-ranks test; the level of significance was set at $P < 0.05$.

RESULTS

Reduced tonic inhibition in gabra5$^{-/-}$ *CA1 and CA3 PCs*

Tonic currents recorded in CA1 PCs of C57/Bl6 and *gabra5*$^{+/+}$ brain slices stored in a submerged chamber before recordings were not statistically different (C57/Bl6 23.2 ± 3.51 pA, $n = 9$; *gabra5*$^{+/+}$ 27.6 ± 5.18, $n = 7$; $P = 0.45$, unpaired *t*-test); therefore the two groups were pooled together and designated as WT (25.1 ± 2.71 pA, $n = 16$). The average input resistance in control conditions was 111.7 ± 4.73 MΩ compared with 125.8 ± 3.9 MΩ in the presence of BMI ($n = 16$, $P < 0.01$, paired *t*-test). CA1 PCs of *gabra5*$^{-/-}$ slices did not show a complete absence of tonic current because a reduced tonic current (60% of control) could still be recorded in these cells that was statistically different from the one recorded in WT (*gabra5*$^{-/-}$ 15.1 ± 1.82 pA, $n = 16$; $P < 0.01$, unpaired *t*-test; Fig. 1, *A* and *C*). In these cells, the input resistances were not significantly different between control and BMI conditions (113.4 ± 6.67 MΩ vs. 119.4 ± 5.9 MΩ; $n = 14$, $P > 0.05$, paired *t*-test). Similar results were found in *gabra5*$^{-/-}$ CA3 PCs with a residual tonic current (53% of control) that was significantly smaller than that recorded in C57/Bl6 CA3 PCs (C57/Bl6 30.1 ± 5.28 pA, $n = 8$ vs. *gabra5*$^{-/-}$ 16.1 ± 2.89 pA, $n = 4$; $P < 0.05$, unpaired *t*-test; Fig. 2).

The health and average life span of slices after preparation depend on the type of storage chamber used (Krimer and Goldman-Rakic 1997). We wanted to compare recordings from slices stored in a modified interface incubating chamber to those stored in a submerged chamber. Tonic currents recorded in CA1 PCs of slices stored in the interface chamber for 1–3 h were larger than those recorded in slices stored in a submerged chamber. Nevertheless, the tonic currents of *gabra5*$^{-/-}$ PCs were still statistically reduced (53% of control) compared with *gabra5*$^{+/+}$ (*gabra5*$^{+/+}$ 43.5 ± 3.30 pA, $n = 12$ vs. *gabra5*$^{-/-}$ 23.1 ± 1.75 pA, $n = 17$; $P < 0.001$, unpaired *t*-test; Fig. 1, *B* and *C*). A two-factor ANOVA between 12 randomly selected samples from WT and *gabra5*$^{-/-}$ PCs in both incubating

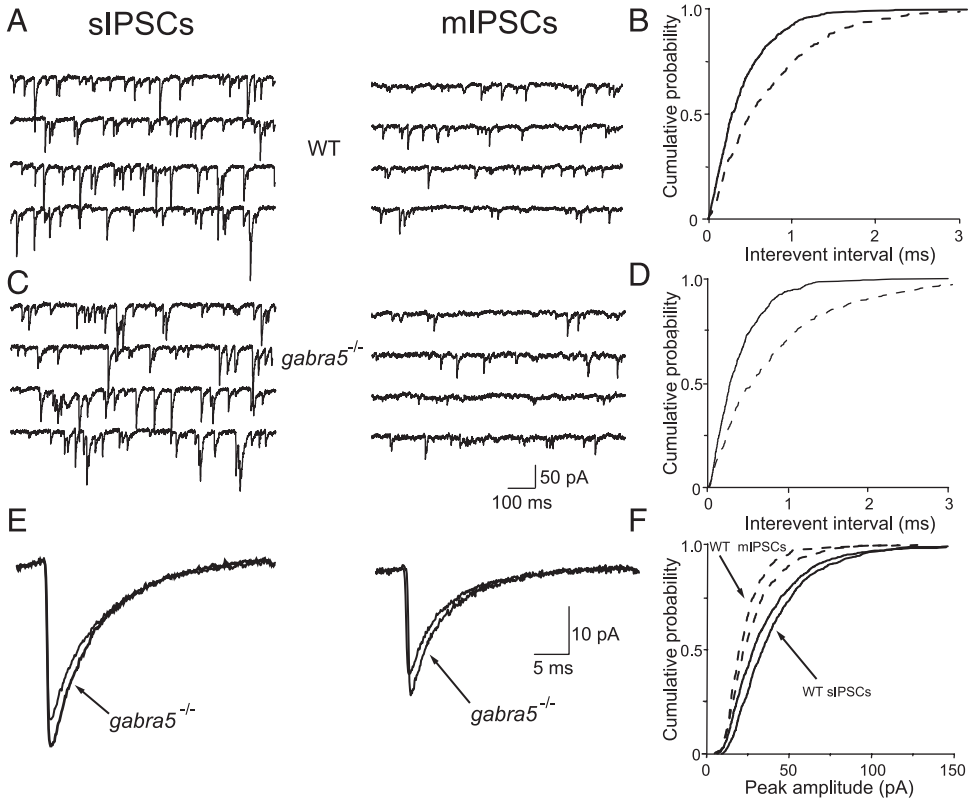

FIG. 3. Phasic inhibition is intact in *gabra5*$^{-/-}$ hippocampal PCs. *A*, *left*: sIPSCs recorded in a WT CA1 PC. *Right*: mIPSCs recorded from a WT CA1 PC ($V_h = -70$ mV). *B*: interevent interval cumulative probability distributions of spontaneous inhibitory postsynaptic currents (sIPSCs, solid line) and miniature (m-)IPSCs (dashed line) from the same cells in *A*. *C*: same as *A* but recorded from a *gabra5*$^{-/-}$ CA1 PC ($V_h = -70$ mV). *D*: same as *B* but recorded from a *gabra5*$^{-/-}$ CA1 PC. *E*, *left*: superimposed averaged sIPSCs recorded from a WT and a *gabra5*$^{-/-}$ (arrow) CA1 PC showing no differences in kinetics. *Right*: superimposed averaged mIPSCs recorded from a WT and a *gabra5*$^{-/-}$ (arrow) CA1 PC showing no differences in kinetics. *F*: cumulative probability plots of peak mIPSCs (dashed lines) and sIPSCs (solid lines) amplitudes from WT (arrows) and *gabra5*$^{-/-}$ CA1 PCs. There are no statistical differences between WT and *gabra5*$^{-/-}$ group averages. All experiments were done in the presence of 3 mM kynurenic acid and 5 μM GABA.

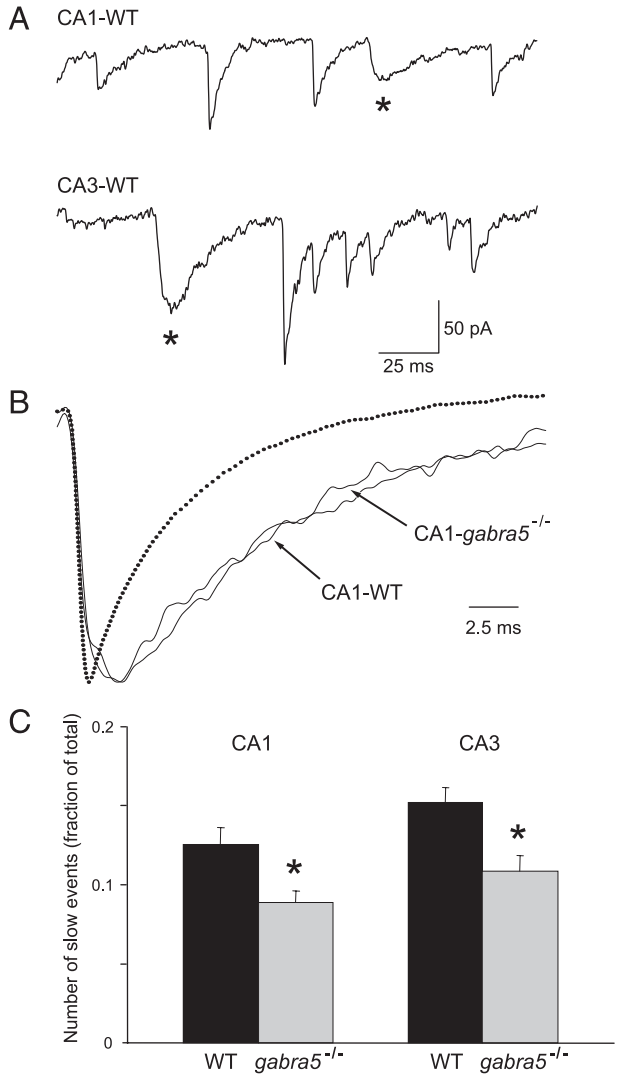

FIG. 4. $IPSC_{slow}$ recorded in CA1 and CA3 PCs. *A*: sample trace recorded from a WT CA1 and CA3 PC ($V_h = -70$ mV). $IPSC_{slow}$ events are indicated by an asterisk. *B*: normalized average sIPSC (dotted line) and $IPSC_{slow}$ in WT (same cell) and in a *gabra5*$^{-/-}$ CA1 PC. Note the difference in kinetics between sIPSC and $IPSC_{slow}$. *C*: average ratios between the number of $IPSC_{slow}$ events and the total number of events in WT CA1 ($n = 11$), *gabra5*$^{-/-}$ CA1 ($n = 11$), WT CA3 ($n = 7$), and *gabra5*$^{-/-}$ CA3 ($n = 6$). Error bars = SE. There is significantly lower fraction of $IPSC_{slow}$ events in *gabra5*$^{-/-}$ PCs than in WT (*$P < 0.05$, unpaired *t*-test).

conditions showed a significant genotype effect regardless of the incubation condition ($P = 4 \times 10^{-6}$, df = 1), a significant effect of the incubation condition regardless of the genotype ($P = 1.1 \times 10^{-5}$, df = 1) and an interaction between genotype and incubation condition ($P = 0.019$, df = 1). The tonic currents recorded in WT PCs in submerged versus interface chamber were significantly different ($P = 3.6 \times 10^{-5}$, one-way ANOVA, Tukey test) but those in *gabra5*$^{-/-}$ PCs were similar between both incubation conditions ($P = 0.29$, one-way ANOVA, Tukey test). In other words, the incubation condition affected more the tonic inhibition recorded in WT than in *gabra5*$^{-/-}$ PCs.

Regardless of the incubation condition, there was a residual tonic current in the absence of GABAR α5 subunits. These results are in contrast to those of Caraiscos et al. (2004) in CA1 PCs, where the loss of α5 subunits was paralleled by a total loss of tonic currents. One difference between the two experimental approaches is that we used steady levels of extracellular GABA (5 μM) to reveal tonic currents instead of enhancing extracellular GABA levels by blocking GABA transaminase by vigabatrin (Caraiscos et al. 2004). Another reason could have been that we used adult mice (1–4 mo old), whereas younger mice (P18–P23) were used in the previous study (Caraiscos et al. 2004). As other GABAR subunits such as the δ start being expressed at a later developmental stage (after P12; Laurie et al. 1992) than α5 subunits (P1–P7; Ramos et al. 2004), compensatory mechanisms by δ subunits may not be in place until later in development. To test this, we performed experiments in P18 *gabra5*$^{-/-}$ CA1 PCs in the presence of 5 μM GABA. A residual tonic current of 22.7 ± 1.30 pA ($n = 4$) was still present in these slices. These results suggest that even at a very young age there is still a residual tonic current in *gabra5*$^{-/-}$ CA1 PCs. Even in the absence of GABA in the aCSF a residual tonic current was still present in adult *gabra5*$^{-/-}$ CA1 PCs (13.9 ± 5.55 pA, $n = 3$).

Phasic inhibition is unaltered in the absence of α5 subunits

Phasic inhibition is mediated by synaptic GABARs (Mody 2001; Semyanov et al. 2004). Because the α5 subunit is known to be localized mostly extrasynaptically in CA1 and CA3 PCs (Brünig et al. 2002), phasic inhibition is not expected to be altered in the absence of these subunits. However, reduced mean peak sIPSC amplitudes recorded at 22°C have been reported in brain slices prepared from P14–P32 *gabra5*$^{-/-}$ mice (Collinson et al. 2002). We also examined whether the absence of α5 subunits alters sIPSCs by recording sIPSCs in

TABLE 2. *Properties of $IPSC_{slow}$ recorded in C57/B16 and gabra5*$^{-/-}$ *pyramidal cells*

	CA1 Pyramidal Cells				CA3 Pyramidal Cells	
	C57/B16	*gabra5*$^{-/-}$	*gabra5*$^{-/-}$Veh	*gabra5*$^{-/-}$ THDOC	C57/B16	*gabra5*$^{-/-}$
Frequency, Hz	3.22 ± 0.28	2.40 ± 0.21*	2.43 ± 0.37	2.49 ± 0.10	4.74 ± 0.60	3.77 ± 0.21
$Ratio_{slow/total}$	0.13 ± 0.01	0.09 ± 0.01*	0.07 ± 0.003	0.09 ± 0.03	0.15 ± 0.01	0.11 ± 0.01*
Peak amplitude, pA	26.4 ± 1.77	23.4 ± 0.48	24.7 ± 0.82	22.2 ± 1.24	30.9 ± 1.63	32.1 ± 2.72
RT10–90, ms	2.56 ± 0.04	2.56 ± 0.04	2.48 ± 0.02	2.42 ± 0.18	2.58 ± 0.08	2.48 ± 0.04
τ_{decay}, ms	6.91 ± 0.26	6.87 ± 0.24	6.30 ± 0.13	6.01 ± 0.36	6.59 ± 0.39	7.10 ± 0.19
n	11	11	3	3	7	6

Values are means ± SE. $IPSC_{slow}$ recorded from CA1 and CA3 PCs from C57/B16 and *gabra5*$^{-/-}$ mice in the presence of 3 mM kynurenic acid and 5 μM GABA, as well as in the CA1 PCs in the presence of vehicle or 100 nM THDOC ($V_h = -70$ mV). $Ratio_{slow/total}$ between C57/B16 CA1 and CA3 PCs was not statistically different ($P = 0.08$). *Signifies statistical difference between genotypes ($P < 0.05$, unpaired *t*-test).

gabra5$^{+/+}$ and *gabra5*$^{-/-}$ CA1 PCs of adult mouse brain slices stored in either a submerged or an interface chamber. In the presence of 3 mM kynurenic acid and 5 μM GABA, the mean frequency of sIPSCs, peak amplitudes, RT10–90, and τ_{decay} were similar between *gabra5*$^{-/-}$ and *gabra5*$^{+/+}$ mice and also did not show an effect of the incubating condition (two-way ANOVA, $P > 0.05$, df = 1, $n = 12$, the same randomly selected cells used to compare the tonic currents; Table 1, Fig. 3). sIPSCs frequency and kinetics recorded in C57/Bl6 and *gabra5*$^{-/-}$ CA3 PCs were also not statistically different ($P > 0.05$, unpaired *t*-test; Table 1). We followed up these experiments by examining mIPSCs in CA1 PCs of the two genotypes using $CdCl_2$ (50 μM) to block presynaptic Ca^{2+} entry. There were no statistical differences in the frequency, peak amplitude, RT10–90, or τ_{decay} of mIPSCs between C57/Bl6 and *gabra5*$^{-/-}$ slices ($P > 0.05$ for each parameter, unpaired *t*-test; Table 1 and Fig. 3). The most plausible explanation for these results is that α5 subunits, even if present at synapses, do not shape the synaptic currents and, consequently, there are no synaptic compensatory mechanisms when this subunit is deleted. If indeed α5 subunits are present at synapses, they may be in a desensitized state or may form heteromeric GABARs with other α subunits, thus overriding the specific properties of α5 subunits.

A proportion of IPSC$_{slow}$ events persist in gabra5$^{-/-}$ animals

$IPSC_{slow}$ have been reported in CA1 PCs in mature rats (Banks et al. 2002), with severalfold slower kinetics than that of $IPSC_{fast}$ (Banks and Pearce 1998; Pearce 1993). To determine whether $IPSC_{slow}$ events in CA1 PCs are attributed to spillover of GABA to extrasynaptic α5 subunit–containing receptors, we compared slow sIPSCs in CA1 and CA3 PCs of WT and *gabra5*$^{-/-}$ brain slices (Fig. 4*A*). We did not find a statistical difference in event kinetics between the two groups (Table 2 and Fig. 4*B*), although there was a statistical difference in the frequency of slow events (WT: frequency 3.22 ± 0.28 Hz, $n = 11$; *gabra5*$^{-/-}$: frequency 2.40 ± 0.21 Hz, $n = 11$; $P < 0.05$, unpaired *t*-test, Table 2) as well as in the $R_{slow/total}$ (WT: 0.13 ± 0.01, $n = 11$ and *gabra5*$^{-/-}$: 0.09 ± 0.01, $n = 11$; $P < 0.05$, unpaired *t*-test; 31% reduction; Table 2 and Fig. 4*C*). When CA3 PCs were compared between the two genotypes, there were no statistical differences in kinetics and in frequency of events (Table 2 and Fig. 4*B*). Nonetheless, there was a significant 27% reduction in $R_{slow/total}$ (C57/Bl6: 0.15 ± 0.01, $n = 7$ and *gabra5*$^{-/-}$: 0.11 ± 0.01, $n = 6$; $P < 0.05$, unpaired *t*-test; Table 2 and Fig. 4*C*), suggesting that at least a fraction of $IPSC_{slow}$ events in the WT result from GABA spillover onto α5 extrasynaptic receptors. The charge mediated by $IPSC_{slow}$ events in C57/Bl6 CA3 PCs was1.17 pC, representing a mere 2.9% of the total inhibitory charge (40.4 pC) of which 74.5% is mediated by the tonic current. In *gabra5*$^{-/-}$ slices $IPSC_{slow}$ events carried a charge of 1.05 pC corresponding to 3.6% of the total inhibitory charge (29.3 pC), of which only 54.8% was mediated by tonic inhibition. Similar results were found in CA1 PCs.

Neuronal hyperexcitability in gabra5$^{-/-}$ hippocampal slices

At this time there are no specific pharmacological blockers of tonic inhibition that will be without effect on phasic currents (Semyanov et al. 2003). Lanthanum has been shown to block GABARs responsible for tonic inhibition (Saxena et al. 1997; Shen et al. 2005), although this cation also has significant effects on synaptic transmission and other cellular events. Therefore the nearly 44% decrease in tonic inhibition in CA1 PCs and the 47% decrease in CA3 PCs with no change in phasic inhibition provide an ideal setting to study the effects of a specific reduction in tonic inhibition on neuronal excitability. To study these two regions independently and to prevent the possibility of the CA3 PCs driving the activity in the CA1 region, we surgically disconnected the two regions at the time the slices were prepared. The aCSF used in these experiments contained neither kynurenic acid nor GABA to record the full excitability of the system. Field recordings in the CA1 pyramidal layer of C57/Bl6 and *gabra5*$^{-/-}$ slices showed no spontaneous activity ($n = 12$ and $n = 9$, respectively). In contrast, spontaneous activity could be recorded in the CA3 region. Spontaneous single-population spikelike events were recorded in both C57/Bl6 and *gabra5*$^{-/-}$ CA3 regions (C57/Bl6 5/12 slices vs. *gabra5*$^{-/-}$ 4/9 slices, χ^2 test, $P > 0.05$,

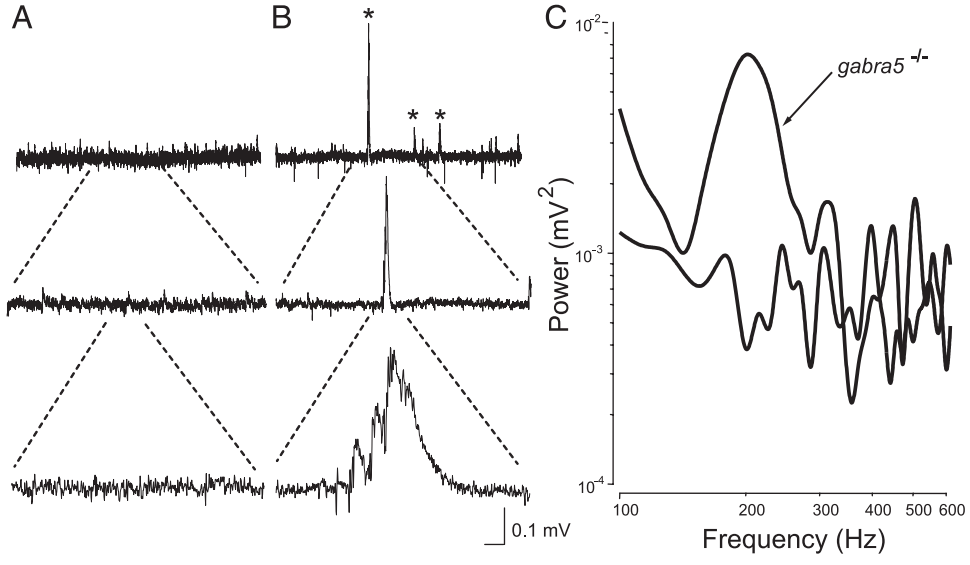

FIG. 5. Decreased tonic inhibition induces hyperexcitability in CA3 pyramidal layer of *gabra5*$^{-/-}$ mice. *A*: spontaneous field recording from the CA3 pyramidal layer of a WT mouse. *Top*: a 3-min sample recording (scale bar: 240 s). *Middle*: a 3-fold higher temporal resolution of the indicated segment (scale bar: 80 ms). *Bottom*: an even higher temporal resolution of the indicated segment (scale bar: 10 ms). Note the absence of spontaneous activity. *B*: spontaneous field recording from the CA3 pyramidal layer of a *gabra5*$^{-/-}$ mouse. Same scale as in *A*. Note the appearance of epileptiform bursts indicated by asterisks. *Bottom*: single epileptiform burst. Note the fast ripple activity during the burst. *C*: power spectrum of the 70-ms sample in *A* and *B* shows the high power at the fast ripple frequency (about 200 Hz) in the *gabra5*$^{-/-}$ (arrow), but not in the WT CA3 pyramidal layer.

df = 1). Interestingly, CA3 *gabra5*$^{-/-}$ slices showed spontaneous epileptiform bursts in five of nine slices with a mean interevent interval of 0.45 ± 0.12 s, mean number of spikes/burst of 2.76 ± 0.71 (n = 3), and a power spectrum peak at 200 Hz (Fig. 5). Such spontaneous bursts were never seen in slices of C57/Bl6 animals unless higher concentrations of extracellular K^+ (3 mM) and lower concentrations of Mg^{2+} (1.8 mM) and Ca^{2+} (1.6 mM) are used (Maier et al. 2003). Our results show that selectively reducing tonic inhibition by half in the CA3 region is sufficient to permit the occurrence of epileptiform bursts with a relatively high frequency (2.2 Hz) in spite of the full activity of phasic inhibition.

Restoring normal levels of tonic current in gabra5$^{-/-}$ decreases the neuronal hyperexcitability

The GABAR δ subunit, which begins expression during late development (Laurie et al. 1992), has been shown to mediate tonic currents in both dentate gyrus and in cerebellar granule cells but not in CA1 PCs (Stell et al. 2003; Wei et al. 2004). Muscimol, a $GABA_A$ receptor agonist, at low concentrations probably binds specifically to the δ subunit–containing GABARs because there is no [^{3}H]muscimol binding in the brains of *gabrd*$^{-/-}$ mice (Mihalek et al. 1999). Therefore we wanted to determine whether muscimol at low concentration (25 nM) in a 5 μM GABA–containing aCSF had any specific effects on tonic inhibition. A two-way ANOVA in seven randomly selected cells in each group showed that the effect of muscimol depended on genotype (P = 0.004, df = 1). Muscimol (25 nM) increased tonic currents only in *gabra5*$^{-/-}$ CA1 PCs, while not affecting those recorded in *gabra5*$^{+/+}$ CA1 PCs (*gabra5*$^{+/+}$ control 45.8 ± 4.46 pA vs. *gabra5*$^{+/+}$ muscimol 48.8 ± 2.88 pA, P = 0.9734; *gabra5*$^{-/-}$ control 23.1 ± 3.70 pA vs. *gabra5*$^{-/-}$ muscimol 57.0 ± 7.24 pA, P = 0.0003; one-way ANOVA, Tukey test; Fig. 6, *A* and *B*). Frequency and kinetics of sIPSCs were not altered by this low concentration of muscimol regardless of genotype (one-way ANOVA for each parameter between the same randomly selected cells used in the tonic analysis, $P > 0.05$, df = 1; Fig. 6, *C* and *D*, Table 3) and the drug did not show an interaction with the genotype (two-way ANOVA, $P > 0.05$, df = 1, for frequency, peak amplitude, RT10–90, and τ_{decay}, between the same randomly selected cells used in the tonic analysis). These results show that 25 nM muscimol selectively increases the residual tonic currents of *gabra5*$^{-/-}$ PCs to wild-type levels without altering phasic inhibition.

We next wanted to test whether restoring the tonic currents to WT levels by this concentration of muscimol will decrease the hyperexcitability observed in the *gabra5*$^{-/-}$ CA3 pyramidal layer. This time, recordings were performed in the presence of 5 μM GABA to mimic the conditions used to record tonic currents. Spontaneous events in CA3 region occurred with a mean frequency of 5.7 ± 1.6 Hz and a mean peak amplitude of 84 ± 6 μV (n = 12; Fig. 7, *A* and *D*). After obtaining a baseline recording, 25 nM muscimol was perfused onto the slices. This produced a significant decrease in both frequency and peak amplitude (4.1 ± 1.5 Hz, P = 0.016; 79 ± 6 μV, P = 0.042, n = 12; Wilcoxon matched-pairs signed-rank test; Fig. 7, *B*, *C*, and *D*). Thus the network hyperexcitability was significantly decreased by 25 nM muscimol, a concentration that restored tonic inhibition in *gabra5*$^{-/-}$ PCs.

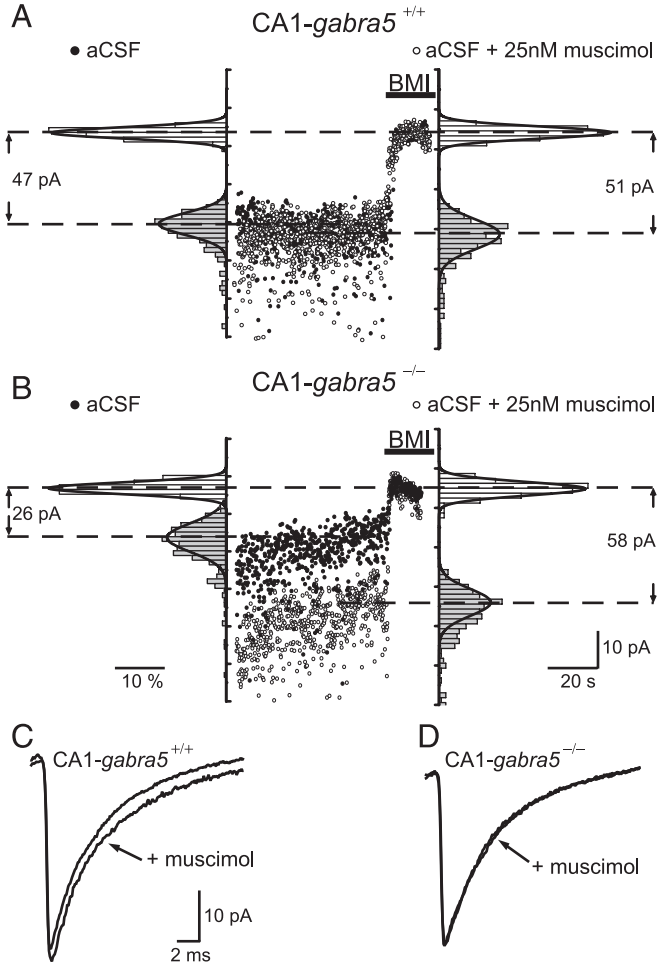

FIG. 6. Muscimol enhances the tonic current in *gabra5*$^{-/-}$ CA1 pyramidal cell but not in *gabra5*$^{+/+}$ CA1. Tonic currents were measured as described in Fig. 1 legend. *A*: superimposed *gabra5*$^{+/+}$ CA1 PC tonic current recordings in the presence of regular artificial cerebrospinal fluid (aCSF, ●, *left*) and 25 nM muscimol (○, *right*). Difference was not statistically different (one-way ANOVA, P = 0.9734, Tukey test). *B*: same as *A*, but recorded from a *gabra5*$^{-/-}$ CA1 PC. Difference in tonic current between aCSF and 25 nM muscimol was statistically different (one-way ANOVA, P = 0.0003, Tukey test). *C*: superimposed averaged sIPSCs from a single *gabra5*$^{+/+}$ CA1 PC in the presence of aCSF and 25 nM muscimol (arrow). *D*: same as *C* but recorded from a single *gabra5*$^{-/-}$ CA1 PC. Frequency and kinetics of sIPSCs were not statistically different between muscimol and regular aCSF in both *gabra5*$^{+/+}$ and *gabra5*$^{-/-}$ (one-way ANOVA, $P > 0.05$, Tukey test).

The $GABA_AR$ δ subunit is responsible for the residual tonic current in gabra5$^{-/-}$ CA1 pyramidal cells

Tonic inhibition in many neurons is mediated by GABARs containing α4 (or α6) and δ subunits highly sensitive to modulation by low, physiological concentrations of neurosteroids such as THDOC (Stell et al. 2003). We therefore wanted to consider the possibility that the residual tonic current seen in *gabra5*$^{-/-}$ PCs was mediated by such GABARs. Recordings were performed in the presence or absence of 100 nM THDOC (submerged incubation). In *gabra5*$^{+/+}$ CA1 PCs the THDOC induced change in tonic inhibition was not significant (THDOC group 40.4 ± 6.23 pA, n = 5 vs. control 25.1 ± 2.71, n = 16; P = 0.091, one-way ANOVA; Fig. 8*A*). A lack of THDOC effect on tonic currents in *gabra5*$^{+/+}$ mice is consistent with our previous findings showing that CA1 PCs are insensitive to this concentration of THDOC (Stell et al.

TABLE 3. *Effects of various drugs on the properties of tonic and phasic inhibition in gabra5$^{+/+}$ and gabra5$^{-/-}$ hippocampal CA1 pyramidal cells*

	Muscimol		THDOC#		THIP		Diazepam	
	gabra5$^{+/+}$	*gabra5*$^{-/-}$	*gabra5*$^{+/+}$	*gabra5*$^{-/-}$	*gabra5*$^{+/+}$	*gabra5*$^{-/-}$	*gabra5*$^{+/+}$	*gabra5*$^{-/-}$
Tonic inhibition								
Tonic current, pA	48.8 ± 2.88	57.0 ± 7.24*	40.4 ± 6.23	38.8 ± 7.21*	223.3 ± 25.20*	170.1 ± 9.81*	60.8 ± 7.05	36.8 ± 5.13
N	7	7	5	8	8	10	6	7
Phasic inhibition								
Frequency, Hz	22.6 ± 4.02	22.7 ± 2.16	18.3 ± 5.12	25.1 ± 4.51	17.2 ± 2.42	18.1 ± 2.19	29.11 ± 3.08	26.2 ± 3.53
Peak amplitude, pA	46.2 ± 3.86	40.8 ± 2.25	40.6 ± 4.25	47.4 ± 2.99	55.0 ± 2.87	53.7 ± 2.87	55.4 ± 3.08	53.0 ± 4.10
Rise Time 10–90, ms	0.75 ± 0.02	0.70 ± 0.02	0.79 ± 0.06	0.70 ± 0.05	0.78 ± 0.03*	0.76 ± 0.03*	0.73 ± 0.03	0.77 ± 0.05
τ_{decay}, ms	6.49 ± 0.37	6.65 ± 0.19	6.16 ± 0.19	6.24 ± 0.17	6.36 ± 0.16	6.12 ± 0.18	7.54 ± 0.27*	7.37 ± 0.35*
n	7		6	9	8		6	

Values are means ± SE. All conditions had in the perfusing solution 3 mM kynurenic acid, 5 μM GABA, and the indicated drug except THIP, where no GABA was added (V_h = −70 mV). Tonic and phasic currents of CA1 PCs are indicated for each condition and genotype. sIPSCs in the presence of THIP were compared to control conditions by the largest amplitude count matching method. *Signifies statistical difference between control conditions of the same genotype (see text for details). # Experiments were performed in slices stored in a submerged chamber. The remaining experiments were performed in slices stored in an interface chamber.

2003). In contrast, *gabra5*$^{-/-}$ CA1 PCs showed a significant increase (257% of control) of the residual tonic current in the presence of THDOC (THDOC group 38.8 ± 7.21 pA, $n = 8$, vs. control 15.1 ± 1.82 pA, $n = 16$; $P = 0.0004$, one-way ANOVA; Fig. 8*B*). Frequency and kinetics of sIPSCs were not altered by THDOC in any of the two genotypes when compared with control conditions (one-way ANOVA for each sIPSC parameter, $P > 0.05$; Table 3). We also wanted to address the contribution of δ subunit–containing GABARs to $IPSC_{slow}$ by comparing the kinetics and frequency of slow events in *gabra5*$^{-/-}$ CA1 PCs when exposed to vehicle or 100 nM THDOC. If the remaining $IPSC_{slow}$ events were mediated by extrasynaptic receptors containing δ subunits, there should be a change in their kinetics in the presence of THDOC; however, this was not the case because we found no significant difference between the groups (Table 2).

We performed another experiment to test the contribution of δ subunit–containing GABARs to the tonic currents recorded in *gabra5*$^{-/-}$ CA1 PCs. THIP (gaboxadol) is a GABA superagonist mostly at δ subunit–containing GABARs (Brown et al. 2002) and we used it to replace GABA in an equimolar concentration in the perfusion solution. *gabra5*$^{+/+}$ CA1 PCs tonic currents measured under this condition were 223.3 ± 25.20 pA ($n = 8$), whereas the *gabra5*$^{-/-}$ CA1 PCs showed a tonic current of 170.1 ± 9.81 pA ($n = 10$), both statistically different from those recorded in the presence of 5 μM GABA (one-way ANOVA, both $P = 1.1 \times 10^{-5}$, Tukey test). A two-way ANOVA from eight randomly selected cells showed no interaction between genotype and THIP. Nevertheless, the THIP/GABA tonic current ratio for the *gabra5*$^{-/-}$ CA1 PCs was 9.88, which was statistically different from the *gabra5*$^{+/+}$ CA1 PC tonic current ratio of 5.74 (one-way ANOVA, $P = 0.0005$, df = 1). To analyze the effect of THIP on phasic inhibitory currents, we had to use the largest-amplitude-count–matching method (Stell 2002) resulting from the high baseline noise in the presence of THIP that prevented the measurement of small-amplitude sIPSCs. THIP caused a statistical difference only in the RT10–90 of both genotypes when compared with 5 μM GABA (*gabra5*$^{+/+}$ control 0.60 ± 0.02 ms vs. *gabra5*$^{+/+}$ THIP 0.78 ± 0.03 ms, $P = 0.0051$; *gabra5*$^{-/-}$ control 0.63 ± 0.05 ms vs. *gabra5*$^{-/-}$ THIP 0.76 ± 0.03 ms, $P = 0.0394$, one-way ANOVA, from the same eight cells randomly selected to determine the ratio of THIP effect; Table 3). These results together with the effects of muscimol and THDOC show that *gabra5*$^{-/-}$ CA1 PCs have replaced the α5 subunit–containing GABARs mediating the tonic inhibition with receptors most likely containing δ subunits.

The $GABA_AR$ γ subunits are necessary for the effects of benzodiazepines (Pritchett et al. 1989). We wanted to test whether GABARs containing γ subunits are involved in generating tonic currents in *gabra5*$^{-/-}$ CA1 PCs. In the presence of a threshold concentration of diazepam (0.5 μM) tonic currents recorded in *gabra5*$^{+/+}$ CA1 PCs were 60.8 ± 7.05 pA ($n = 6$), whereas in *gabra5*$^{-/-}$ CA1 PCs were 36.8 ± 5.13 pA ($n = 7$). A two-way ANOVA between six randomly selected cells showed no interaction between genotype and diazepam ($P = 0.888$, df = 1). There was no statistical difference between the control values and diazepam for both *gabra5*$^{+/+}$ and *gabra5*$^{-/-}$ (*gabra5*$^{+/+}$ control: 45.5 ± 4.67 pA; *gabra5*$^{+/+}$ diazepam: 60.8 ± 7.05 pA; *gabra5*$^{-/-}$ control: 24.0 ± 2.64 pA; *gabra5*$^{-/-}$ diazepam: 37.8 ± 5.97 pA; $P > 0.05$, one-way ANOVA, Tukey test, $n = 6$). In contrast to its lack of effect on the tonic currents, this concentration of diazepam significantly increased the τ_{decay} of sIPSCs, whereas frequency and other properties were not affected (τ_{decay}: *gabra5*$^{+/+}$ control 6.50 ± 0.11 ms vs. *gabra5*$^{+/+}$ diazepam 7.54 ± 0.27 ms; *gabra5*$^{-/-}$ control 6.41 ± 0.22 ms vs. *gabra5*$^{-/-}$ diazepam 7.37 ± 0.35 ms; one-way ANOVA for each category, $P < 0.05$ for τ_{decay}, $n = 6$, the same cells used to compare tonic currents). A two-way ANOVA showed a significant drug effect regardless of the genotype for the RT10–90 ($P = 0.0386$, df = 1) and τ_{decay} ($P = 0.0007$, df = 1). Thus the low concentration of diazepam affected sIPSCs kinetics but failed to induce a statistical difference in tonic currents. The small, albeit not statistically significant, increase in the *gabra5*$^{-/-}$ tonic currents by diazepam points to a portion of the residual tonic current being mediated by GABARs containing α1–3 and γ subunits in addition to the diazepam-insensitive δ subunit–containing receptors. Perhaps this question can best be answered in a *gabrad*$^{-/-}$/*gabra5*$^{-/-}$ double knockout mouse, which is presently unavailable.

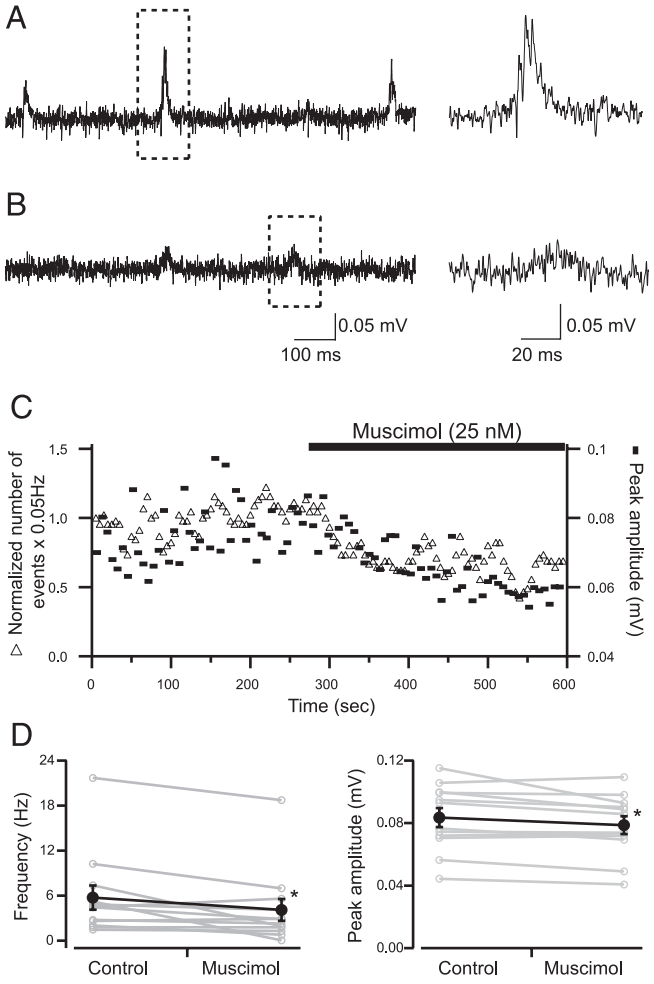

FIG. 7. Muscimol decreases the hyperexcitability of *gabra5*$^{-/-}$ CA3 pyramidal layer. *A*: spontaneous field recording from the CA3 PC layer of a *gabra5*$^{-/-}$ mouse in the presence of aCSF and 5 μM GABA showing epileptiform bursts. *Right*: expanded event seen in the dashed box. *B*: same slice as in *A* but after the perfusion of 25 nM muscimol. *Right*: expanded event from dashed box. *C*: spontaneous field recording from the same *gabra5*$^{-/-}$ CA3 pyramidal layer shown above plotting the normalized frequency of events as well as the amplitude of events (>0.04 mV). *Top bar* shows when 25 nM muscimol started. Note the decrease in frequency and amplitude of the epileptiform events in the presence of 25 nM muscimol. *D*: effect of 25 nM muscimol on absolute frequency (*left*) and peak amplitude (*right*) of epileptiform bursts. Gray dots and lines indicate individual recordings; black dots indicate means ± SE. (*) Statistical significance ($P < 0.05$; Wilcoxon matched-paired signed-rank test, $n = 12$).

DISCUSSION

In this study we examined the tonic and phasic inhibitory currents of hippocampal PCs in the absence of α5 subunits, a key component of extrasynaptic GABARs. Our results can be summarized as follows. *1*) In the absence of the GABAR α5 subunits, the tonic inhibition of hippocampal CA1 and CA3 PCs is about half that found in the WT, but phasic inhibition is unaltered. *2*) This specific reduction of tonic inhibition is sufficient to induce epileptiform hyperexcitability in the CA3 region that can be reversed by pharmacologically increasing the tonic current. *3*) In the absence of α5 subunits, expression of GABAR δ subunits is responsible for the residual tonic inhibition in *gabra5*$^{-/-}$ PCs. *4*) Some $IPSCs_{slow}$ are attributed to spillover of GABA onto extrasynaptic receptors.

There is mounting evidence for extrasynaptic GABARs having subunit compositions different from that of synaptic receptors (Brickley et al. 2001; Nusser et al. 2002; Stell et al. 2003; Wisden et al. 2002). The α5 subunit is thought of as a specific subunit forming extrasynaptic receptors in hippocampal PCs (Brünig et al. 2002; Caraiscos et al. 2004; Sperk et al. 1997). Even if present at synapses, our experiments support the exclusive participation of these subunits in the generation of tonic inhibition. The similar properties of sIPSCs and mIPSCs in CA1 and CA3 PCs in WT and *gabra5*$^{-/-}$ mice argue against the participation of α5 subunits in phasic inhibition, in contrast to a previous report where recordings were carried out at room temperature (Collinson et al. 2002). According to our results showing similar sIPSC frequencies in WT and *gabra5*$^{-/-}$ PCs, it is also unlikely that an α5 subunit–mediated tonic inhibition dampens the excitability of interneurons responsible for generating sIPSCs in PCs. Furthermore, the similar properties of mIPSCs between C57/Bl6 and *gabra5*$^{-/-}$ are consistent with the absence of any compensatory GABAR subunit changes at PC synapses in *gabra5*$^{-/-}$ animals. Taken together, our data support the idea that α5 subunits are not functional at GABA synapses on CA1 and CA3 PCs, but may be involved in generating some slow IPSCs. If α5 subunits are present at synapses, the α5-containing GABARs must be in a nonfunctional (probably desensitized) state or they may combine with other α subunits (e.g., α1–3) that overshadow the properties of the α5 subunits. Thus in the *gabra5*$^{-/-}$ mice, synaptic α5 subunits may be replaced by other α subunits without altering the general properties of the phasic currents. Similar to findings in rat CA1 PCs (Banks et al. 1998; Pearce 1993), we observed $IPSC_{slow}$ at low frequencies in CA1 and CA3 PCs of both WT and *gabra5*$^{-/-}$ mice. Their reduced ratio in *gabra5*$^{-/-}$ PCs is consistent with some $IPSC_{slow}$ events resulting from GABA spillover onto extrasynaptic α5 subunit–containing receptors, whereas the rest of $IPSC_{slow}$ events may result from GABA spillover onto extrasynaptic receptors containing other subunits. Another possibility is that some of the $IPSC_{slow}$ may be mediated by a specific class of interneuron as previously proposed in the rat (Banks et al. 1998, 2002). In any case, the total charge mediated by $IPSC_{slow}$ is minimal (about 3%) in comparison with the charge transfer caused by tonic inhibition. Therefore the decrease in the ratio of $IPSC_{slow/total}$ must have only a negligible contribution to the hyperexcitability found in the CA3 layer of the hippocampus.

In the absence of any changes in phasic inhibition in CA1/3 PCs, the *gabra5*$^{-/-}$ mice appear to be an ideal preparation to study the effects of selective decreases in tonic inhibition, otherwise difficult to accomplish by pharmacological means. In both CA1 and CA3 PCs of *gabra5*$^{-/-}$ mice the tonic current was about 56 and 53%, respectively, of that recorded in WT. These results are in contrast with those reported by Caraiscos et al. (2004) in CA1 PCs, where the loss of α5 subunits was paralleled by a total loss of tonic currents. In our experiments, CA1 PCs in P18 *gabra5*$^{-/-}$ mice (as used by Caraiscos et al. 2004) showed a residual tonic current in the presence of 5 μM GABA, as well as in adult *gabra5*$^{-/-}$ mice in the absence of any externally added GABA. It remains to be determined whether the difference between the two contradicting results arises from different methods used to reveal the tonic inhibition (5 μM GABA vs. vigabatrin) or from other experimental reasons. Nevertheless, based on our findings, we used hip-

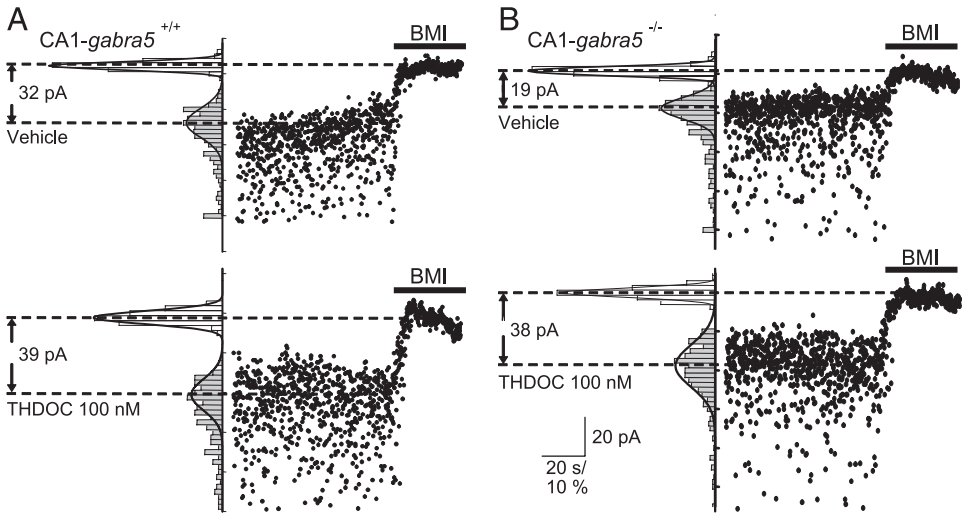

FIG. 8. Modulation of GABAR-mediated tonic current by tetrahydrodeoxycorticosterone (THDOC) in *gabra5*$^{-/-}$ but not in *gabra5*$^{+/+}$ CA1 PCs. Tonic currents were measured as described in the legend of Fig. 1 in the presence of vehicle or 100 nM THDOC added to the aCSF. *A*: tonic recordings from *gabra5*$^{+/+}$ CA1 PC. *B*: tonic recordings from *gabra5*$^{-/-}$ CA1 PC. Average tonic currents recorded in the presence of THDOC were significantly larger in *gabra5*$^{-/-}$ CA1 PCs than in *gabra5*$^{+/+}$ (see Table 3).

pocampal slices of *gabra5*$^{-/-}$ mice to study the effects of a specific reduction in tonic inhibition on network excitability.

The hyperexcitability found in field recordings from the CA3 pyramidal layer in *gabra5*$^{-/-}$ slices indicates that a specific reduction in tonic inhibition can tip over the balance of neuronal excitability leading to epileptiform events. In contrast, the lack of spontaneous field activity in the CA1 region of *gabra5*$^{-/-}$ slices may mean that the nearly 50% reduction in tonic inhibition is not sufficient to allow the expression of hyperexcitability in this region. Thus the residual tonic inhibition appears to be effective in maintaining normal excitability in a relatively less hyperexcitable region of the hippocampus. Consistent with its specific activation of extrasynaptic receptors of a certain subunit composition, a low concentration of muscimol (25 nM) was sufficient to significantly decrease the amplitude and frequency of the epileptiform events in CA3 region *gabra5*$^{-/-}$ slices. It is not known whether similar changes in tonic inhibition or compensatory mechanisms occur when the levels of α5 subunits become decreased in PCs in the pilocarpine model of epilepsy (Houser and Esclapez 2003; Scimemi et al. 2005). One would expect such change to lead to a compromised tonic inhibition like that we found in *gabra5*$^{-/-}$ mice. Our findings on the heightened network excitability in the face of a specific reduction of tonic inhibition are consistent with previous studies. Because extrasynaptic receptors outnumber those found at synapses (Banks and Pearce 2000; Nusser et al. 1995; Wei et al. 2003) and are activated by ambient GABA levels (Mody 2001), it is not surprising that this pool of receptors plays an important role in controlling the background inhibitory input of a cell and its gain control (Chance et al. 2002). Tonic inhibition affects the input–output function of cerebellar granule cells in vivo and in vitro (Chadderton et al. 2004; Mitchell and Silver 2003). Moreover, the α5 subunit has been shown to alter the dynamic profile of gamma oscillations to changes in network drive (Towers et al. 2004).

In a battery of pharmacological experiments, we have identified δ subunit–containing GABARs as the mediators of the residual tonic current in *gabra5*$^{-/-}$ PCs. The δ subunit has been well established as an extrasynaptic receptor in dentate and cerebellar granule cells and as a mediator of tonic currents (Nusser et al. 1998; Stell et al. 2003; Wei et al. 2003). However, only small amounts of this subunit mRNA and immunoreactivity are detectable in CA1 PCs (Fritschy and Möhler 1995; Pirker et al. 2000; Sperk et al. 1997; Wisden et al. 1992), and CA1 PC tonic currents are insensitive to the neurosteroid THDOC, which specifically enhances tonic currents where δ subunits are expressed (Stell et al. 2003). Consistent with these previous findings, phasic and tonic currents were unaffected by 100 nM THDOC in WT CA1 PCs. In contrast, 100 nM THDOC enhanced the tonic currents in *gabra5*$^{-/-}$ CA1 PCs by 174%. This concentration of THDOC produced a nearly 150% potentiation of the tonic currents in δ subunit–containing dentate gyrus granule cells and a nearly 255% increase in δ subunit–containing cerebellar granule cells (Stell et al. 2003). Thus THDOC potentiation of the residual tonic current recorded in the absence of α5 subunits was commensurate with its effect on cells where tonic inhibition is mediated by δ subunits in combination with α4 or α6 subunits. Immunostaining for δ subunits did not show a difference between *gabra5*$^{+/+}$ and *gabra5*$^{-/-}$ (data not shown), probably arising from a lack of sensitivity of this method to detect small changes in subunit expression. However, our further pharmacological experiments were consistent with the compensatory upregulation of δ subunit–containing GABARs in the absence of α5 subunits.

Muscimol (25 nM) significantly increased tonic currents only in *gabra5*$^{-/-}$ CA1 PCs, whereas tonic currents of *gabra5*$^{+/+}$ CA1 PCs were unaffected. Furthermore, the ratio of tonic currents recorded in THIP or GABA was significantly higher in *gabra5*$^{-/-}$ CA1 PCs than that in *gabra5*$^{+/+}$ CA1 PCs, which also suggests a higher expression of δ subunits in *gabra5*$^{-/-}$ mice. The only inconsistent pharmacological finding is that threshold concentrations of diazepam (0.5 μM) that enhanced synaptic currents failed to produce a significant increase in the tonic currents of *gabra5*$^{+/+}$ CA1 PCs. This would be expected from a lower diazepam sensitivity of an α5/γ subunit containing extrasynaptic GABARs than that at the synapses or the concentration of extracellular GABA is not high enough to fully activate diazepam-sensitive receptors. Our combined pharmacological evidence strongly suggests that in the absence of α5 subunits, a residual tonic current in both CA1 and CA3 PCs is mediated by the GABARs containing δ subunits, possibly in combination with α4 subunits. The com-

pensation for the tonic current is not full and the decreased tonic inhibition translates into network hyperexcitability in the CA3 region of *gabra5*$^{-/-}$ mice.

While experimenting with optimal methods for slice storage and recording, we also discovered a significant effect of the slice storage conditions specifically affecting the tonic currents recorded in WT PCs. The larger tonic currents recorded in *gabra5*$^{+/+}$ slices, but not in *gabra5*$^{-/-}$ slices, stored in an interface chamber compared with those stored in a submerged chamber, mean that specific conditions in the interface chamber favor the activation of α5 subunit–containing receptors. A decreased GABA uptake, an altered phosphorylation/dephosphorylation process, or a lower metabolical distress of the cells in the interface chamber may all affect the activation of α5 subunit–containing GABARs. The lack of statistical difference between tonic currents in *gabra5*$^{-/-}$ slices stored in the two different storage chambers could be a result of the different properties of the receptors mediating tonic currents in the absence of α5 subunits. Regardless of the mechanisms, our studies identify slice storage conditions as an important factor in controlling the amount of tonic inhibition present in hippocampal PCs.

Interestingly, the *gabra5*$^{-/-}$ mice have been shown to perform better in various learning and memory tests (Collinson et al. 2002). If indeed the tonic inhibition mediated by α5 subunit–containing GABARs impedes learning and memory, there might have been an evolutionary pressure to diminish the levels of this subunit, especially because the α5 distribution in the adult brain is very restricted. One possible reason for the enduring presence of α5 subunits in hippocampal PCs could be that the advantages in learning/memory stemming from its loss may be counterbalanced by a hyperexcitable epileptic phenotype. The α5 subunit has also been found in the human hippocampus with a distinct localization to the CA1 and CA3 regions (Wainwright et al. 2000). The ability to modulate tonic inhibition mediated by these receptors by specific pharmacological means may allow striking a balance between the needs for increased excitation required for better learning and those for controlling the hyperexcitability.

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