*J Neurophysiol* 98: 2244–2254, 2007.
First published August 22, 2007; doi:10.1152/jn.00482.2007.

# $\alpha$5GABA$_A$ Receptors Regulate the Intrinsic Excitability of Mouse Hippocampal Pyramidal Neurons

**Robert P. Bonin,[1] Loren J. Martin,[2] John F. MacDonald,[1] and Beverley A. Orser[1,2,3]**
[1]*Department of Physiology and* [2]*Institute of Medical Science, University of Toronto; and* [3]*Department of Anesthesia, Sunnybrook Health Science Center, Toronto, Ontario, Canada*

Submitted 27 April 2007; accepted in final form 17 August 2007

**Bonin RP, Martin LJ, MacDonald JF, Orser BA.** $\alpha$5GABA$_A$ receptors regulate the intrinsic excitability of mouse hippocampal pyramidal neurons. *J Neurophysiol* 98: 2244–2254, 2007. First published August 22, 2007; doi:10.1152/jn.00482.2007. GABA$_A$ receptors generate both phasic and tonic forms of inhibition. In hippocampal pyramidal neurons, GABA$_A$ receptors that contain the $\alpha$5 subunit generate a tonic inhibitory conductance. The physiological role of this tonic inhibition is uncertain, although $\alpha$5GABA$_A$ receptors are known to influence hippocampal-dependent learning and memory processes. Here we provide evidence that $\alpha$5GABA$_A$ receptors regulate the strength of the depolarizing stimulus that is required to generate an action potential in pyramidal neurons. Neurons from $\alpha$5 knock-out ($\alpha$5$-/-$) and wild-type (WT) mice were studied in brain slices and cell cultures using whole cell and perforated-patch-clamp techniques. Membrane resistance was 1.6-fold greater in $\alpha$5$-/-$ than in WT neurons, but the resting membrane potential and chloride equilibrium potential were similar. Membrane hyperpolarization evoked by an application of exogenous GABA was greater in WT neurons. Inhibiting the function of $\alpha$5GABA$_A$ receptor with nonselective (picrotoxin) or $\alpha$5 subunit-selective (L-655,708) compounds depolarized WT neurons by ~3 mV, whereas no change was detected in $\alpha$5$-/-$ neurons. The depolarizing current required to generate an action potential was twofold greater in WT than in $\alpha$5$-/-$ neurons, whereas the slope of the input-output relationship for action potential firing was similar. We conclude that shunting inhibition mediated by $\alpha$5GABA$_A$ receptors regulates the firing of action potentials and may synchronize network activity that underlies hippocampal-dependent behavior.

## INTRODUCTION

The ionotropic $\gamma$-aminobutyric acid (GABA) type A (GABA$_A$) receptor is the major inhibitory neurotransmitter receptor in the mammalian brain. GABA$_A$ receptors are pentameric complexes that contain various combinations of $\alpha$1–6, $\beta$1–3, $\gamma$1–3, $\delta$, $\rho$1–3, $\varepsilon$, $\theta$, and $\pi$ subunits (Rudolph et al. 2001). A diverse array of GABA$_A$ receptor subtypes occurs, each with unique developmental and regional patterns of expression and distinct pharmacological and biophysical properties (for a review, see Whiting et al. 1999). The physiological properties of regionally distinct GABA$_A$ receptors are of great interest because drugs that target specific receptor subtypes may offer potential therapeutic advantages.

GABA$_A$ receptors that contain the $\alpha$5 subunit are of particular interest because these receptors have been strongly implicated in cognitive processes such as learning and memory. Genetically modified mice lacking the GABA$_A$ receptor $\alpha$5 subunit have better hippocampal-dependent learning than those with the subunit (Collinson et al. 2002). Furthermore, expression of the $\alpha$5 subunit is unexpectedly reduced in transgenic mice expressing a point mutation of the $\alpha$5 subunit (H105R) (Crestani et al. 2002). These $\alpha$5H105R mice show enhanced memory performance for trace fear conditioning. In addition, inverse agonists that selectively inhibit the activity of $\alpha$5GABA$_A$ receptors improve memory performance in animal models (Chambers et al. 2003). On the basis of these results, compounds are now being developed that selectively inhibit $\alpha$5GABA$_A$ receptor function for the purpose of enhancing cognition (Dawson et al. 2006; Sternfeld et al. 2004). An increase in $\alpha$5GABA$_A$ receptor activity has been implicated in the memory-impairing properties of benzodiazepines and volatile and injectable anesthetics (Bai et al. 2001; Caraiscos et al. 2004b; Cheng et al. 2006). However, the mechanisms by which activation of $\alpha$5GABA$_A$ receptors constrains learning and memory have not yet been elucidated.

The distinct cellular distribution of $\alpha$5GABA$_A$ receptors may contribute to their unique effects on behavior. The $\alpha$5GABA$_A$ receptor subunit has a highly restricted pattern of distribution, being predominantly expressed in hippocampal pyramidal neurons where it is incorporated into ~20% of all GABA$_A$ receptors (Sur et al. 1999). Immunocytochemical studies have revealed an abundance of the $\alpha$5 subunit in the hippocampal CA1 region with lower levels of expression in CA2 and CA3 regions (Fritschy and Mohler 1995; Pirker et al. 2000; Serwanski et al. 2006; Sur et al. 1999; Wisden et al. 1992). Staining for the $\alpha$5 subunit was homogenous across the dendritic and cell body regions of the hippocampal formation (Prenosil et al. 2006). The $\alpha$5GABA$_A$ receptor also shows distinct expression patterns at the subcellular level. Immunofluorescence studies with light microscopy have shown that $\alpha$5GABA$_A$ receptors are primarily expressed in the extrasynaptic regions of pyramidal neurons (Brunig et al. 2002; Christie et al. 2002; Crestani et al. 2002). Higher-resolution electron microscopy studies have shown that some $\alpha$5GABA$_A$ receptors are located in the peri-synaptic and synaptic regions of cultured pyramidal neurons (Serwanski et al. 2006). The reasons for and physiological importance of this pattern of subcellular distribution of $\alpha$5GABA$_A$ receptors are unknown.

Increasing evidence indicates that distinct subpopulations of extrasynaptic GABA$_A$ receptors generate a persistent tonic inhibition, whereas synaptic receptors generate inhibitory postsynaptic potentials (Semyanov et al. 2004). A tonic

Address for reprint requests and other correspondence: B. A. Orser, Dept of Physiology, Rm. 3318, Medical Sciences Bldg., 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada (E-mail: beverley.orser@utoronto.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

GABAergic conductance has been identified in hippocampal pyramidal neurons in both dissociated cell culture (Bai et al. 2001; Caraiscos et al. 2004b) and slice models (Caraiscos et al. 2004a; Maguire et al. 2005; Stell and Mody 2002). We previously showed that the tonic conductance in pyramidal neurons is predominantly generated by GABA$_A$ receptors that contain the $\alpha$5 subunit (Caraiscos et al. 2004a). Studies using neonatal mouse and rat hippocampal slices role have confirmed that extrasynaptic $\alpha$5GABA$_A$ receptors generate a tonic conductance in pyramidal neurons (Cheng et al. 2006; Glykys and Mody 2006; Marchionni et al. 2007; Prenosil et al. 2006). In contrast, $\alpha$5GABA$_A$ receptors do not make a major contribution to GABA-mediated synaptic currents as indicated by the lack of difference in miniature inhibitory postsynaptic currents between $\alpha$5 subunit knock out neurons ($\alpha$5$-/-$) and WT neurons (Caraiscos et al. 2004a).

Few studies to date have investigated the effects of reduced $\alpha$5GABA$_A$ receptor function on the excitability of mature hippocampal pyramidal neurons. Field recordings in the CA3 pyramidal layer have revealed differences in the frequency of spontaneous single-population "spike-like events" in slices from $\alpha$5$-/-$ mice and control mice (Glykys and Mody 2006). Spontaneous epileptiform bursts were observed in slices from $\alpha$5$-/-$ mice but not controls unless the extracellular concentrations of $K^+$ or $Mg^{2+}$ were altered. A residual tonic conductance identified in $\alpha$5$-/-$ pyramidal neurons was generated, in part, by $\delta$ subunit-containing GABA$_A$ receptors (Scimemi et al. 2005). Others have studied gamma frequency oscillations evoked by the application of kainate in $\alpha$5$-/-$ and WT slices (Towers et al. 2004). The power of the gamma oscillations was greater in $\alpha$5$-/-$ than in WT slices, and interventions aimed at increasing the extracellular concentration of GABA were more effective at reducing the power of gamma oscillations in WT than in $\alpha$5$-/-$ slices. Pharmacological block of $\alpha$5-mediated tonic inhibition altered the frequency of action potential firing in CA1 pyramidal neurons from rat slice (Marchionni et al. 2007). However, a detailed analysis of the mechanistic basis for $\alpha$5GABA$_A$ receptor regulation of pyramidal cell excitability that impacts network oscillation and memory behavior has not been reported.

The results summarized in the preceding text raise several fundamental questions that must be answered if we are to understand how tonic inhibition regulates hippocampal function. Do $\alpha$5GABA$_A$ receptors alter the minimal depolarizing current required to generate an action potential or rheobase in pyramidal neurons? Does the residual tonic inhibitory conductance in $\alpha$5$-/-$ pyramidal neurons or some other unidentified factor fully compensate and hence normalize pyramidal cell firing? Deletion of a gene that encodes a GABA$_A$ subunit that underlies a tonic inhibitory conductance does not necessarily lead to alteration in neuronal excitability. For example, cerebellar-specific $\alpha$6$\delta$ subunit-containing GABA$_A$ receptors generate a tonic conductance (Brickley et al. 1996). The tonic conductance is reduced in cerebellar neurons from $\alpha$6 null mutant mice; nevertheless, neuronal excitability is unchanged due to a compensatory increase in voltage-dependent K+ conductance (Brickley et al. 2001). Thus it is important to determine if selective reduction in the function of $\alpha$5GABA$_A$ receptors (by either genetic manipulations or pharmacological inhibition) increase the intrinsic excitability of hippocampal pyramidal neurons. Finally, does selective activation of $\alpha$5GABA$_A$ receptors in neurons cause membrane hyperpolarization or shunting inhibition? These questions were addressed using patch-clamp recordings from pyramidal neurons in dissociated cultures and hippocampal slices.

## METHODS

### *Hippocampal cell culture and slice preparation*

The experiments reported here were approved by the Animal Care Committee of the University of Toronto. All experiments were conducted with hippocampal tissue harvested from $\alpha$5$-/-$ or WT mice. The generation of the $\alpha$5$-/-$ mice has been previously described (Collinson et al. 2002). Briefly, all mice were of mixed genetic background (approximately half C57/BL6 and half 129SvEv), and $\alpha$5$-/-$ and WT mice were generated by crossing $\alpha$5$+/-$ mice. Cultures of hippocampal neurons were prepared as previously described (MacDonald et al. 1989) from $\alpha$5$-/-$ mice and WT littermates on postnatal day 1. Mice were killed by decapitation. Cells were maintained in culture for 14–21 days before recordings. Extracellular solutions for cultured neurons contained (in mM): 140 NaCl, 1.3 $CaCl_2$, 2.0 KCl, 25 HEPES, and 33 glucose; the pH was adjusted to 7.4 with 10 N NaOH and the osmolarity adjusted to 300–310 mosM with sucrose. The extracellular solution was applied to neurons by a computer-controlled multi-barreled perfusion system (SF-77B; Warner Instruments, Hamden, CT).

Hippocampal slices were prepared from 3- to 6-wk-old WT and $\alpha$5$-/-$ mice as previously described (Caraiscos et al. 2004a). Slices (350 $\mu$m) containing transverse hippocampal sections were placed in ice-cold oxygenated (95% $O_2$-5% $CO_2$) artificial cerebrospinal fluid (composition in mM: 124 NaCl; 3.0 KCl, 1.3 $MgCl_2$, 2.6 $CaCl_2$, 1.25 $NaH_2PO_4$, 26 $NaHCO_3$, and 10 D-glucose) adjusted to 300–310 mosM. The CA1 region was isolated from the CA3 region by a surgical cut. Studies were performed in a submerged chamber, and hippocampal CA1 pyramidal neurons were visually identified using a microscope equipped with differential interference contrast infrared optics.

### *Electrophysiology*

Data were acquired with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA) controlled with pClamp 9.0 software (Molecular Devices) via a Digidata 1322 interface (Molecular Devices). Membrane capacitance was measured using the membrane test protocol in pClamp 9.0. Access resistance was monitored throughout the experiments by applying a brief 10-mV or 10-pA hyperpolarizing step during voltage- and current-clamp experiments, respectively. Cells were eliminated from further analysis if the access resistance changed by >20%. The liquid junction potential and pipette capacitance were corrected before establishing the whole cell configuration using pClamp 9.0 software. Patch pipettes were pulled from thin-walled borosilicate glass capillary tubes and had open tip resistances of 3–5 M$\Omega$ when filled with an intracellular solution that contained (in mM) 140 CsCl, 10 HEPES, 10 EGTA, 4 Mg-ATP, 1 $CaCl_2$, and 5 QX-314. The pH was adjusted to 7.3 with CsOH and the osmolarity was adjusted to 295–305 mosM. Whole cell currents were recorded with the holding potential clamped at $-60$ mV except when indicated otherwise. We restricted our study to pyramidal neurons to measure the influence of $\alpha$5GABA$_A$ receptor activity on neuronal excitability. Although a tonic GABAergic conductance has been identified in stratum radiatum and s. oriens interneurons from guinea pig hippocampi (Semyanov et al. 2003) and in murine dentate gyrus granule cells (Stell et al. 2003), it was primarily mediated by GABA$_A$ receptors that did not contain the $\alpha$5 subunit. The neuron cultures used in this study contained a heterogeneous population of neurons and glia cells. We selected pyramidal neurons on the basis of morphology. The pyramidal neurons selected were 15–20 $\mu$m in diameter with promi-

nent apical dendrites and pyramidal somas. The duration of the action potential, as measured by the spike half-width, is a defining functional characteristic that distinguishes pyramidal cells from other cell types in the hippocampus. Pyramidal neurons generate action potentials with longer durations than those generated by interneurons (Lacaille et al. 1987). Accordingly, we restricted our study population to pyramidal neurons that generated action potentials with half-widths >1 ms.

Perforated-patch recordings were performed in some experiments by including gramicidin D (Sigma-Aldrich Canada, Oakville, ON, Canada) in the patch pipettes. The pipette solution contained (in mM) 150 K-gluconate and 10 HEPES with the pH adjusted to 7.3 with KOH. The osmolarity of the solution was adjusted to 290–300 mosM with sucrose. Gramicidin stock solution (10 mg/ml in methanol) was diluted in K-gluconate solution to a final concentration of 50–100 $\mu$g/ml just before the experiment. The tip of the patch pipette was filled with a gramicidin-free K-gluconate solution and was backfilled with the gramicidin-containing solution. A high-resistance (G$\Omega$) seal was formed between the electrode and the cell membrane by applying gentle suction after the pipette touched the cell. The electrode was voltage clamped at −60 mV, and membrane perforation was monitored by applying 10-mV hyperpolarizing pulses every 20 s. A perforated-patch with an access resistance of <30 M$\Omega$ was achieved within 30 min of establishing the G$\Omega$ seal. Capacitive transients, access resistance and leak conductance were carefully monitored throughout the experiments to confirm the stability of the perforated-patch. To further confirm patch stability, the membrane patch was ruptured at the end of the recording, which caused a sudden change in capacitance and access resistance. For current-clamp experiments, patch pipettes had open tip resistances of 4–6 M$\Omega$ when filled with an intracellular solution that contained (in mM) 145 K-gluconate, 5 Na-gluconate, 2 KCl, 10 HEPES, 1 EGTA, 4 Mg-ATP, and 1 $CaCl_2$ with the pH adjusted to 7.3 with KOH and the osmolarity adjusted to 300–310 mosM with sucrose.

In all experiments the ionotropic glutamate antagonists 6-cyano-7-nitro-quinoxaline-2,3-dione (10 $\mu$M) and 2-amino-4-phosphonovaleric acid (40 $\mu$M) were added to the extracellular solution. In some experiments, tetrodotoxin (TTX; 300 nM; Alomone Labs, Jerusalem, Israel) was added to the extracellular solution to block voltage-sensitive sodium channels. Aqueous stock solution of all drugs was prepared with distilled water except for L-655,708 (Tocris, Ellisville, MO), which was prepared in dimethyl sulfoxide. Concentrations of dimethyl sulfoxide equivalent to those present in the drug solution did not affect the tonic or synaptic currents. All drugs and chemicals were purchased from Sigma-Aldrich Canada except where indicated otherwise.

### *Data analysis*

Tonic current and spontaneous IPSCs (sIPSCs) were measured using voltage-clamp techniques. Voltage-clamp data were sampled at 10 kHz and low-pass filtered at 2 kHz using an 8-pole Bessel filter. The amplitude of the tonic current was measured as the difference in the holding current before and during application of the $GABA_A$ receptor antagonist bicuculline methiodide (100 $\mu$M) (Bai et al. 2001). This reduction in holding current has been previously shown to not be a result of bicuculline inhibition of $K^+$ channels (Bai et al. 2001). The root mean square (rms) of current noise was calculated from 30-s recorded segments that lacked miniature IPSCs (mIPSCs) using MiniAnalysis 5.0 software (Synaptosoft, Decatur, GA). Spontaneous IPSCs were recorded in the absence of TTX using a gap-free voltage-clamp protocol. To determine the amplitude and time course of the sIPSCs, 25–75 events were randomly selected from each recording and analyzed using MiniAnalysis software as previously described (Yeung et al. 2003). Decay of IPSCs was fitted with a double-exponential function, and a weighted time constant was used to compare groups. The decay was considered to be monoexponential when tau1 was similar to tau2 (within 10%) (Collinson et al. 2002).

Current-clamp recordings were used to measure resting membrane potential and evoked action potentials. Data were sampled at 50 kHz and low-pass filtered at 10 kHz using an 8-pole Bessel filter. A high sampling rate was employed to allow accurate determination of the time course of action potentials. The resting membrane potential was determined as the potential measured over a stable 5-s period. Membrane resistance, action potential threshold, and waveform of the action potentials were measured by applying 2-pA incremental current steps (250 ms at 0.8 Hz) with one current step per sweep. The action potential threshold was defined as the inflection point in the voltage preceding the action potential. The duration and frequency of the current steps were selected to minimize the opening of slowly activating voltage-gated conductances. Action potentials that occurred at current steps that were 0, 2, and 4 pA above the threshold were averaged off-line and analyzed with Clampfit 9.0 (Molecular Devices). The frequency of action potentials could not be compared when current steps of equal magnitude were applied because of genotypic differences in the firing threshold. Accordingly, the slope of the input-output relationship (the gain) was studied by means of current steps that varied in 20-pA increments. Starting at 40 pA below threshold and increasing to 60 pA above threshold (at 1 step per sweep), six 2-s current steps were applied at 0.05 Hz. Membrane resistance was calculated using linear regression to fit the relationship between membrane potential at subthreshold levels and current amplitude. The frequency of action potential firing at each current level was determined and the gain calculated with the equation frequency = slope · (injected current – threshold current) + frequency at threshold. The slope of this relationship (gain) was used for comparisons between genotypes.

### *Statistics*

Statistical analyses were performed using Prism 4 (Graphpad, San Diego, CA). Statistical comparisons used 2-tailed paired or unpaired Student *t*-test or one-way ANOVA followed by Tukey's post hoc tests where appropriate. A $P$ value <0.05 was considered significant. All data are shown as means ± SE.

## RESULTS

To determine whether the expression of $\alpha$5$GABA_A$ receptors altered the passive properties of hippocampal pyramidal neurons grown in dissociated cultures, membrane capacitance and input resistance were measured in WT and $\alpha5-/-$ neurons. Series resistance was typically <12 M$\Omega$ (9.6 ± 0.1 M$\Omega$, $n = 28$). Membrane capacitance was similar in the two types of neurons (39.3 ± 1.6 pF, $n = 16$ vs. 37.8 ± 2.2 pF, $n = 17$; $P >$ 0.05). In contrast, membrane resistance measured under current-clamp conditions was 1.6-fold greater in $\alpha5-/-$ than in WT neurons (370 ± 10 M$\Omega$, $n = 15$ vs. 237 ± 8 M$\Omega$, $n = 15$; $P < 0.0001$). Next, the amplitude of the tonic current in cultured pyramidal neurons was studied using whole cell voltage-clamp methods with CsCl-based intracellular fluid (ICF). The $GABA_A$ receptor antagonist bicuculline (100 $\mu$M) was applied and the change in holding current ($I_{Hold}$) that was required to maintain the membrane potential at −60 mV was measured (Fig. 1*A*). Notably, a low-amplitude residual tonic conductance was detected in $\alpha5-/-$ neurons (WT: 11.4 pA ± 1.2, $n = 13$ vs. 5.3 ± 0.9 pA; $n = 13$; $P < 0.05$) as has been previously reported (Caraiscos et al. 2004a). The change in $I_{Hold}$, normalized to membrane capacitance, was 2.6-fold greater in WT than in $\alpha5-/-$ neurons (0.42 ± 0.05 pA/pF, $n = 13$ vs. 0.16 ± 0.02 pA/pF, $n = 13$,; $P < 0.001$; Fig. 1*B*).

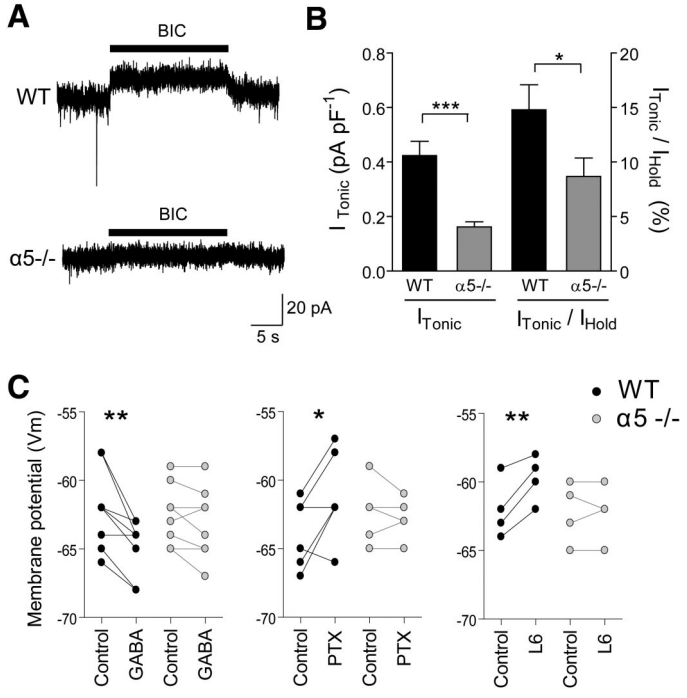


FIG. 1. Increased tonic GABAergic current in cultured wild-type (WT) neurons. *A*: sample traces show a shift in the holding current during an application of the GABAergic antagonist bicuculline (BIC; 100 μM), which reveals a persistent inward GABAergic current. *B*: average tonic conductance (*left*) recorded in WT and α5−/− neurons (normalized to membrane capacitance) shows a larger tonic current in WT ($n = 13$) than in α5−/− neurons ($n = 13$; $P < 0.005$). The tonic current contributes a larger proportion of the total holding current at −60 mV (*right*) in WT neurons than in α5−/− neurons ($P < 0.05$). *C*: resting membrane potential ($V_m$) of cultured pyramidal neurons was sensitive to modulation of α5GABA$_A$ receptor activity. Using a K-gluconate ICF, GABA hyperpolarized $V_m$ ($n = 8$; $P < 0.01$), whereas picrotoxin (PTX; $n = 6$; $P < 0.05$) and L-655,708 (L6; $n = 4$; $P < 0.05$) depolarized $V_m$ in WT neurons. The Vm of α5−/− neurons was insensitive to GABA ($n = 8$), PTX ($n = 5$) or L6 ($n = 4$; $P > 0.05$).

The percent contribution of the bicuculline-sensitive conductance to the total $I_{Hold}$ was also considerably greater in WT than in α5−/− neurons (14.8 ± 2.30%; $n = 13$ vs. 8.67 ± 1.70%, $n = 13$; $P < 0.05$; Fig. 1*B*). The current noise was greater in WT than in α5−/− neurons as indicated by the rms of $I_{Hold}$ ($\sigma$: 3.74 ± 0.11 pA, $n = 10$ vs. 3.39 ± 0.10 pA, $n = 10$; $P < 0.05$). During the application of bicuculline, the rms noise of $I_{Hold}$ was similar in WT and α5−/− neurons (100 μM; $\sigma$: 3.08 ± 0.16 pA, $n = 10$ vs. 3.12 ± 0.09 pA, $n = 10$; $P > 0.05$). This latter finding indicated that under control conditions, the greater noise in WT neurons was attributable to activation of GABA$_A$ receptors.

### *Activation of α5GABA$_A$ receptors alters membrane potential*

The resting membrane potential ($V_m$) was measured after formation of the whole cell configuration using current-clamp methods with a K-gluconate-based ICF to maintain low intracellular chloride concentrations. Resting membrane potential was similar in WT and α5−/− neurons (−61.7 ± 0.7 mV, $n = 16$ vs. −62.1 ± 0.5 mV, $n = 17$; $P > 0.05$). To examine the contribution of GABA$_A$ receptors to $V_m$, picrotoxin was added to the extracellular solution (Fig. 1*C*). Picrotoxin (100 μM), depolarized the WT neurons by 2.67 ± 1.02 mV ($n = 6$, $P < 0.05$), whereas the change in the $V_m$ of α5−/− neurons (0.40 ± 0.51 mV) was not significant ($n = 5$; $P > 0.05$). To study the specific contribution of α5GABA$_A$ receptors, the α5 subunit-selective inverse agonist, L-655,708 was applied. A previous study showed that L-655,708 (50 μM) attenuated a tonic conductance in hippocampal pyramidal neurons, whereas postsynaptic inhibitory currents were unaffected (Caraiscos et al. 2004a). L-655,708 (20 μM) depolarized WT neurons by 2.25 ± 0.48 mV ($n = 4$; $P < 0.01$), whereas no change in $V_m$ was detected in α5−/− neurons (0.00 ± 0.41 mV change, $n = 4$; $P > 0.05$; Fig. 1*C*). Picrotoxin and L-655,708 produced a similar change, which suggests that GABAergic regulation of $V_m$ is primarily determined by the tonic conductance under the current experimental conditions.

GABA$_A$ receptors that generate a tonic conductance have a higher affinity for GABA than those that underlie inhibitory postsynaptic currents (Semyanov et al. 2004). α5GABA$_A$ receptors show intermediate GABA sensitivity ($EC_{50}$ value of 19 μM), which permits these receptor to respond to low, ambient concentrations of transmitter (Caraiscos et al. 2004a). In the preceding experimental conditions, no exogenous GABA was applied, and the extracellular concentration of GABA was unknown. The brain concentration of GABA in the extracellular space, measured with microdialysis techniques, ranges from 0.1 to 2.9 μM (Attwell et al. 1993; Lerma et al. 1986; Tossman et al. 1986). To mimic physiological conditions, $V_m$ was measured during an application of GABA (1 μM). GABA consistently hyperpolarized the WT neurons by −2.75 ± 0.70 mV ($n = 8$; $P < 0.01$), whereas no hyperpolarization was detected in α5−/− neurons (0.63 ± 0.38 mV, $n = 8$; $P > 0.05$). The hyperpolarizing response in WT neurons was consistent with a GABA-induced increase in chloride influx.

### *GABA hyperpolarizes WT but not α5−/− neurons*

We next determined whether a deficit of α5GABA$_A$ receptors was associated with an alteration in the electrochemical gradient for chloride ions under resting conditions. The intracellular concentration of chloride ions in hippocampal pyramidal neurons changes during neuronal maturation because of an increase in the expression of the potassium-chloride co-transporter, KCC2 (Attwell et al. 1993; Lerma et al. 1986; Rivera et al. 1999). In immature hippocampal neurons, low levels of KCC2 result in a depolarizing chloride gradient. After 13–14 days in vitro, KCC2 protein levels increase and the chloride gradient becomes hyperpolarizing (Khirug et al. 2005). The firing of action potentials can also alter intracellular chloride concentrations on a short time scale (Woodin et al. 2003). To minimize the perturbation of the chloride gradient and the washout of intracellular macromolecules, we made perforated-patch recordings using the ionophore gramicidin D (Fig. 2*A*). During formation of the perforated-patch, the membrane was voltage clamped at −60 mV. Series resistance after formation of the perforated-patch was typically <20 MΩ (18.6 ± 1.3 MΩ; $n = 12$). GABA (10 μM) was applied using a rapid perfusion system and the holding potential was increased from −40 to −100 mV in 10-mV steps. The reversal potential for the GABA-evoked current was estimated by constructing a current-to-voltage plot and calculating the *x* intercept (Fig. 2*B*). The GABA current showed a reversal potential consistent with a hyperpolarizing chloride conductance and outward rectification as previously described for GABA$_A$ receptors (Curmi et al.

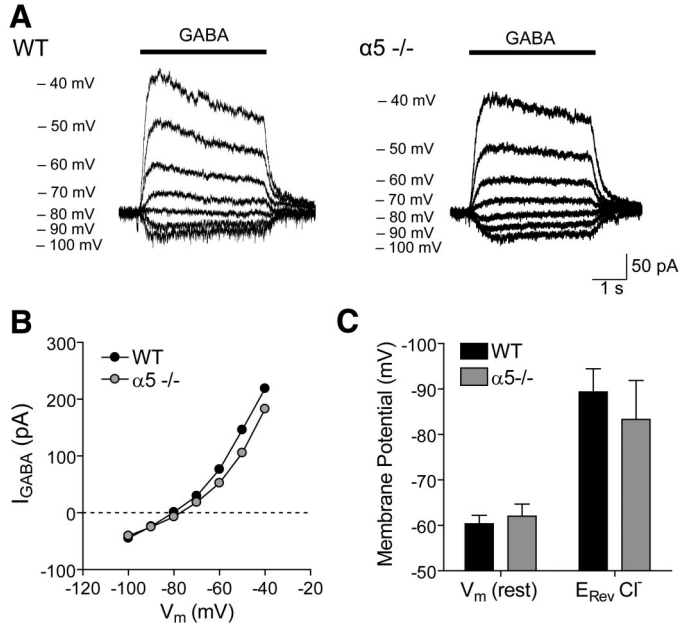


FIG. 2. GABA evoked current is hyperpolarizing in cultured WT and $\alpha5-/-$ neurons. The resting membrane potential and reversal potential for current evoked by GABA current were measured in cultured hippocampal pyramidal neurons from WT and $\alpha5-/-$ mice using gramicidin perforated-patch. *A*: sample recordings of current evoked by an application of GABA (10 $\mu$M) at several holding potentials. *B*: maximum GABA-evoked current recorded at various holding potentials is shown. The reversal potential, defined as the holding potential at which GABA evoked no net current, is indicated by the dotted line. *C*: resting membrane potential and reversal potential of GABA-evoked current was similar in WT neurons ($n = 3$) and $\alpha5-/-$ neurons ($n = 3$; $P > 0.05$).

1993). No difference was detected in reversal potential for WT and $\alpha5-/-$ neurons ($-82.8 \pm 3.1$ mV, $n = 7$ vs. $-79.6 \pm 5.2$ mV, $n = 5$ respectively, $P > 0.05$; Fig. 2*C*).

The perforated-patch technique was also used to measure $V_m$. No difference in $V_m$ was detected between WT and $\alpha5-/-$ neurons ($-63.2 \pm 1.4$ mV, $n = 7$ vs. $-62.9 \pm 1.6$ mV, $n = 5$; $P > 0.05$; Fig. 2*C*). Notably, the resting $V_m$ measured using either whole cell or perforated-patch techniques did not differ in WT and $\alpha5-/-$ neurons (Fig. 1*C*). Together, the results show that an increase in $\alpha5GABA_A$ receptor function causes an influx of chloride ions and membrane hyperpolarization in cultured pyramidal neurons.

### *$\alpha5GABA_A$ receptors regulate excitability of pyramidal neurons*

A primary measure of neuronal excitability is the magnitude of the depolarizing input required to trigger an action potential or rheobase. The excitability of WT and $\alpha5-/-$ hippocampal pyramidal neurons was compared by applying depolarizing steps (2 pA for 250 ms at 0.8 Hz; Fig. 3*A*). To minimize series resistance, experiments were performed using the whole cell patch-clamp technique. To maintain the gradient for chloride ions close to physiological values for mature neurons, a potassium gluconate-based intracellular solution was used in the recording electrode. The amplitude of the depolarizing current was increased until an action potential was detected in response to three successive depolarizing steps. The current required to generate the first action potential was recorded as the threshold current.

The absolute current required to generate an action potential was almost twofold greater in WT than in $\alpha5-/-$ neurons ($100.4 \pm 12.0$ pA; $n = 15$ vs. $56.6 \pm 7.4$ pA; $n = 14$; $P < 0.05$). The current required to generate an action potential, normalized to cell capacitance was 1.6-fold greater in WT neurons than in $\alpha5-/-$ neurons ($2.63 \pm 0.31$ pA/pF, $n = 15$ vs. $1.61 \pm 0.19$ pA/pF, $n = 14$; $P < 0.0001$; Fig. 3*B*). Notably, the membrane potential at which action potentials fired was similar in WT and $\alpha5-/-$ neurons ($-42.7 \pm 1.6$ mV, $n = 9$ vs. $-42.4 \pm 1.3$ mV, $n = 10$; $P > 0.05$). To determine whether changes in voltage-dependent sodium and potassium conductances that generated the action potential contributed to the increased excitability of $\alpha5-/-$ neurons, the characteristics of the action potentials and afterhyperpolarizating responses were studied. No differences were detected in the frequency or waveform of action potentials recorded in WT and $\alpha5-/-$ neurons: the peak amplitude, rise time, decay time, and afterhyperpolarization amplitude were similar (Table 1).

### *Excitability of CA1 pyramidal neurons in hippocampal slices is similar to that of cultured neurons*

Neurons grown in dissociated culture express a complement of $GABA_A$ receptors similar to that observed in vivo (Brunig

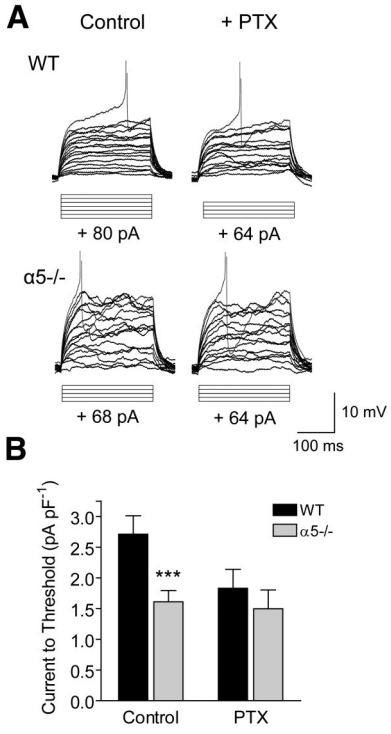


FIG. 3. Picrotoxin occluded the difference in excitability in cultured WT and $\alpha5-/-$ neurons. The amplitude of the depolarizing current required to generate action potentials was determined in WT and $\alpha5-/-$ neurons. *A*: sample recordings illustrate that the magnitude of excitatory current (more depolarizing current steps) required to trigger an action potential was greater in WT neurons than $\alpha5-/-$ neurons (*left*). The depolarizing current that generated action potentials is indicated below each sample trace. The application of picrotoxin (PTX; 1 $\mu$M) reduced the magnitude of depolarizing current in WT neurons but not $\alpha5-/-$ neurons (right). *B*: magnitude of current required to trigger an action potential (normalized to membrane capacitance) was greater in WT neurons ($n = 15$) than in $\alpha5-/-$ neurons ($n = 14$; $P < 0.001$). The depolarizing current required to activate an action potential was similar after the application of PTX (WT: $n = 6$ vs. $\alpha5-/-$: $n = 6$; $P > 0.05$).

TABLE 1. *Action potential characteristics*

| | WT (10) | α5−/− (10) | *P* Value |
|---|---|---|---|
| Peak amplitude, mV | 74.9 ± 2.6 | 81.6 ± 2.6 | *0.172* |
| Half-width, ms | 1.40 ± 0.10 | 1.62 ± 0.12 | *0.220* |
| Action potential threshold, mV | −41.6 ± 1.6 | −42.0 ± 0.9 | *0.767* |
| AHP amplitude, mV | −16.9 ± 1.6 | −14.9 ± 1.6 | *0.628* |
| Maximum rise slope, mV ms$^{-1}$ | 201.9 ± 20.6 | 198.3 ± 24.1 | *0.952* |
| Maximum decay slope, mV ms$^{-1}$ | −69.3 ± 6.7 | −57.2 ± 4.4 | *0.181* |

Properties of action potentials measured from cultured type (WT) and α5−/− pyramidal neurons. Values are means ± SE. Parentheses enclose *n* values.

et al. 2002). However, cell cultures lack the complex architecture and synaptic connectivity of hippocampal slices. Consequently, we next studied neuronal excitability in mature CA1 pyramidal neurons in slices prepared from 4-wk-old α5−/− and WT mice. A K-gluconate-based ICF was employed to maintain low intracellular chloride concentrations. Series resistance for the recordings was 20.4 ± 1.3 MΩ ($n$ = 11). Membrane capacitance was similar in WT neurons and α5−/− neurons (32.8 ± 2.54 pF, $n$ = 6 vs. 30.9 ± 3.61 pF, $n$ = 5; $P >$ 0.05). The resting membrane potential was also similar in WT neurons and α5−/− neurons (−60.8 ± 1.0 mV, $n$ = 6 vs. −61.0 ± 1.3 mV, $n$ = 5; $P >$ 0.05). Notably, the $V_m$ was similar to that measured in cultured neurons. The depolarizing current required to generate an action potential was twofold greater in WT than in α5−/− neurons (1.91 ± 0.15 pA/pF, $n$ = 6 vs. 1.05 ± 0.36 pA/pF, $n$ = 5; $P <$ 0.05). The amplitude of excitatory current required to generate action potentials in WT and α5−/− neurons was similar for neurons studied in cell cultures and brain slices. These findings indicate that the cell culture model reflects the properties of extrasynaptic GABA$_A$ receptors in hippocampal slices. The culture preparation offers more effective concentration clamp and drug solution exchange conditions than brain slices. Thus the remaining pharmacological experiments were conducted using cultured neurons.

### *WT and α5−/− neurons differ in sensitivity to GABA$_A$ receptor blockade*

Unidentified compensatory changes can occur after the genetic deletion of proteins that critically regulate neuronal excitability. We next addressed whether the enhanced excitability of α5−/− pyramidal neurons was due to alterations in the activity of GABA$_A$ receptors alone. We reasoned that the difference in excitability between α5−/− and WT neurons should be occluded by an application of picrotoxin provided no compensatory changes, independent of GABA function, occurred after deletion of the α5GABA$_A$ receptor subunit. Picrotoxin (100 μM) abolished the differences in excitability between WT and α5−/− neurons resulting in a similar amplitude of the depolarizing current required to generate action potentials (1.83 ± 0.31 pA/pF, $n$ = 6 vs. 1.50 ± 0.31 pA/pF, $n$ = 5; $P >$ 0.05; Fig. 3*B*). Notably, in WT neurons, picrotoxin reduced the current required to generate an action potential to 71.6 ± 6.3% of control ($n$ = 6, $P <$ 0.05 vs. control) but had no effect in α5−/− neurons (94.8 ± 3.0% of control, $n$ = 5, $P >$ 0.05 vs. control; $P <$ 0.05 vs. WT; Fig. 3*A*). These results suggest that a GABAergic conductance is primarily responsible for differences in the excitability of WT and α5−/− neurons.

To further ensure that the difference in current required to generate action potentials was attributable to reduced expression of α5GABA$_A$ receptors, we tested the effect of the α5GABA$_A$ receptor-selective inverse agonist L-655,708 (20 μM; Fig. 4*A*). In WT neurons, L-655,708 reduced the depolarizing current to 76.0 ± 7.0% of control ($n$ = 4, $P <$ 0.05 vs. control), whereas L-655,708 was ineffective in α5−/− neurons (95.7 ± 3.7% of control, $n$ = 4; $P >$ 0.05 vs. control; $P <$ 0.05 vs. WT; Fig. 4*B*). The similarity in the effects of L-655,708 and picrotoxin in reducing the current required to generate an action potential in WT but not α5−/− neurons suggests that the α5GABA$_A$ receptor-mediated tonic conductance is a primary determinant by which GABAergic inhibition regulates the excitability of pyramidal neurons.

### *Low concentrations of GABA further distinguish α5−/− and WT neurons*

We next addressed whether an increase in the concentration of GABA to physiological levels would further distinguish the

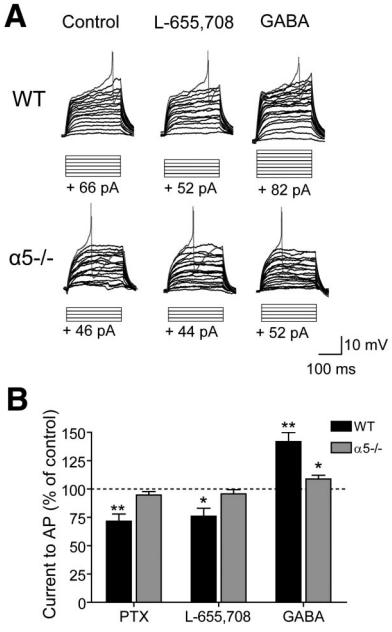


FIG. 4. GABA$_A$ receptor antagonists modify the excitability of cultured WT but not α5−/− neurons. *A*: sample traces of current-clamp recordings from WT and α5−/− neurons show the magnitude of excitatory current required to trigger action potentials in the presence of GABAergic drugs. The depolarizing current that generated action potentials is indicated below each sample trace. WT neurons were highly sensitive to GABAergic modulation with the α5GABA$_A$ receptor selective inverse agonist L-655,708 (L6; 20 μM) reducing the current required and application of a low GABA concentration (1 μM) greatly increasing the current required to generate action potentials. Conversely, α5−/− neurons were much less sensitive to these GABAergic drugs. *B*: graph summarizes the changes in current required to generate an action potential after drug treatment. Note that the data are normalized to control. Picrotoxin (PTX; 1 μM) reduced the firing threshold in WT neurons ($n$ = 6; $P <$ 0.05 vs. control) but not α5−/− neurons ($n$ = 5; $P >$ 0.05 vs. control; $P <$ 0.05 WT versus α5−/− neurons). L655-708 also reduced the firing threshold current in WT neurons ($n$ = 4; $P <$ 0.05 vs. control) but not α5−/− neurons ($n$ = 4; $P >$ 0.05 vs. control; $P <$ 0.01 WT vs. α5−/−). An application of exogenous GABA increased the firing threshold in both WT neurons ($n$ = 8; $P <$ 0.01 vs. control) and α5−/− neurons ($n$ = 8; $P >$ 0.05 vs. control) but had greater effect on WT neurons ($P <$ 0.01 WT vs. α5−/−).

firing patterns of WT and $\alpha5-/-$ neurons. GABA (1 $\mu$M) was added to the extracellular solution, and the magnitude of the depolarizing current required to generate an action potential was determined (Fig. 4*A*). GABA (1 $\mu$M) increased the current required to generate action potentials by >40% in WT neurons (141.9 ± 8.1% of control, $n = 8$, $P < 0.01$) but by only 9% in $\alpha5-/-$ neurons (108.8 ± 3.4% of control, $n = 7$, $P < 0.05$; Fig. 4*B*). Thus low concentrations of GABA increase the action potential threshold current of WT neurons ~4.5 times more efficaciously than was the case for $\alpha5-/-$ neurons (WT vs. $\alpha5-/-$ neurons, $P < 0.01$). These results suggest that extracellular GABA, acting through $\alpha5GABA_A$ receptors, is capable of strongly regulating the excitability of pyramidal neurons.

### *Spontaneous IPSCs in WT and α5−/− neurons*

It has been previously reported that $\alpha5GABA_A$ receptors increase the amplitude of spontaneous TTX-sensitive IPSCs (sIPSCs) recorded in hippocampal slices from 14- to 32-day-old mice (Collinson et al. 2002). We previously demonstrated that $\alpha5GABA_A$ receptors do not contribute to mIPSCs, which are thought to be purely synaptic events (Caraiscos et al. 2004b). However, it is possible that a larger release of transmitter from presynaptic terminals recruits extrasynaptic receptors through spillover from neighboring synapses (Semyanov et al. 2003). Here, sIPSCs were recorded in cultured hippocampal pyramidal neurons in the absence of TTX (Fig. 5*A*). To facilitate detection of GABAergic events, a CsCl-based ICF was employed to raise the chloride reversal potential to approximately −3 mV. The frequency of sIPSC was similar in WT and $\alpha5-/-$ neurons (0.70 ± 0.05 Hz, $n = 7$ vs. 0.76 ± 0.05 Hz, $n = 7$; $P > 0.05$; Fig. 5*B*). The time constant of sIPSC decay was also similar between WT and $\alpha5-/-$ neurons (43.3 ± 3.76 ms, $n = 7$ vs. 47.6 ± 2.9 ms, $n = 7$, $P > 0.05$; Fig. 5*C*). Finally, the amplitude of sIPCSs was similar in WT and $\alpha5-/-$ neurons (519 ± 137 pA, $n = 7$ vs. 530 ± 180 pA, $n = 7$; $P > 0.05$). Spontaneous IPSCs were also recorded in WT and $\alpha5-/-$ CA1 pyramidal neurons in hippocampal slices. L-655,708 (20 $\mu$M) was added to the perfusate to further determine the contribution of $\alpha5GABA_A$ receptors to synaptic events. No differences in sIPSC amplitude, kinetics or frequency were detected between genotypes under control conditions or following an application of L-655,708 (Table 2). These data suggest that $\alpha5GABA_A$ receptors do not contribute appreciably to the amplitude or duration of sIPSCs in cultured neurons. Alternatively, another subpopulation of receptors might fully compensate for the $\alpha5GABA_A$ receptors.

### *α5GABA$_A$ receptors do not influence the gain of the input-output relationship*

A change in the firing rates of neurons in response to sustained depolarizing input provides a measure of neuronal excitability. It has been previously demonstrated that during tonic excitation, shunting inhibition does not change the slope or "gain" of the relationship (Brickley et al. 1996; Chance et al. 2002). The input (depolarizing current) to output (action potential frequency) relationship was assessed in WT and $\alpha5-/-$ pyramidal neurons by injecting sustained, suprathreshold current steps (Fig. 6*A*). The input-output relationships were linear, and the slopes were compared between WT and $\alpha5-/-$ neurons (Fig. 6*B*). Threshold current was significantly greater in WT than in $\alpha5-/-$ neurons (106 ± 19 pA, $n = 12$ vs. 69 ± 10 pA, $n = 12$; $P < 0.01$), but the frequency of action potentials at the threshold current was similar (2.67 ± 0.43 vs. 2.58 ± 0.50 Hz, $n = 12$; $P > 0.05$). The slope for the input-output relationship was similar in WT and $\alpha5-/-$ neurons (0.244 ± 0.001 Hz/pA, $n = 12$ vs. 0.252 ± 0.011 Hz/pA, $n = 12$; $P > 0.05$; Fig. 6*B*). These data confirm that tonic inhibition generated by $\alpha5GABA_A$ receptors does not alter the gain of the input-output relationship.

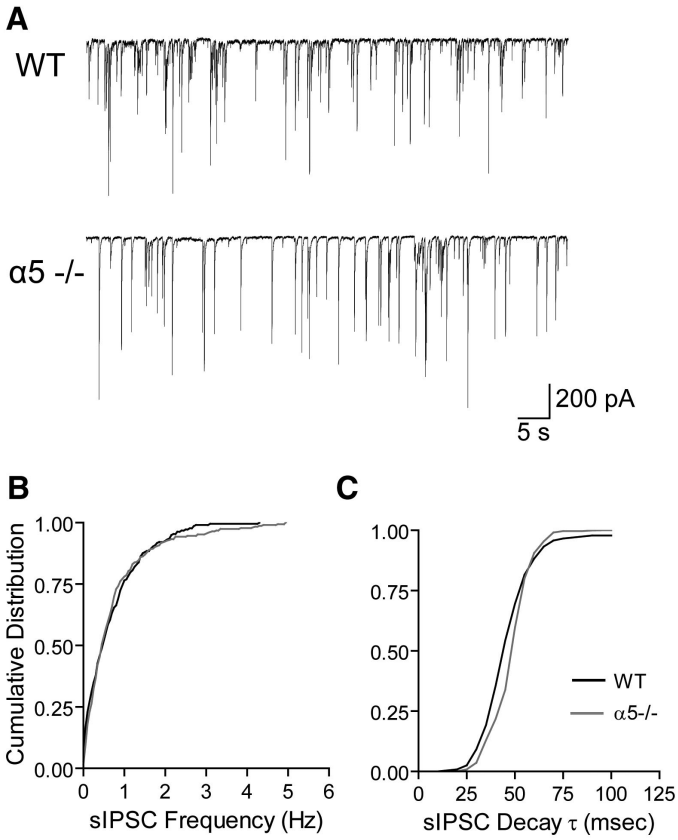


FIG. 5. Deletion of $\alpha5GABA_A$ receptors does not modify spontaneous inhibitory postsynaptic currents (IPSCs) in cultured pyramidal neurons. *A*: sample trace of spontaneous IPSCs (sIPSCs) recorded in cultured WT neurons and $\alpha5-/-$ neurons. *B*: normalized cumulative distribution of the frequency of sIPSCs is shown. No difference was detected between currents recorded in WT ($n = 7$) and $\alpha5-/-$ neurons ($n = 7$, $P > 0.05$). *C*: normalized cumulative distribution curves of the decay time of sIPSC were similar for currents recorded in WT neurons ($n = 7$) and $\alpha5-/-$ neurons ($n = 7$, $P > 0.05$).

## DISCUSSION

This study provides direct evidence that $\alpha5GABA_A$ receptors regulate the firing of action potentials in pyramidal neurons. Notably, the magnitude of depolarizing current required to trigger action potentials was greater in WT than in $\alpha5-/-$ neurons. The absence of $\alpha5GABA_A$ receptors did not alter the gain or frequency of action potential firing during a prolonged depolarizing pulse. Furthermore, reduced expression of $\alpha5GABA_A$ receptors did not influence spontaneous inhibitory synaptic transmission. Thus a tonic conductance generated by $\alpha5GABA_A$Rs contributes to the lower excitability of WT neurons relative to that of $\alpha5-/-$ neurons. These in vitro findings are consistent with behavioral studies indicating that

TABLE 2. *Properties of spontaneous IPSCs recorded in CA1 pyramidal neurons in hippocampal slices*

| | WT (5) | | α5−/− (5) | |
|---|---|---|---|---|
| | ACSF | 20 μM L6 | ACSF | 20 μM L6 |
| Peak amplitude, pA | 48.4 ± 3.1 | 45.6 ± 4.2 | 52.6 ± 4.2 | 49.6 ± 4.2 |
| Rise time, ms | 1.4 ± 0.2 | 1.5 ± 0.2 | 1.4 ± 0.2 | 1.6 ± 0.6 |
| Weighted decay tau, ms | 9.4 ± 1.4 | 8.8 ± 1.4 | 9.0 ± 1.3 | 9.0 ± 1.2 |
| Frequency, Hz | 2.9 ± 1.2 | 2.8 ± 0.5 | 2.7 ± 1.2 | 2.9 ± 0.6 |

Values are means ± SE. Parentheses enclose *n* values. IPSC, inhibitory post synaptic current, ACSF, artificial cerebrospinal fluid; L6, L-655, 708.

reduced expression of α5GABA$_A$ receptor is not fully compensated in α5−/− mice (Cheng et al. 2006; Collinson et al. 2002).

### *Adaptive changes in α5−/− neurons*

Picrotoxin did not alter the excitability of α5−/− neurons in the absence of exogenous GABA. This result is unexpected, as it suggests that under the current experimental conditions, tonic rather than synaptic GABAergic inhibition plays the predominant role in regulating excitability. Consistent with our results, charge transfer attributed to the tonic conductance is several times greater than that attributable to synaptic inhibition (Caraiscos et al. 2004a; Hamann et al. 2002; Scimemi et al. 2005). Furthermore, α5−/− neurons fail to generate a fully compensatory or residual GABAergic conductance sufficient to depress excitability. The residual tonic conductance in α5−/− neurons (Caraiscos et al. 2004a; Glykys and Mody 2006) could be generated by GABA$_A$ receptors that contain subunits other than α5 and or unidentified compensatory changes. It is unlikely to result from an increase in the number of αβγ subunit-containing GABA$_A$ receptors because the activity of [3H]Ro15-1788, a radioligand with high affinity to benzodiazepine sites of the α1-, α2-, α3-, and α5-containing receptors was reduced by 16% more in hippocampal membrane prepared from α5−/− mice than in the same tissue from WT mice (Collinson et al. 2002). This reduction is predicted from the proportion of α5GABA$_A$ receptors relative to other benzodiazepine-sensitive receptors in the hippocampus (Sur et al. 1999). A pharmacological study of α5−/− neurons showed that a residual tonic conductance is generated at least in part by δGABA$_A$ receptors (Glykys and Mody 2006; Scimemi et al. 2005). GABA$_A$ receptors that exhibit constitutive opening might also contribute to the residual tonic conductance in pyramidal neurons (McCartney et al. 2007). However, for several reasons, it is unlikely that constitutively active α5GABA$_A$ receptors generate a sizeable tonic conductance in hippocampal neurons. First, neurons and glia grown in dissociated co-cultures release a soluble transmitter that activates a tonic GABAergic conductance (Valeyev et al. 1993;Vautrin et al. 2000). Several release mechanisms, including spillover of GABA from synapses, reverse operation of GABA transporters and nonvesicular release mechanisms, and calcium-independent release of transmitter contribute to the extracellular GABA (Kullmann et al. 2005). Second, we and others found that increasing the flow of extracellular solution to the surface of neurons rapidly and reversibly reduced the baseline current and fluctuation in the baseline noise (Bai et al. 2001). This change in holding current produced by changing the flow was eliminated by bicuculline. Fluctuation analysis showed that the variance in the baseline current signal was exponentially distributed with estimated kinetics comparable to those activated by submicromolar concentrations of exogenous GABA. The kinetics of channels activated by endogenous GABA displayed a potential sensitivity comparable to those activated by exogenous GABA (Bai et al. 2001). Finally, acutely isolated hippocampal pyramidal neurons did not generate a detectable tonic GABAergic current in the absence of exogenous GABA (Bai et al. 2001).

As mentioned in the INTRODUCTION, genetic deletion of a GABA$_A$ receptor subunit that contributes to a tonic conductance does not necessarily lead to altered neuronal excitability. Targeted deletion of the gene that encodes for the α6 subunit resulted in complete loss of α6δ subunits expressed on the surface of cerebellar granule neurons (Jones et al. 1997) and a reduction in tonic conductance (Brickley et al. 2001). However, the leak conductance, neuronal response to depolarizing current injection, and excitatory synaptic input were similar in α6−/− and WT neurons (Brickley et al. 2001). Adaptive changes were attributed to an increase in voltage-independent $K^+$ conductance generated by TASK-1 channels. As well, Northern blot analysis of whole cerebella showed a 20% increase in TASK-1 messenger RNA in α6−/− mice. Reduced expression of postsynaptic GABA$_A$ receptors also resulted in adaptive changes in subunit expression. GABA$_A$ receptors containing the α1 subunit represent nearly half of the receptors in the brain (Kralic et al. 2002; McKernan and Whiting 1996)

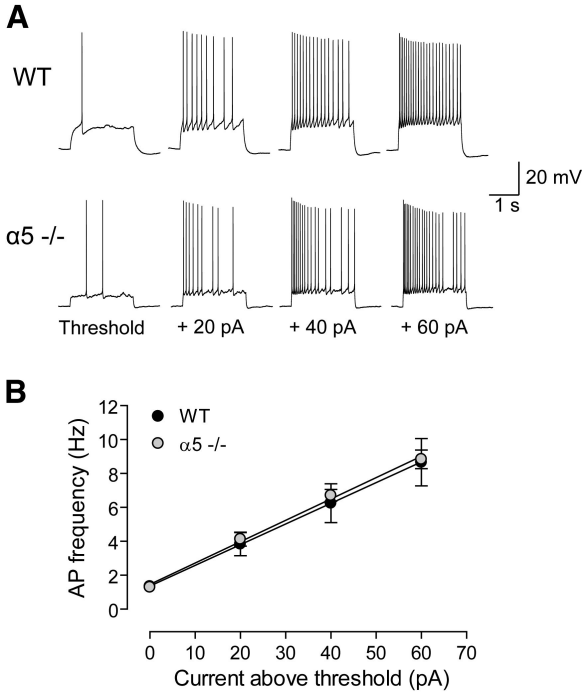


FIG. 6. α5GABA$_A$ receptors do not regulate the frequency of action potentials in cultured neurons. The input-output relationship between the stimulus intensity and frequency of action potentials in WT and α5−/− hippocampal pyramidal neurons is shown. *A*: sample traces of action potentials recorded in WT and α5−/− neurons in response to injection of depolarizing current steps. The depolarizing steps were increased in 20-pA increments after the firing of the 1st potential. *B*: relationship between the excitatory input frequency and frequency of action potentials is similar for potentials recorded in WT neurons ($n = 12$) and α5−/− neurons ($n = 12$; $P > 0.05$).

and are primarily localized to postsynaptic clusters (Sassoe-Pognetto et al. 2000). Deletion of the $\alpha$1 subunit gene resulted in a marked reduction in the expression of $GABA_A$ receptors but only moderate behavioral changes. Adaptive changes in $\alpha1-/-$ mice include the reorganization of neuronal circuits and replacement of the $\alpha$1 subunit with the $\alpha$3 and $\alpha$4 subunits (Kralic et al. 2006). In $\alpha1-/-$ cerebellar granule cells, a tonic conductance was increased by 62%, whereas the activity of the GABA transporter was reduced (Ortinski et al. 2006). Our results indicate that homeostatic mechanisms fail to fully compensate for a reduction in $\alpha5GABA_A$ receptor activity as the excitability of $\alpha5-/-$ neurons is increased. Demonstrable compensatory changes in $\alpha6-/-$ and $\alpha1-/-$ neurons but not $\alpha5-/-$ pyramidal neurons indicate important regulatory differences in the production and trafficking of various $GABA_A$ receptor subunits. Also $\alpha5GABA_A$ receptors appear to play a unique role in the hippocampus that is not substituted for by other $GABA_A$ receptor subtypes.

Pharmacological compounds that potentiated or inhibited the tonic conductance produced hyperpolarization or depolarization of WT neurons, respectively. These compounds had no demonstrable effect on $\alpha5-/-$ neurons even though a small residual tonic GABAergic current was present. Despite this residual conductance, the application of exogenous GABA failed to hyperpolarize $\alpha5-/-$ neurons. This may be attributed to high-affinity $\delta GABA_A$ receptors that are thought to underlie the residual tonic conductance in $\alpha5-/-$ neurons (Scimemi et al. 2005). Saturation of a small population of $\delta GABA_A$ receptors by ambient GABA would prevent further depolarization by exogenous GABA. Finally, the resting membrane potential was similar between genotypes. An inconsistency exists between the similarity in the resting membrane potential and difference in the picrotoxin-induced depolarizing current in WT but not $\alpha5-/-$ neurons. Several factors might account for this discrepancy. For example, there might be a difference in the resting membrane potential that we failed to detect because the difference was small and the sample size inadequate. However, we favor the explanation that there may have been no detectable difference in $V_m$ because the permeability of the tonic anion channels is low relative to that of other ion species that determine $V_m$.

### *Synaptic inhibition and $\alpha5GABA_A$ receptors*

The contribution of $\alpha5GABA_A$ receptors to synaptic transmission remains controversial. A subpopulation of these receptors is expressed in both the synaptic and the extrasynaptic regions of neurons (Serwanski et al. 2006) and may contribute to tonic and phasic GABAergic currents. There was no difference in the amplitude or kinetics of sIPSCs recorded in WT or $\alpha5-/-$ neurons. Consistent with this result, a previous report showed that miniature IPSCs, which result from the release of single quanta of transmitter and the exclusive activation of postsynaptic receptors, were not influenced by deletion of the $\alpha$5 subunit (Caraiscos et al. 2004a; Cheng et al. 2006). Several studies have shown that $\alpha5GABA_A$ receptors in cultured neurons do not contribute to synaptic transmission; however, the low density of neurons and the absence of glia from the cultures may prevent sufficient GABA from accumulating in the extracellular space. In hippocampal slices, rhythmic activity may cause the release of GABA from interneurons and activation of GABA receptors on neighboring neurons (Hamann et al. 2002; Scanziani 2000). Also nonvesicular release of GABA (Rossi et al. 2003) and reduced activity of GABA transporters contributes further to extracellular GABA (Semyanov et al. 2003). No change in the frequency, amplitude, or decay of sIPSCs in the CA1 and CA3 regions of hippocampal slices were detected from WT and $\alpha5-/-$ mice (Glykys and Mody 2006). Similarly, monosynaptically evoked IPSPs were similar in CA3 pyramidal neurons in slices from WT and $\alpha5-/-$ mice (Towers et al. 2004). Contrasting these results, a modest reduction in the amplitude of evoked IPSCs in hippocampal slices from $\alpha5-/-$ mice (87% of WT) has been reported (Collinson et al. 2002). Also a subpopulation of slowly activating sIPSCs that occur at low frequency in CA3 and CA1 pyramidal neurons were reported to be more numerous in WT than in $\alpha5-/-$ neurons (Glykys and Mody 2006). A study of diazepine-sensitive receptors that used single-, double-, and triple-mutant mice showed that $\alpha5GABA_A$ receptors do not contribute to fast synaptic transmission on the soma or dendrites of CA1 pyramidal neurons, but perisynaptic $\alpha5GABA_A$ receptors do contribute to slow IPSCs (Prenosil et al. 2006). Collectively, these results indicate that although a small proportion of $\alpha5GABA_A$ receptors are expressed at synapses the total inhibitory charge transfer generated by synaptic $\alpha5GABA_A$ receptors is negligible. Thus synaptic $\alpha5GABA_A$ receptors may make a small contribution to network behavior, whereas extrasynaptic $GABA_A$ receptors appear to play a significant role.

### *Tonic inhibition and excitability*

Activation of $\alpha5GABA_A$ receptors caused only a modest hyperpolarization of WT pyramidal neurons. A tonic conductance can reduce excitability via shunting mechanisms that are independent of changes in membrane potential (Brickley et al. 1996; Mitchell and Silver 2003). Shunting inhibition influences the input-output activity of neurons by increasing the excitatory drive required to generate an action potential. Typically, shunting inhibition does not change the slope or gain of the input-output relationship when the neurons are stimulated by nonfluctuating excitatory input (Chance et al. 2002; Mitchell and Silver 2003). We showed that activation of $\alpha5GABA_A$ receptors did not change the gain of the input-output relationship. These results indicate that $\alpha5GABA_A$ receptors cause a robust shunting inhibition and only modest membrane hyperpolarization. However, it should be appreciated that we examined the impact of the tonic inhibitory conductance under experimental conditions where the level of excitatory input was persistent and low. Under more physiological conditions, excitatory input is greater and fluctuates widely because of transient activation of the postsynaptic receptors. The influence of a tonic inhibitory conductance on neuronal excitability with a fluctuating or "noisy" excitatory synaptic input has been studied previously by others using computer simulations and an in vitro cerebellar preparation (Mitchell and Silver 2003). Under these conditions, the effect of tonic inhibition on neuronal firing is nonlinear and depends on the nature of the excitatory synaptic input. A tonic shunt induced a change in both the gain and threshold of the action potential firing during noisy synaptic input. Such a change in the firing rate has been proposed to increase the storage capacity of the cerebellum

(Hamman et al. 2002). In contrast to the results for the cerebellum, an increase in tonic inhibition in the hippocampus appears to reduce learning. Our behavioral studies suggest that an increase in $\alpha$5GABA$_A$ receptor activity impairs performance for hippocampal-dependent memory tasks (Cheng et al. 2006).

Hippocampal gamma frequency oscillations have been proposed to underlie the encoding of spatial information and formation of episodic memory (Lisman 1999). In particular, it has been proposed that oscillations originating in CA1 and CA3 regions regulate cognition (Csicsvari et al. 2003). Several studies have investigated the function of tonic inhibition in CA1 and CA3 pyramidal neurons in regulating network activity (Towers et al. 2004; Whittington et al. 1996). The role of $\alpha$5GABA$_A$ receptors in modifying kainate-induced gamma oscillations (20–80 Hz) was investigated using hippocampal slices from WT and $\alpha5-/-$ mice (Towers et al. 2004). The power of network oscillations in response to increased excitatory drive was greater in hippocampal slices prepared from $\alpha5-/-$ mice than in those prepared from WT mice. Also the frequency range over which the oscillations occurred was lower, which resulted in very strong oscillations over a narrow bandwidth in the $\alpha5-/-$ slices. In addition, raising the extracellular concentration of GABA caused a greater reduction in the power of gamma oscillations in WT slices than in $\alpha5-/-$ slices. Computer simulation of a neuronal network indicated that reduced in tonic conductance in the dendritic membrane of the principal cells accurately reproduced the increased gamma power observed in $\alpha5-/-$ slices (Towers et al. 2004). The power of the gamma oscillations was dependent on membrane resistance and shunting inhibition, which is consistent with the results presented here. Hippocampal oscillations are capable of regulating the firing of pyramidal neurons (Cobb et al. 1995). Thus a complex interaction of synchronous network activity and individual neuronal activity exists in the hippocampus with both elements being regulated by tonic inhibition. Our data now offer realistic parameters that can be incorporated into computational models that further investigate hippocampal network synchrony.

In summary, we have shown that tonic inhibition in hippocampal pyramidal neurons regulates spike firing. Together with the results of previous studies, these findings raise the intriguing possibility that a reduction of tonic inhibition, and the simultaneous enhancement of oscillatory activity and neuronal firing, facilitates network co-ordination in the hippocampus in $\alpha5-/-$ slices and improves the performance for learning and memory tasks in $\alpha5-/-$ mice.

## ACKNOWLEDGMENTS

The authors thank E. Czerwinski for assistance with the cell cultures and Dr. Michael Jackson for assistance with the preparation of the manuscript.

## GRANTS

The research was supported by Canadian Institutes of Health Research Operating Grant MOP-38028 to B. A. Orser and a Canada Research Chair to B. A. Orser.

## REFERENCES

**Attwell D, Barbour B, Szatkowski M.** Nonvesicular release of neurotransmitter. *Neuron* 11: 401–407, 1993.

**Bai D, Zhu G, Pennefather P, Jackson MF, MacDonald JF, Orser BA.** Distinct functional and pharmacological properties of tonic and quantal inhibitory postsynaptic currents mediated by gamma-aminobutyric acid(A) receptors in hippocampal neurons. *Mol Pharmacol* 59: 814–824, 2001.

**Brickley SG, Cull-Candy SG, Farrant M.** Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABA$_A$ receptors. *J Physiol* 497: 753–759, 1996.

**Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M.** Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature* 409: 88–92, 2001.

**Brunig I, Scotti E, Sidler C, Fritschy JM.** Intact sorting, targeting, and clustering of gamma-aminobutyric acid A receptor subtypes in hippocampal neurons in vitro. *J Comp Neurol* 443: 43–55, 2002.

**Caraiscos VB, Elliott EM, You T, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA.** Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by {alpha}5 subunit-containing {gamma}-aminobutyric acid type A receptors. *Proc Natl Acad Sci USA* 101: 3662–3667, 2004a.

**Caraiscos VB, Newell JG, You T, Elliott EM, Rosahl TW, Wafford KA, MacDonald JF, Orser BA.** Selective enhancement of tonic GABAergic inhibition in murine hippocampal neurons by low concentrations of the volatile anesthetic isoflurane. *J Neurosci* 24: 8454–8458, 2004b.

**Chambers MS, Atack JR, Broughton HB, Collinson N, Cook S, Dawson GR, Hobbs SC, Marshall G, Maubach KA, Pillai GV, Reeve AJ, MacLeod AM.** Identification of a novel, selective GABA(A) alpha5 receptor inverse agonist which enhances cognition. *J Med Chem* 46: 2227–2240, 2003.

**Chance FS, Abbott LF, Reyes AD.** Gain modulation from background synaptic input. *Neuron* 35: 773–782, 2002.

**Cheng VY, Martin LJ, Elliott EM, Kim JH, Mount HT, Taverna FA, Roder JC, MacDonald JF, Bhambri A, Collinson N, Wafford KA, Orser BA.** Alpha5GABA$_A$ receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. *J Neurosci* 26: 3713–3720, 2006.

**Christie SB, Miralles CP, de Blas AL.** GABAergic innervation organizes synaptic and extrasynaptic GABA$_A$ receptor clustering in cultured hippocampal neurons. *J Neurosci* 22: 684–697, 2002.

**Cobb SR, Burl EH, Halasy K, Paulsen O, Somogyi P.** Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378: 75–78, 1995.

**Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW.** Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABA$_A$ receptor. *J Neurosci* 22: 5572–5580, 2002.

**Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, Bluthmann H, Mohler H, Rudolph U.** Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. *Proc Natl Acad Sci USA* 99: 8980–8985, 2002.

**Csicsvari J, Jamieson B, Wise KD, Buzsaki G.** Mechanisms of gamma oscillations in the hippocampus of the behaving rat. *Neuron* 37: 311–322, 2003.

**Curmi JP, Premkumar LS, Birnir B, Gage PW.** The influence of membrane potential on chloride channels activated by GABA in rat cultured hippocampal neurons. *J Membr Biol* 136: 273–280, 1993.

**Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM, Choudhury HI, McDonald LM, Pillai G, Rycroft W, Smith AJ, Sternfeld F, Tattersall FD, Wafford KA, Reynolds DS, Seabrook GR, Atack JR.** An inverse agonist selective for {alpha}5 subunit-containing GABAA receptors enhances cognition. *J Pharmacol Exp Ther* 316: 1335–1345, 2006.

**Fritschy JM, Mohler H.** GABA$_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J Comp Neurol* 359: 54–94, 1995.

**Glykys J, Mody I.** Hippocampal network hyperactivity following selective reduction of tonic inhibition in GABAA receptor {alpha}5 subunit deficient mice. *J Neurophysiol* 95: 2796–2807, 2006.

**Hamann M, Rossi DJ, Attwell D.** Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. *Neuron* 33: 625–633, 2002.

**Jones A, Korpi ER, McKernan RM, Pelz R, Nusser Z, Makela R, Mellor JR, Pollard S, Bahn S, Stephenson FA, Randall AD, Sieghart W, Somogyi P, Smith AJ, Wisden W.** Ligand-gated ion channel subunit partnerships: GABAA receptor alpha6 subunit gene inactivation inhibits delta subunit expression. *J Neurosci* 17: 1350–1362, 1997.

**Khirug S, Huttu K, Ludwig A, Smirnov S, Voipio J, Rivera C, Kaila K, Khiroug L.** Distinct properties of functional KCC2 expression in immature

mouse hippocampal neurons in culture and in acute slices. *Eur J Neurosci* 21: 899–904, 2005.

**Kralic JE, Korpi ER, O'Buckley TK, Homanics GE, Morrow AL.** Molecular and pharmacological characterization of GABA(A) receptor alpha1 subunit knockout mice. *J Pharmacol Exp Ther* 302: 1037–1045, 2002.

**Kralic JE, Sidler C, Parpan F, Homanics GE, Morrow AL, Fritschy JM.** Compensatory alteration of inhibitory synaptic circuits in cerebellum and thalamus of gamma-aminobutyric acid type A receptor alpha1 subunit knockout mice. *J Comp Neurol* 495: 408–421, 2006.

**Kullmann DM, Ruiz A, Rusakov DM, Scott R, Semyanov A, Walker MC.** Presynaptic, extrasynaptic and axonal $GABA_A$ receptors in the CNS: where and why? *Prog Biophys Mol Biol* 87: 33–46, 2005.

**Lacaille JC, Mueller AL, Kunkel DD, Schwartzkroin PA.** Local circuit interactions between oriens/alveus interneurons and CA1 pyramidal cells in hippocampal slices: electrophysiology and morphology. *J Neurosci* 7: 1979–1993, 1987.

**Lerma J, Herranz AS, Herreras O, Abraira V**, **Martin DR.** In vivo determination of extracellular concentration of amino acids in the rat hippocampus. A method based on brain dialysis and computerized analysis. *Brain Res* 384: 145–155, 1986.

**Lisman JE.** Relating hippocampal circuitry to function: recall of memory sequences by reciprocal dentate-CA3 interactions. *Neuron* 22: 233–242, 1999.

**MacDonald JF, Mody I, Salter MW.** Regulation of *N*-methyl-D-aspartate receptors revealed by intracellular dialysis of murine neurons in culture. *J Physiol* 414: 17–34, 1989.

**Maguire JL, Stell BM, Rafizadeh M**, **Mody I.** Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci* 8: 797–804, 2005.

**Marchionni I, Omrani A, Cherubini E.** In the developing rat hippocampus a tonic $GABA_A$-mediated conductance selectively enhances the glutamatergic drive of principal cells. *J Physiol* 581: 515–528, 2007.

**McCartney MR, Deeb TZ, Henderson TN, Hales TG.** Tonically active $GABA_A$ receptors in hippocampal pyramidal neurons exhibit constitutive GABA-independent gating. *Mol Pharmacol* 71: 539–548, 2007.

**McKernan RM, Whiting PJ.** Which $GABA_A$-receptor subtypes really occur in the brain? *Trends Neurosci* 19: 139–143, 1996.

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

**Mody I.** Distinguishing between GABA(A) receptors responsible for tonic and phasic conductances. *Neurochem Res* 26: 907–913, 2001.

**Ortinski PI, Turner JR, Barberis A, Motamedi G, Yasuda RP, Wolfe BB, Kellar KJ, Vicini S.** Deletion of the GABA(A) receptor alpha1 subunit increases tonic GABA(A) receptor current: a role for GABA uptake transporters. *J Neurosci* 26: 9323–9331, 2006.

**Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G.** GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* 101: 815–850, 2000.

**Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy JM, Vogt KE.** Specific subtypes of $GABA_A$ receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. *J Neurophysiol* 96: 846–857, 2006.

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

**Rossi DJ, Hamman M, Attwell D.** Multiple modes of GABAergic inhibition of rat cerebellar granule cells. *J Physiol* 548: 97–110, 2003.

**Rudolph U, Crestani F, Mohler H.** GABA(A) receptor subtypes: dissecting their pharmacological functions. *Trends Pharmacol Sci* 22: 188–194, 2001.

**Sassoe-Pognetto M, Panzanelli P, Sieghart W, Fritschy JM.** Colocalization of multiple GABA(A) receptor subtypes with gephyrin at postsynaptic sites. *J Comp Neurol* 420: 481–498, 2000.

**Scanziani M.** GABA spillover activates postsynaptic GABA(B) receptors to control rhythmic hippocampal activity. *Neuron* 25: 673–681, 2000.

**Scimemi A, Semyanov A, Sperk G, Kullmann DM, Walker MC.** Multiple and plastic receptors mediate tonic $GABA_A$ receptor currents in the hippocampus. *J Neurosci* 25: 10016–10024, 2005.

**Semyanov A, Walker MC, Kullmann DM.** GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. *Nat Neurosci* 6: 484–490, 2003.

**Semyanov A, Walker MC, Kullmann DM, Silver RA.** Tonically active $GABA_A$ receptors: modulating gain and maintaining the tone. *Trends Neurosci* 27: 262–269, 2004.

**Serwanski DR, Miralles CP, Christie SB, Mehta AK, Li X, de Blas AL.** Synaptic and nonsynaptic localization of $GABA_A$ receptors containing the alpha5 subunit in the rat brain. *J Comp Neurol* 499: 458–470, 2006.

**Stell BM, Brickley SG, Tang CY, Farrant M, Mody I.** Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by {delta} subunit-containing GABAA receptors. *Proc Natl Acad Sci USA* 100: 14439–14444, 2003.

**Stell BM, Mody I.** Receptors with different affinities mediate phasic and tonic $GABA_A$ conductances in hippocampal neurons. *J Neurosci* 22: 1–5, 2002.

**Sternfeld F, Carling RW, Jelley RA, Ladduwahetty T, Merchant KJ, Moore KW, Reeve AJ, Street LJ, O'Connor D, Sohal B, Atack JR, Cook S, Seabrook G, Wafford K, Tattersall FD, Collinson N, Dawson GR, Castro JL, MacLeod AM.** Selective, orally active gamma-aminobutyric acidA alpha5 receptor inverse agonists as cognition enhancers. *J Med Chem* 47: 2176–2179, 2004.

**Sur C, Fresu L, Howell O, McKernan RM, Atack JR.** Autoradiographic localization of alpha5 subunit-containing GABAA receptors in rat brain. *Brain Res* 822: 265–270, 1999.

**Tossman U, Jonsson G, Ungerstedt U.** Regional distribution and extracellular levels of amino acids in rat central nervous system. *Acta Physiol Scand* 127: 533–545, 1986.

**Towers SK, Gloveli T, Traub RD, Driver JE, Engel D, Fradley R, Rosahl TW, Maubach K, Buhl EH, Whittington MA.** Alpha 5 subunit-containing $GABA_A$ receptors affect the dynamic range of mouse hippocampal kainate-induced gamma frequency oscillations in vitro. *J Physiol* 559: 721–728, 2004.

**Valeyev AY, Cruciani RA, Lange GD, Smallwood VS, Barker JL.** $Cl^-$ channels are randomly activated by continuous GABA secretion in cultured embryonic rat hippocampal neurons. *Neurosci Lett* 155: 199–203, 1993.

**Vautrin J, Maric D, Sukhareva M, Schaffner AE, Barker JL.** Surface-accessible GABA supports tonic and quantal synaptic transmission. *Synapse* 37: 38–55, 2000.

**Wall MJ, Usowicz MM.** Development of the quantal properties of evoked and spontaneous synaptic currents at a brain synapse. *Nat Neurosci* 1: 675–682, 1998.

**Whiting PJ, Bonnert TP, McKernan RM, Farrar S, Le Bourdelles B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Thompson SA, Wafford KA.** Molecular and functional diversity of the expanding GABA-A receptor gene family. *Ann NY Acad Sci* 868: 645–653, 1999.

**Whittington MA, Jefferys JG, Traub RD.** Effects of intravenous anaesthetic agents on fast inhibitory oscillations in the rat hippocampus in vitro. *Br J Pharmacol* 118: 1977–1986, 1996.

**Wisden W, Laurie DJ, Monyer H, Seeburg PH.** The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* 12: 1040–1062, 1992.

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

**Yeung YT, Canning KJ, Zhu G, Pennefather P, MacDonald JF, Orser BA.** Tonically activated $GABA_A$ receptors in hippocampal neurons are high-affinity, low-conductance sensors for extracellular GABA. *Mol Pharm* 63: 2–8, 2003.