J Neurophysiol 89: 1378–1386, 2003.
First published December 4, 2002; 10.1152/jn.0899.2002.

Intact Synaptic GABAergic Inhibition and Altered Neurosteroid Modulation of Thalamic Relay Neurons in Mice Lacking δ Subunit

DARRELL M. PORCELLO,[1] MOLLY M. HUNTSMAN,[1] ROBERT M. MIHALEK,[2] GREGG E. HOMANICS,[2,3] AND JOHN R. HUGUENARD[1]

[1]*Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, California 94305; and* [2]*Department of Anesthesiology and* [3]*Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261*

Submitted 8 October 2002; accepted in final form 26 November 2002

Porcello, Darrell M., Molly M. Huntsman, Robert M. Mihalek, Gregg E. Homanics, and John R. Huguenard. Intact synaptic GABAergic inhibition and altered neurosteroid modulation of thalamic relay neurons in mice lacking δ subunit. *J Neurophysiol* 89: 1378–1386, 2003. First published December 4, 2002; 10.1152/jn.0899.2002. Robust GABA-mediated inhibitory postsynaptic currents (IPSCs) in neurons of the thalamic relay (TC) nuclei are important in sustaining oscillatory activity within thalamic and thalamocortical circuits. The biophysical properties and pharmacological sensitivities of these IPSCs both depend on the subunit combination of postsynaptic γ-aminobutyric acid-A ($GABA_A$) receptors. Recombinant $GABA_A$ receptors containing the δ subunit (heavily expressed in TC nuclei) have been shown to exhibit slowed desensitization rates and high affinity for GABA in heterologous expression systems. We tested whether the $GABA_A$-mediated synaptic inhibition in TC neurons would be affected by loss of the δ subunit. Spontaneous and evoked IPSCs were recorded from neurons in the ventral basal complex (VB) of the thalamus from brain slices of wild-type ($\delta^{+/+}$) and homozygous δ subunit deficient mice ($\delta^{-/-}$). Spontaneous IPSCs (sIPSCs) from $\delta^{-/-}$ mice had no significant differences in amplitude, duration, or frequency compared with their $\delta^{+/+}$ counterparts. However, baseline noise (63% of control) and the relative contribution of the slow component to overall decay (79% of control) were significantly lower in $\delta^{-/-}$ VB recordings. Evoked IPSCs (eIPSCs) in $\delta^{-/-}$ neurons showed no difference in peak amplitude, but had an accelerated slow decay component (40- vs. 55-ms time constant). We further tested whether neurosteroid modulation of $GABA_A$ receptors was dependent on the presence of the δ subunit, as previously reported in recombinant systems. Pregnenolone sulfate (PS) significantly reduced eIPSC peak amplitude (−30%) and increased duration in $\delta^{-/-}$, but not in $\delta^{+/+}$ mice. sIPSCs were not affected in any neurons, $\delta^{-/-}$ or $\delta^{+/+}$. In contrast, 3-alpha,5-alpha-tetrahydrodeoxycorticosterone (THDOC) increased the durations of eIPSCs and sIPSCs in both $\delta^{-/-}$ and $\delta^{+/+}$ VB neurons. Our findings show that although the δ subunit confers a striking PS insensitivity to eIPSCs in VB neurons, it plays only a minor role in the synaptic inhibition of VB neurons. This suggests δ subunit containing $GABA_A$ receptors may be functionally limited to an extrasynaptic locus in VB neurons.

INTRODUCTION

The biophysical and pharmacological diversity of ionotropic GABA receptors ($GABA_A$Rs) is a well-established principle leading to heterogeneous inhibitory synaptic transmission. $GABA_A$Rs are pentameric heteromers assembled from a large multigene family of subunits (α_{1-6}, β_{1-4}, γ_{1-4}, δ, ε, π, and ρ_{1-3}). Throughout the mammalian brain, $GABA_A$Rs exist as multiple isoforms, generally containing two α, two β, and one γ or δ subunit (Farrar et al. 1999; McKernan and Whiting 1996). The subunit-dependent kinetics and pharmacology of $GABA_A$Rs (Macdonald and Olsen 1994), along with developmental- and regional-specific expression patterns of subunits (Laurie et al. 1992; Pirker et al. 2000; Wisden et al. 1992), suggest distinct functional roles for $GABA_A$Rs isoforms in the brain. Recombinant receptors containing the δ subunit are characterized by high affinity for GABA and slow desensitization rates (Adkins et al. 2001; Saxena and Macdonald 1994), which may favor signaling by low, persistent amounts of transmitter. The replacement of the γ2 with the δ subunit in $GABA_A$R constructs alters the modulation due to endogenous (Mensah-Nyagan et al. 1999) and synthetic neurosteroids such as alphaxalone and pregnenolone sulfate (PS) (Adkins et al. 2001; Wohlfarth et al. 2002; Zhu et al. 1996), which have behavioral, anti-convulsant, and hypnotic actions in rodents (Lambert et al. 1995; Macdonald and Olsen 1994; Vallee et al. 1997). While the above findings obtained with recombinant receptors have advanced our knowledge of the δ subunit in general, it is uncertain whether they can be faithfully translated to native $GABA_A$Rs in neurons that express the δ subunit.

Native δ-containing $GABA_A$Rs develop postnatally (Laurie et al. 1992) and are most prominently observed in the cerebellum, followed by forebrain regions of the dentate gyrus and dorsal thalamus (Pirker et al. 2000; Wisden et al. 1992). In cerebellar granule cells, δ subunits are co-assembled with α6 subunits into $GABA_A$Rs that are largely restricted to extrasynaptic sites (Nusser et al. 1998). The complete loss of δ-containing $GABA_A$Rs in cerebellar granule cells, resulting from genetic inactivation of the α6 subunit ($\alpha6^{-/-}$) (Jones et al. 1997), is associated with the disruption of a tonic inhibitory current (Brickley et al. 2001) produced by resting GABA levels. While such a δ subunit dependent tonic inhibition may also exist in forebrain (Nusser and Mody 2002), there is presently no subcellular anatomical evidence supporting an

Address for reprint requests: J. R. Huguenard, Department of Neurology and Neurological Sciences, Stanford University Medical Center, Stanford, CA 94305-5122 (E-mail: John.Huguenard@Stanford.EDU).

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.

exclusively extrasynaptic location for δ-containing $GABA_A$Rs in dentate granule or thalamocortical (TC) relay neurons. A possible synaptic function for δ-containing $GABA_A$Rs in forebrain has been suggested by results from mice lacking the δ subunit itself ($\delta^{-/-}$). Studies of $\delta^{-/-}$ mice have reported faster decay times for both spontaneous and evoked synaptic $GABA_A$ currents recorded from dentate granule neurons (Li et al. 1998; Mihalek et al. 1999), but this contrasts with no difference in cerebellar granule cells (Vicini et al. 2002). Because dentate granule and TC neurons both assemble δ-containing $GABA_A$Rs with the α4 subunit (Korpi et al. 2002; Peng et al. 2002; Sur et al. 1999) and undergo a similar reorganization of remaining $GABA_A$R subunits after the loss of the δ subunit (Korpi et al. 2002; Peng et al. 2002), we tested for similar alterations in the synaptic inhibition onto TC neurons from $\delta^{-/-}$ mice.

GABA-mediated inhibition in TC neurons resulting from activity of the reticular thalamic nucleus (RTN) has been shown to be involved in the generation of synchronous activity including sleep spindles and slow-wave sleep (McCormick and Bal 1997; Steriade et al. 1993). A hypersynchronous state of the intrathalamic circuit, reminiscent of several rodent models of absence, can be experimentally induced through either an enhancement of the inhibitory output from RTN onto TC nuclei (Huguenard and Prince 1994) or a reduction of the intra-RTN GABAergic inhibition (Huntsman et al. 1999). These two opposing roles of GABAergic inhibition within the intrathalamic circuit make it necessary for any potential anti-absence therapy directed against $GABA_A$Rs to differentiate between RTN → TC and intra-RTN connections. With intra-RTN connections devoid of the δ subunit (Peng et al. 2002; Wisden et al. 1992; but see Browne et al. 2001), a functional postsynaptic presence of δ-containing $GABA_A$Rs in TC neurons may provide a novel method to differentiate the two intrathalamic pathways. Additionally, a class of pharmacological compounds, neurosteroids, may be promising in targeting δ-containing synapses. $\delta^{-/-}$ mice have attenuated behavioral responses to neurosteroids (Mihalek et al. 1999), which have also been shown to influence specific recombinant $GABA_A$Rs (Brown et al. 2002; Zhu et al. 1996). In this paper, we study the synaptic physiology and neurosteroid sensitivities of TC neurons in $\delta^{-/-}$ mice, to further understand the above findings.

METHODS

Thalamic slice preparation

C57BL/6J × Strain 129Sv/SvJ wild-type ($\delta^{+/+}$) and knockout ($\delta^{-/-}$) adult mice (Mihalek et al. 1999), of either sex, were anesthetized with 50 mg/kg pentobarbital and decapitated. Brains were blocked, removed, and immediately transferred to ice-cold, oxygen-equilibrated (95% O_2-5% CO_2), sucrose-cutting solution (in mM: 234 sucrose, 11 glucose, 24 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$ and 0.5 $CaCl_2$). After being submerged for 2 min, brains were glued to a petri dish filled with the same cutting solution as above and sectioned into 200-μm-thick horizontal slices on a Vibratome (TPI, St. Louis, MO). Slices were bisected and trimmed to only thalamus and parts of adjacent striatum before being placed into an incubator containing artificial cerebral spinal fluid (ACSF, in mM: 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 10 glucose, 26 $NaHCO_3$) continuously bubbled with 95% O_2-5% CO_2 at 35°C at least 1 h prior to recording.

Electrophysiology

In the recording chamber, slices were gently weighted down under nylon netting and superfused with a constant flow of ACSF (2 ml/min) equilibrated with 95% O_2-5% CO_2. Glass electrodes (KG-33 borosilicate glass, ID 1.0 mm, OD 1.5 mm; Garner Glass, Claremont, CA) were pulled in multiple stages to a resistance of 2.5–3.3 MΩ using a Flaming-Brown micropipette puller (model P-87, Sutter Instruments, Novato, CA) and filled with a cesium chloride solution [in mM: 135 CsCl, 5 lidocaine-*N*-ethyl bromide (QX-314, Sigma-RBI, St. Louis, MO), 2 $MgCl_2$, 10 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA, Sigma-RBI), pH = 7.3]. Voltage-clamp recordings were made from identified neurons within the boundaries of either the ventral posterior medial or the ventral posterior lateral thalamic nucleus (referred to as the ventral basal complex, VB) through the use of fixed-stage upright microscope (Axioskop, Carl Zeiss MicroImaging, Thornwood, NY) equipped with an insulated 63× objective, Nomarski optics, and an infrared-sensitive video camera (Cohu, San Diego, CA). All recordings were obtained with either a List-Medical EPC-7 (Darmstadt, Germany) or an Axopatch 200B (Axon Instruments, Foster City, CA) patch-clamp amplifier. Evoked inhibitory postsynaptic potentials (eIPSCs) were elicited via a bipolar tungsten electrode placed into the RTN. Once a minimal response was obtained, the test stimulation consisted of a brief, single pulse, at the same intensity but with a 1.5× duration. An inter-trial interval of at least 5 s was used. All recordings were made at room temperate (23°C) with a holding potential of −60 mV. Using the conditions described above, all $GABA_A$-mediated currents appeared as inward events in our recordings.

Pharmacology

$GABA_A$ receptor-mediated currents were pharmacologically isolated by bath application of the ionotropic excitatory amino acid receptor antagonists: 6,7-dinitro-quinoxaline-2,3-dione (DNQX, 20 μM final, Sigma-RBI) and 2-amino-5-phosphonopentanoic acid (AP-5, 100 μM final, Sigma-RBI) in physiological saline. $GABA_B$ receptors were blocked internally using Cs^+ (135 mM) and QX-314 (5 mM) in the pipette solution. PS (Sigma-RBI) was dissolved in distilled H_2O at a 1:1,000 stock concentration and used at a final concentration of 10 μM. 3-Alpha,5-alpha-tetrahydrodeoxycorticosterone (THDOC, Sigma-RBI) was dissolved in DMSO at a 1:4,000 stock concentration and used at a final concentration of 250 nM.

Data collection and analysis

Continuous records of spontaneous inhibitory postsynaptic potentials (sIPCSs) were filtered at 1 kHz and collected with Axotape v.2 or PClamp v.8 (Axon Instruments). sIPSC events were sorted and separated with the customized software Detector v.5.14 (J. R. Huguenard) to obtain raw amplitude, duration, and frequency measurements. Single sIPSC events that decayed completely to baseline before the start of another event (type 1) were hand-sorted, averaged, and curve-fitted with PClamp v.8 (Axon Instruments) to generate decay time constants and amplitudes. eIPSCs were collected with Clampex v.5.5 or PClamp v.8 and curve-fitted with Clampfit v.8. Data were further analyzed with Origin v.6.1 (MicroCal Software, Northhampton, MA) and statistical significance was measured using a Student's *t*-test.

RESULTS

Spontaneous IPSC comparisons between $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons

Continuous recordings of sIPSCs were made from VB neurons ($\delta^{+/+}$: $n = 32$, $\delta^{-/-}$: $n = 33$) at room temperature (Fig. 1, summary data provided in Table 1). We regularly observed

a difference in baseline physiological noise between $\delta^{+/+}$ (Fig. 1*A1*) and $\delta^{-/-}$ (Fig. 1*A2*) recordings. $\delta^{-/-}$ VB neuron showed a 40% lower baseline noise compared with $\delta^{+/+}$ VB neurons ($\delta^{+/+}$: 4.8 ± 0.3 pA; $\delta^{-/-}$: 3.0 ± 0.3 pA, $P \ll 0.001$, Fig. 1*C*). By contrast there were with no differences in sIPSC rise time, frequency, or duration (Fig. 1*C*). sIPSC decay was quantified by fitting ensemble-averaged type 1 events with a single or more commonly ($\delta^{+/+}$: 93%; $\delta^{-/-}$: 81%, $P > 0.05$) double-exponential decay function. No significant differences were observed between $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons (Fig. 1*B*) in either mean sIPSC amplitude ($\delta^{+/+}$: −32.3 ± 3.0 pA; $\delta^{-/-}$: −31.5 ± 3.3 pA, $P = 0.85$, Fig. 1*C*) or decay rate [weighted decay time constant ($\tau_{d,w}$), $\delta^{+/+}$: 9.2 ± 0.5 ms; $\delta^{-/-}$: 8.6 ± 0.4 ms, $P = 0.39$, Fig. 1*C*]. For the neurons in which double exponential fits were obtained, there was a modest, but significant, decrease in the percentage of total sIPSC amplitude represented by the slow component in $\delta^{-/-}$ VB neurons ($\delta^{+/+}$: 29.3 ± 4.1%; $\delta^{-/-}$: 23.0 ± 3.3%, $P < 0.05$). This slight change in amplitude of the slow component was however not associated with a significant decrease in overall sIPSP duration, as measured by $\tau_{d,w}$ (see Table 1).

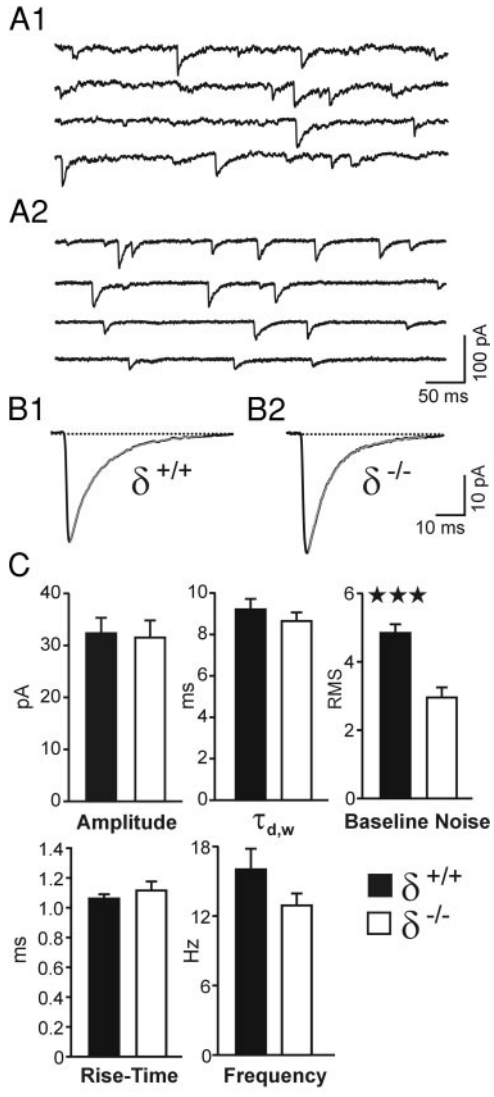

FIG. 1. Spontaneous inhibitory postsynaptic potentials (sIPSC) in ventral basal complex (VB) neurons of wild-type ($\delta^{+/+}$) and knockout animals ($\delta^{-/-}$). Representative traces from individual (*A1*) $\delta^{+/+}$ and (*A2*) $\delta^{-/-}$ VB neurons. *B1*, *B2*: corresponding average sIPSCs for the 2 neurons shown in (*A*). Average sIPSC waveforms contain only events that have fully decayed before another event begins (type 1 events). The mean sIPSC duration from a single neuron (measured by the weighted decay time constant, $\tau_{d,w}$) was obtained from an exponential fit of the average sIPSC waveform (overlaid gray line, *B*). *C*: black bars in the summary histograms refer to $\delta^{+/+}$ control animals, while white bars indicate $\delta^{-/-}$ knockout animals. All error bars show ± SE. For this and all subsequent figures: $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.

TABLE 1. *sIPSC and eIPSC characteristics for $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons*

	$\delta^{+/+}$	$\delta^{-/-}$
Spontaneous IPSCs		
Amplitude$_1$ (fast component), pA	19.9 ± 2.1	25.7 ± 3.4
Amplitude$_2$ (slow component), pA	10.2 ± 1.3	7.9 ± 1.4
Decay time constant$_1$ (fast component), ms	5.3 ± 0.4	5.5 ± 0.3
Decay time constant$_2$ (slow component), ms	18.5 ± 2.2	20.6 ± 1.8
Frequency, Hz	16.0 ± 1.8	12.9 ± 1.0
Weighted time constant ($\tau_{d,w}$), ms	9.2 ± 0.5	8.6 ± 0.4
Rise-time, ms	1.1 ± 0.03	1.1 ± 0.06
Baseline noise, RMS	4.8 ± 0.3 pA	3.0 ± 0.3**
Evoked IPSCs		
Amplitude$_1$ (fast component), pA	−886.7 ± 132.4	−807.4 ± 176.3
Amplitude$_2$ (slow component), pA	−219.4 ± 40.8	−237.8 ± 71.5
Decay time constant$_1$ (fast component), ms	11.8 ± 1.6	9.8 ± 0.7
Decay time constant$_2$ (fast component), ms	55.0 ± 5.5	39.9 ± 3.2*
Weighted time constant ($\tau_{d,w}$), ms	20.1 ± 2.5	16.9 ± 1.7

Values are means ± SE. $\delta^{+/+}$, wild-type; $\delta^{-/-}$, knockout; VB, ventral basal complex; sIPSC, spontaneous inhibitory post-synaptic potentials; eIPSC, evoked inhibitory post-synaptic potentials; $\tau_{d,w}$, weighted decay time constant; RMS, root-mean-square. $*P < 0.05$, $**P < 0.005$.

Evoked IPSC comparisons between $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons

To investigate the population of receptors that might be distal to the synapse, we evoked IPSCs by stimulating GABAergic fibers in RTN (Fig. 2*A*, summary data provided in Table 1). As with previous studies attempting to assess extrasynaptic $GABA_B$ receptors (Kim et al. 1997; Scanziani 2000), a synchronously evoked response was used to trigger sufficient GABA release and spillover to activate potentially extrasynaptic δ-containing $GABA_A$ receptors (Roepstorff and Lambert 1994). Robust, monosynaptic eIPSCs were elicited in both $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons ($\delta^{+/+}$: $n = 15$, $\delta^{-/-}$: $n = 19$). Average eIPSC peak amplitude was similar in both groups ($\delta^{+/+}$: −1106.2 ± 164.9 pA; $\delta^{-/-}$: −1045.3 ± 226.9 pA, $P = 0.84$). Double exponential fits of averaged $\delta^{+/+}$ and $\delta^{-/-}$ eIPSCs revealed a trend toward faster eIPSCs from $\delta^{-/-}$ VB neuron recordings compared with $\delta^{+/+}$ controls (Table 1), but this trend was not significant for the eIPSC decay constant of the fast component (τ_1). However, the slower component (τ_2) was significantly faster in $\delta^{-/-}$ VB neurons ($\delta^{+/+}$: 55.0 ± 5.5 ms; $\delta^{-/-}$: 39.9 ± 3.2 ms, $P < 0.05$).

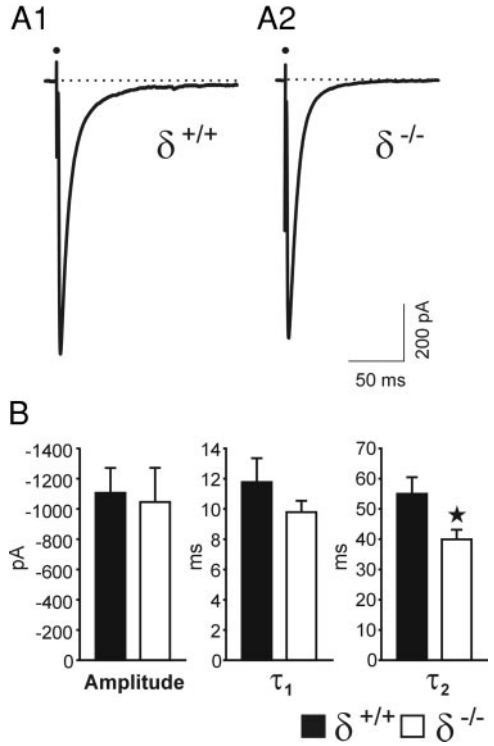

FIG. 2. Evoked inhibitory postsynaptic potentials (eIPSCs) in VB neurons of $\delta^{+/+}$ and $\delta^{-/-}$ animals. Representative average traces from individual (*A1*) $\delta^{+/+}$ and (*A2*) $\delta^{-/-}$ VB neurons. Dots (●) in this figure, and all subsequent figures containing eIPSCs, indicate the time of RTN stimulation. Average traces from each $\delta^{+/+}$ and $\delta^{-/-}$ VB neuron were fit to a double exponential curve to obtain decay kinetics for both the fast (τ_2) and the slow (τ_1) component. *B*: black bars in the summary histograms refer to $\delta^{+/+}$ control animals, while white bars indicate $\delta^{-/-}$ knockout animals. All error bars show SE.

Pregnenolone sulfate modulation of $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons

Having shown a subtle difference in both sIPSCs and eIPSCs, we next investigated modulation by neurosteroids in $\delta^{-/-}$ VB neurons. PS is a known negative allosteric modulator of $GABA_A$ receptors lacking the δ subunit (Zhu et al. 1996). In wild-type neurons ($n = 12$), neither amplitude nor decay of averaged eIPSC waveforms were affected by 10 μM PS (Fig. 3, summary data provided in Table 2). In contrast, PS caused a significant transformation of eIPSCs in $\delta^{-/-}$ VB neurons (Fig. 4, $n = 9$). Both τ_1 ($\delta^{-/-}$: control: 9.7 ± 0.9 ms, PS: 13.1 ± 1.2 ms, $P < 0.01$) and τ_2 ($\delta^{-/-}$: control: 47.9 ± 11.2 ms, PS: 79.4 ± 17.3 ms, $P < 0.005$) of $\delta^{-/-}$ average eIPSCs were substantially increased after 10 μM PS. On average, 10 μM PS applications produced a 30% decrease in eIPSC amplitude of $\delta^{-/-}$ VB neurons (Fig. 4B, $\delta^{-/-}$: control: −1771.3 ± 490.9 pA, PS: −1142.6 ± 272.6 pA, $P < 0.05$). These effects were not accompanied by an alteration in sIPSC frequency ($\delta^{-/-}$: control: 13.2 ± 6.4 Hz, PS: 15.2 ± 7.6 Hz, $P = 0.38$). No effects of PS on sIPSCs were observed in either $\delta^{+/+}$ or $\delta^{-/-}$ VB neurons (Fig. 5, *A* and *B*).

PS (10 μM) caused no significant changes in either sIPSC amplitude ($\delta^{+/+}$: 97.9 ± 2.7% of control, $n = 7$, $P = 0.33$; $\delta^{-/-}$: 96.5 ± 5.4% of control, $n = 12$, $P = 0.34$) or $\tau_{d,w}$ ($\delta^{+/+}$: 117.0 ± 10.2% of control, $P = 0.22$; $\delta^{-/-}$: 106.0 ± 5.0% of control, $P = 0.26$). The lack of effect of PS on sIPSCs in $\delta^{-/-}$ VB neurons is contrasted with the significant effects on eIPSCs in the same cells (Fig. 6) and 10 μM PS also had no effect on baseline noise for either group ($\delta^{+/+}$, control: 4.5 ± 0.5 pA vs. PS: 4.6 ± 0.5 pA, $P = 0.57$; $\delta^{-/-}$, control: 2.5 ± 0.4 pA vs. PS: 2.3 ± 0.4 pA, $P = 0.35$).

THDOC modulation of $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons

Unlike PS, THDOC, a neurosteroid shown to potentiate GABA responses, had similar effects in the two animals types tested. In both $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons, 250 nM THDOC almost doubled the $\tau_{d,w}$ of average eIPSC waveforms (Fig. 7*B2*, $\delta^{+/+}$: 180.9 + 8.4% of control, $n = 3$, $P < 0.05$; $\delta^{-/-}$: 211.8 + 35.2% of control, $n = 4$, $P < 0.05$), while having no effect on eIPSC amplitude (Fig. 7*B1*, $\delta^{+/+}$: 87.3 + 1.9% of control, $P = 0.07$; $\delta^{-/-}$: 93.8 + 9.0% of control, $P = 0.22$). In several $\delta^{+/+}$ VB neurons, the lack of PS effect on eIPSCs could be observed preceding the positive modulation of eIPSC duration by THDOC (Fig. 8). Although the increase in eIPSC duration due to THDOC appears to be mirrored in sIPSCs (Fig. 8*A2*, Fig. 5C), we were unable to show significance due to the high variability of the effect. We observed no change in baseline noise for THDOC applications in either group ($\delta^{+/+}$, control: 4.7 ± 0.7 pA vs. THDOC: 4.8 ± 0.5 pA, $n = 5$, $P = 0.52$; $\delta^{-/-}$, control: 2.7 ± 0.2 pA vs. THDOC: 2.9 ± 0.4 pA, $n = 5$, $P = 0.62$).

DISCUSSION

In this study we have demonstrated subtle changes in the phasic, GABAergic inhibition onto VB neurons, along with an

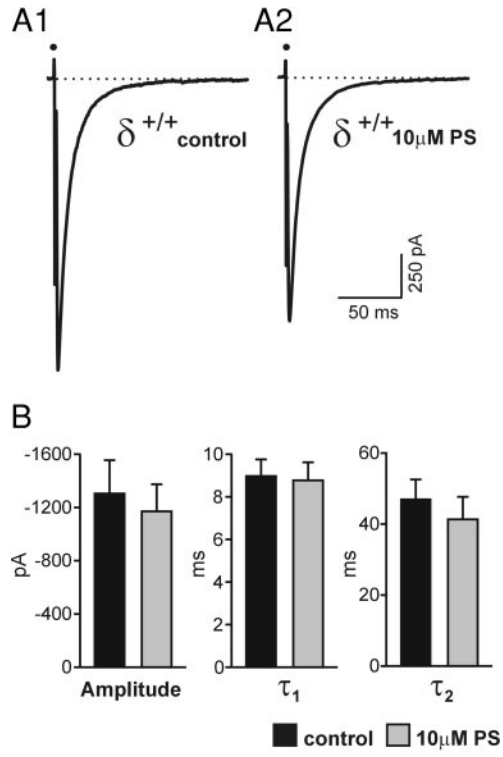

FIG. 3. 10 μM pregnenolone sulfate (PS) has no effect on VB eIPSCs in $\delta^{+/+}$ animals. Average traces from a representative $\delta^{+/+}$ VB neuron (*A1*) before and (*A2*) after 10 μM PS. *B*: black bars in the summary histograms refer to eIPSPs in control conditions, while gray bars indicate those in 10 μM PS. All error bars show SE.

TABLE 2. *10 μM PS modulation of eIPSCs in $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons*

	Control	10 μM PS	% Change from Control
		$\delta^{+/+}$ *eIPSCs*	
Total amplitude, pA	−1300 ± 250	−1170 ± 200	−10%
Decay time constant$_1$ (fast component), ms	9.0 ± 0.8	8.8 ± 0.8	−2%
Decay time constant$_2$ (slow component), ms	46.9 ± 5.6	41.3 ± 6.4	−12%
Weighted time constant ($\tau_{d,w}$), ms	16.7 ± 2.2	16.0 ± 1.8	−4%
		$\delta^{-/-}$ *eIPSCs*	
Total amplitude, pA	−1770 ± 490	−1140 ± 270	−35%*
Decay time constant$_1$ (fast component), ms	9.8 ± 0.9	13.1 ± 1.2	+34%**
Decay time constant$_2$ (slow component), ms	47.9 ± 11.2	79.4 ± 17.3	+66%***
Weighted time constant ($\tau_{d,w}$), ms	15.7 ± 1.7	20.0 ± 2.3	+27%***

Values are means ± SE. PS, pregnenolone sulfate. For other abbreviations, see Table 1. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

increased sensitivity to PS, in mice lacking the δ subunit. Although sIPSCs of $\delta^{-/-}$ VB recordings showed no significant difference in total amplitude, duration, or frequency compared with $\delta^{+/+}$ controls, we did observe a minor, but significant, decrease in the relative amplitude of the slow decay component of sIPSCs. This change in the late decay of sIPSCs was paralleled by a change in eIPSCs of $\delta^{-/-}$ VB recordings with a significantly shortened duration of the slow decay component relative to controls. $\delta^{-/-}$ VB recordings also exhibited a substantial decrease in background physiological noise when compared with $\delta^{+/+}$ recordings. While synaptic $\delta^{-/-}$ GABAergic inhibition was left relatively intact, neurosteroid modulation was partially affected. PS significantly increased duration and decreased amplitude of eIPSCs recorded from $\delta^{-/-}$ VB neurons but had no effect on $\delta^{+/+}$ eIPSCs. In contrast, another neurosteroid, THDOC, produced similar changes in the eIPSCs and sIPSCs of $\delta^{+/+}$ and $\delta^{-/-}$ mice. These differences between $\delta^{+/+}$ and $\delta^{-/-}$ mice, and a role for the δ subunit in the neurosteroidal modulation of native neurons, are discussed in the following text.

sIPSC and eIPSC of $\delta^{-/-}$ VB neurons are intact

The loss of the δ subunit results in significant anatomical reorganization of $GABA_A$Rs in VB neurons. In thalamus, where δ subunits are co-assembled with α4 subunits (Sur et al.

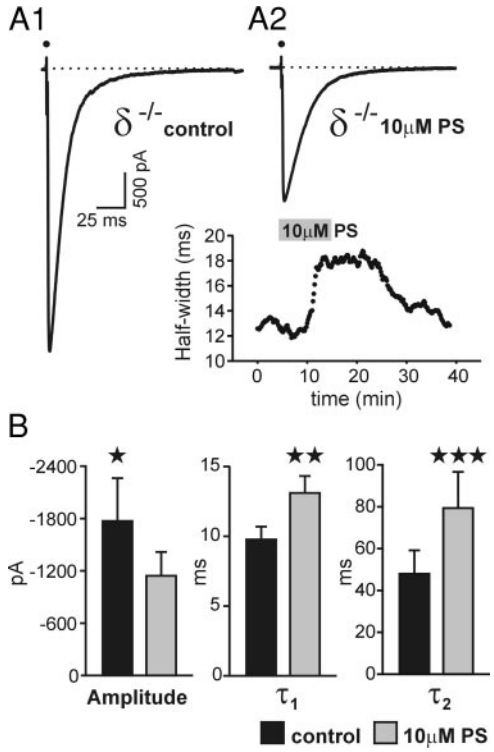

FIG. 4. Loss of the δ subunit confers a PS susceptibility on eIPSCs of $\delta^{-/-}$ VB neurons. Average eIPSC traces from a representative $\delta^{-/-}$ VB neuron (*A1*) before and (*A2*) after 10 μM PS. Inset shows the time course of the eIPSC half-width change due to the 10 μM PS application (gray bar) on the VB neuron from (*A*). *B*: black bars in the summary histograms refer to eIPSCs in control conditions, while gray bars indicate those in 10 μM PS. All error bars show SE.

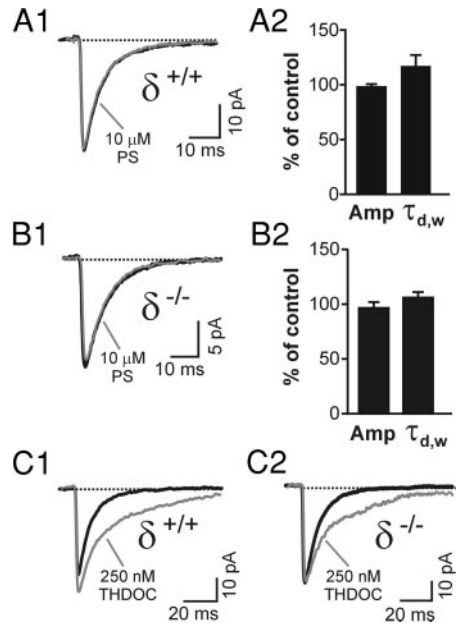

FIG. 5. Effects of PS and THDOC on sIPSCs from either $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons. Average sIPSC waveforms for representative (*A1*) $\delta^{+/+}$ and (*B1*) $\delta^{-/-}$ VB neurons in control conditions (black trace) and in 10 μM PS (overlaid gray trace). *A2*, *B2*: neither group showed a significant percentage change from control sIPSC amplitude or duration in 10 μM PS. 250 nM 3-alpha,5-alpha-tetrahydrodeoxycorticosterone (THDOC) increased sIPSC duration in both (*C1*) $\delta^{+/+}$ and (*C2*) $\delta^{-/-}$ VB neurons. All error bars show SE.

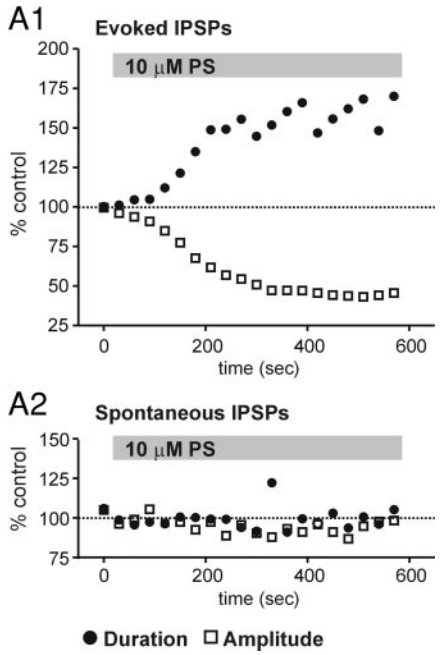

FIG. 6. Time course of PS effects on sIPSCs and eIPSCs simultaneously recorded from a $\delta^{-/-}$ VB neuron. *A1*: eIPSC and *A2*: sIPSC amplitudes (□) and durations (●) were averaged together in bins of 30 s and normalized to control values, over a 10-min application of 10 μM PS (gray bars). eIPSC duration was measured as a weighted decay time constant ($\tau_{d,w}$), while sIPSC duration was obtained from event half-widths. No significant changes in sIPSC rise time were observed for the duration of this experiment, demonstrating stability in recording conditions.

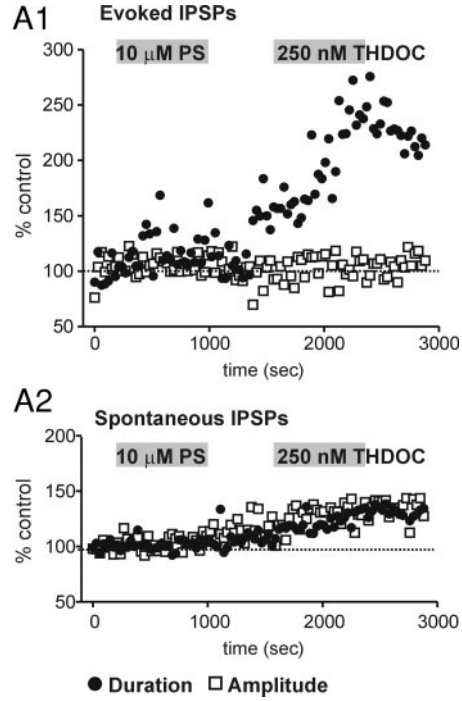

FIG. 8. Time course of PS and THDOC effects on sIPSCs and eIPSCs simultaneously recorded from a $\delta^{+/+}$ VB neuron. *A1*: eIPSC and *A2*: sIPSC amplitudes (□) and durations (●) were averaged together in bins of 30 s and normalized to control values, over a 10-min application of 10 μM PS (first gray bar) followed by 10-min application of 250 nM THDOC (second gray bar). eIPSC duration was measured as a weighted decay time constant ($\tau_{d,w}$), while sIPSC duration was obtained from event half-widths.

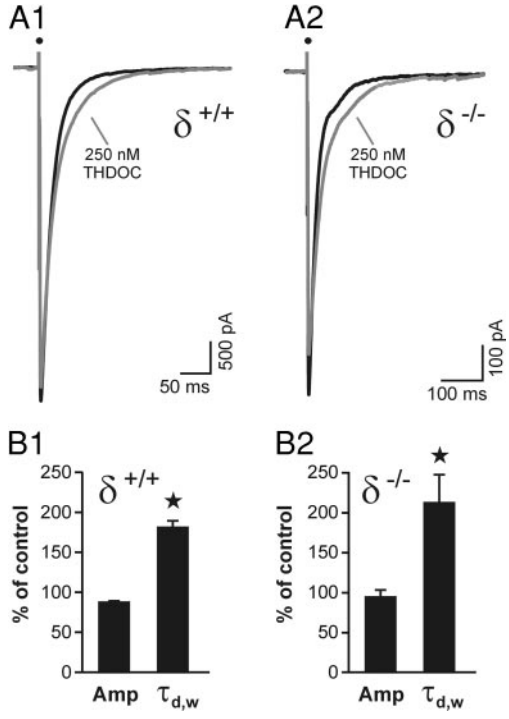

FIG. 7. THDOC has a similar effect on eIPSCs from $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons. Average eIPSC traces from a representative (*A1*) $\delta^{-/-}$ and (*A1*) $\delta^{+/+}$ VB neuron before (black traces) and after (overlaid gray traces) 250 nM THDOC. Percentage change from control eIPSC amplitude and duration are shown for both (*A1*) $\delta^{-/-}$ and (*A1*) $\delta^{+/+}$ VB neurons. Duration was measured as a weighted decay time constant ($\tau_{d,w}$). All error bars show SE.

1999), the absence of δ-containing $GABA_A$Rs is accompanied by a significant downregulation of α4 protein (Peng et al. 2002). This decrease in $GABA_A$Rs is partially offset by the increased presence of $\alpha4\gamma2$-containing $GABA_A$Rs in $\delta^{-/-}$ forebrain regions, as supported by an upregulation of the γ2 subunit and higher levels of α4 protein immunoprecipitated by γ2 antibody (Korpi et al. 2002; Peng et al. 2002). The lack of substantial differences between synaptic $GABA_A$ currents of $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons suggests that δ subunits do not contribute to synaptic GABA receptors, as previously reported in cerebellar granule cells (Nusser et al. 1998). However, if δ-containing $GABA_A$Rs are functionally active at $\delta^{+/+}$ RTN → VB inhibitory synapses, compensation by the γ2 subunit may be a sufficient rescue for the δ knockout. The modest amplitude and duration changes we observed in the slow decay components of $\delta^{-/-}$ sIPSCs and eIPSCs, respectively, could be attributed to the biophysical differences in desensitization rates and channel opening frequencies previously reported for recombinant $GABA_A$Rs containing the δ or γ2 subunits (Saxena and Macdonald 1994).

Although δ subunits co-assemble with different α subunits in thalamus (α4) and cerebellum (α6) (Nusser et al. 1998; Sur et al. 1999), both recombinant constructs demonstrate the high agonist affinity (Adkins et al. 2001; Saxena and Macdonald 1996) ideal for detecting extrasynaptic signals consisting of low levels of GABA (Attwell et al. 1993). If GABA spillover (Brickley et al. 1999) does occur at RTN → VB inhibitory synapses, the contribution of extrasynaptic δ-containing $GABA_A$Rs would lead to slow-rising, low-amplitude, and

long-lasting sIPSCs and eIPSCs similar to those observed in cerebellar granule cell recordings (Rossi and Hamann 1998). The removal of this slow IPSC component from $\delta^{-/-}$ recordings, through either a complete loss or a subunit reorganization of extrasynaptic $GABA_A$Rs, may also account for the changes we observed in the slow decay component of mean $\delta^{-/-}$ sIPSC and eIPSC waveforms (Roepstorff and Lambert 1994). Furthermore, the significant reduction in background noise we observed in $\delta^{-/-}$ recordings appears to be qualitatively similar to the decrease in noise from $\alpha6^{-/-}$ cerebellar granule cells (Brickley et al. 2001) that lose all δ-containing $GABA_A$Rs, predominantly from extrasynaptic areas (Nusser et al. 1998, 1999). Although the net loss of $GABA_A$Rs in $\delta^{-/-}$ VB neurons is significantly less than the 50% decrease observed in $\alpha6^{-/-}$ cerebellar granule cells (Nusser et al. 1999), the compensatory increase of γ2-containing $GABA_A$Rs in $\delta^{-/-}$ thalamus could also contribute to lower background noise because the receptors would have a reduction in overall GABA affinity (Korpi et al. 2002), and they would be less sensitive to resting levels of GABA. These compensatory responses among $GABA_A$Rs subunits may have also been responsible for the alterations in neurosteroid sensitivities we observed in $\delta^{-/-}$ VB neurons.

Neurosteroid modulation changes in $\delta^{-/-}$ VB neurons

In contrast to the attenuated neurosteroid sensitivity initially reported for $\delta^{-/-}$ mice using sleep time assays (Mihalek et al. 1999), here we have reported an elevated PS sensitivity in $\delta^{-/-}$ VB neurons. PS, a sulfated neurosteroid present in the brain (Wang et al. 1997), is known for its inhibitory actions on $GABA_A$Rs (Majewska et al. 1988) at an EC_{50} close to 10 μM (Park-Chung et al. 1999; Shen et al. 2000). Believed to reduce $GABA_A$R channel-opening frequency (Akk et al. 2001; Mienville and Vicini 1989) and increase the rate of desensitization (Shen et al. 2000), PS applications in native neurons can result in reduced peak amplitude and accelerated decay of $GABA_A$-mediated responses (Shen et al. 2000). In our experiments using PS we observed a similar reduction in eIPSC amplitude, accompanied by a previously undescribed *increase* in eIPSC duration (Fig. 4). The increased effectiveness of 10 μM PS at reducing $\delta^{-/-}$ VB eIPSC amplitude is consistent with previous studies reporting a higher PS sensitivity in recombinant $GABA_A$Rs after substituting the δ subunit with the γ2 subunit (Zhu et al. 1996). However, we cannot completely account for the simultaneous lengthening of $\delta^{-/-}$ VB eIPSC duration by PS.

Dual modulation by a sulfated neurosteroid has been previous reported with 11-ketopregnenolone sulfate and recombinant $GABA_A$Rs (Park-Chung et al. 1999). PS may also act at distinct positive and negative modulatory sites at $\delta^{-/-}$ VB $GABA_A$Rs, resulting in the eIPSCs modulation described above. Another explanation for the dual modulation may be a potential inhibitory PS effect on neurotransmitter release (Teschmacher et al. 1997; ffrench-Mullen et al. 1994) at the RTN → VB synapse, coupled with a small potentiation of postsynaptic $\delta^{-/-}$ VB $GABA_A$Rs. An alteration in presynaptic RTN neurons, which do not express the δ subunit, may indicate a change in $\delta^{-/-}$ mice *independent* of $GABA_A$Rs. If these changes occurred in a PS-sensitive component of RTN neurons affecting release, such as NMDA receptors (Wu et al. 1991) or high-voltage activated Ca^{2+} channels (ffrench-Mullen et al. 1994), they could have substantial consequences for synaptic VB currents evoked with RTN stimulation.

While a PS effect on neurotransmitter release at $\delta^{-/-}$ RTN → VB synapses is a distinct possibility, it was not supported by a resultant change in $\delta^{-/-}$ VB sIPSCs. The lack of effect on $\delta^{-/-}$ VB sIPSCs suggests the absence of the δ subunit cannot be the sole determining factor of PS modulation in $GABA_A$Rs. Therefore eIPSCs and sIPSCs of $\delta^{-/-}$ VB neurons may originate from different $GABA_A$R populations based either on subunit composition or on interactions within the native neuronal environment. Given the complex response of $\delta^{-/-}$ VB neurons to PS, more work is needed to determine the factors involved.

Another neurosteroid, THDOC, showed no difference between effects in $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons. THDOC is structurally distinct from PS (Park-Chung et al. 1999) and has been shown to prolong $GABA_A$-mediated currents (Cooper et al. 1999; Zhu and Vicini 1997). As predicted from previous work in native neurons (Cooper et al. 1999), 250 nM THDOC increased sIPSC and eIPSC duration in both $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons without significantly affecting amplitude (Figs. 7 and 8). The potentiation due to 100–10,000 nM THDOC is substantially increased in recombinant $GABA_A$Rs after the γ2 subunit is substituted for the δ subunit (Zhu et al. 1996). Interestingly, we observed far less of a difference in THDOC eIPSC potentiation between $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons (Fig. 7), a finding which agrees with recent results obtained with recombinant receptors (Brown et al. 2002; Wohlfarth et al. 2002). A more extensive screening with THDOC would be required to establish whether differences are statistically significant. It may be possible that a THDOC concentration lower than 250 nM is required to observe any δ subunit specific differences in neurons. sIPSCs from cerebellar granule cells show a significantly reduced potentiation due to 100 nM THDOC in $\delta^{-/-}$ mice compared with wild-type controls (Vicini et al. 2002).

The similar potentiation of both eIPSCs and sIPSCs in $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons by THDOC contrasts with the effects of PS, which depended on both the presence of δ and the response type (eIPSCs vs. sIPSC). These contrasting sensitivities to THDOC and PS support the hypothesis that positive and negative neurosteroid $GABA_A$ modulators act at different binding sites (Park-Chung et al. 1999; Shen et al. 2000).

Future directions

The biophysical and pharmacological differences between $\delta^{+/+}$ and $\delta^{-/-}$ VB neurons demonstrated in the present experiments including the following: *1*) the reduction in background noise; *2*) minor changes restricted to the slow decay component of synaptic currents; and *3*) altered pharmacological sensitivities in eIPSCs but not sIPSCs with one neurosteroid; suggest δ-containing $GABA_A$Rs are distant from RTN → VB synaptic areas. Unlike $\alpha6^{-/-}$ mice where there is a clear and complete loss of δ-containing extrasynaptic $GABA_A$Rs in cerebellar granule cells, $\delta^{-/-}$ mice undergo a mixture of $GABA_A$Rs loss and compensation with the γ2 subunit, which challenges the interpretation of $\delta^{-/-}$ physiological relevance. A pharmacological block, or inducible genetic manipulation, which selectively renders membrane-inserted, δ-containing $GABA_A$Rs in-

active, might provide a more reliable method for determining the role of the δ subunit in thalamus.

The authors thank C. Ferguson for expert technical assistance and V. Sohal for reading an initial draft of this manuscript.

This work was supported by National Institutes of Health Grants GM-52035, AA-10422, and NS-34774.

REFERENCES

Adkins CE, Pillai GV, Kerby J, Bonnert TP, Haldon C, McKernan RM, Gonzalez JE, Oades K, Whiting PJ, and Simpson PB. $\alpha4\beta3\delta$ $GABA_A$ receptors characterized by fluorescence resonance energy transfer-derived measurements of membrane potential. *J Biol Chem* 276: 38934–38939, 2001.

Akk G, Bracamontes J, and Steinbach JH. Pregnenolone sulfate block of $GABA_A$ receptors: mechanism and involvement of a residue in the M2 region of the α subunit. *J Physiol* 532: 673–684, 2001.

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

Brickley SG, Cull-Candy SG, and Farrant M. Single-channel properties of synaptic and extrasynaptic $GABA_A$ receptors suggest differential targeting of receptor subtypes. *J Neurosci* 19: 2960–2973, 1999.

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

Brown N, Kerby J, Bonnert TP, Whiting PJ, and Wafford KA. Pharmacological characterization of a novel cell line expressing human $\alpha4\beta3\delta$ $GABA_A$ receptors. *Br J Pharmacol* 136: 965–974, 2002.

Browne SH, Kang J, Akk G, Chiang LW, Schulman H, Huguenard JR, and Prince DA. Kinetic and pharmacological properties of $GABA_A$ receptors in single thalamic neurons and $GABA_A$ subunit expression. *J Neurophysiol* 86: 2312–2322, 2001.

Cooper EJ, Johnston GA, and Edwards FA. Effects of a naturally occurring neurosteroid on $GABA_A$ IPSCs during development in rat hippocampal or cerebellar slices. *J. Physiol* 521(Pt. 2): 437–449, 1999.

Farrar SJ, Whiting PJ, Bonnert TP, and McKernan RM. Stoichiometry of a ligand-gated ion channel determined by fluorescence energy transfer. *J Biol Chem* 274: 10100–10104, 1999.

ffrench-Mullen JM, Danks P, and Spence KT. Neurosteroids modulate calcium currents in hippocampal CA1 neurons via a pertussis toxin-sensitive G-protein-coupled mechanism. *J. Neurosci* 14: 1963–1977, 1994.

Huguenard JR and Prince DA. Intrathalamic rhythmicity studied in vitro: nominal T-current modulation causes robust antioscillatory effects. *J Neurosci* 14: 5485–5502, 1994.

Huntsman MM, Porcello DM, Homanics GE, DeLorey TM, and Huguenard JR. Reciprocal inhibitory connections and network synchrony in the mammalian thalamus. *Science* 283: 541–543, 1999.

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, and Wisden W. Ligand-gated ion channel subunit partnerships: $GABA_A$ receptor $\alpha6$ subunit gene inactivation inhibits delta subunit expression. *J Neurosci* 17: 1350–1362, 1997.

Kim U, Sanchez-Vives MV, and McCormick DA. Functional dynamics of GABAergic inhibition in the thalamus. *Science* 278: 130–134, 1997.

Korpi ER, Mihalek RM, Sinkkonen ST, Hauer B, Hevers W, Homanics GE, Sieghart W, and Luddens H. Altered receptor subtypes in the forebrain of $GABA_A$ receptor delta subunit-deficient mice: recruitment of $\gamma2$ subunits. *Neuroscience* 109: 733–743, 2002.

Lambert JJ, Belelli D, Hill-Venning C, and Peters JA. Neurosteroids and $GABA_A$ receptor function. *Trends Pharmacol Sci* 16: 295–303, 1995.

Laurie DJ, Wisden W, and Seeburg PH. The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci* 12: 4151–4172, 1992.

Li Z, DeLorey TM, Olsen RW, Mihalek RM, Homanics GE, and Spigelman I. Altered IPSPs in dentate granule cells of mice lacking the delta subunit of the $GABA_A$ receptor. *Soc Neurosci Abstr* 24: 1833, 1998.

Macdonald RL and Olsen RW. $GABA_A$ receptor channels. *Annu Rev Neurosci* 17: 569–602, 1994.

Majewska MD, Mienville JM, and Vicini S. Neurosteroid pregnenolone sulfate antagonizes electrophysiological responses to GABA in neurons. *Neurosci Lett* 90: 279–284, 1988.

McCormick DA and Bal T. Sleep and arousal: thalamocortical mechanisms. *Annu Rev Neurosci* 20: 185–215, 1997.

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

Mensah-Nyagan AG, Do-Rego JL, Beaujean D, Luu-The V, Pelletier G, and Vaudry H. Neurosteroids: expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system. *Pharmacol Rev* 51: 63–81, 1999.

Mienville JM and Vicini S. Pregnenolone sulfate antagonizes $GABA_A$ receptor-mediated currents via a reduction of channel opening frequency. *Brain Res* 489: 190–194, 1989.

Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, and Homanics GE. Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. *Proc Natl Acad Sci USA* 96: 12905–12910, 1999.

Nusser Z, Ahmad Z, Tretter V, Fuchs K, Wisden W, Sieghart W, and Somogyi P. Alterations in the expression of $GABA_A$ receptor subunits in cerebellar granule cells after the disruption of the $\alpha6$ subunit gene. *Eur J Neurosci* 11: 1685–1697, 1999.

Nusser Z and Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol* 87: 2624–2628, 2002.

Nusser Z, Sieghart W, and Somogyi P. Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci* 18: 1693–1703, 1998.

Park-Chung M, Malayev A, Purdy RH, Gibbs TT, and Farb DH. Sulfated and unsulfated steroids modulate gamma-aminobutyric acid A receptor function through distinct sites. *Brain Res* 830: 72–87, 1999.

Peng Z, Hauer B, Mihalek RM, Homanics GE, Sieghart W, Olsen RW, and Houser CR. $GABA_A$ receptor changes in delta subunit-deficient mice: altered expression of $\alpha4$ and $\gamma2$ subunits in the forebrain. *J Comp Neurol* 446: 179–197, 2002.

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

Roepstorff A and Lambert JD. Factors contributing to the decay of the stimulus-evoked IPSC in rat hippocampal CA1 neurons. *J Neurophysiol* 72: 2911–2926, 1994.

Rossi DJ and Hamann M. Spillover-mediated transmission at inhibitory synapses promoted by high affinity alpha6 subunit $GABA_A$ receptors and glomerular geometry. *Neuron* 20: 783–795, 1998.

Saxena NC and Macdonald RL. Assembly of $GABA_A$ receptor subunits: role of the delta subunit. *J Neurosci* 14: 7077–7086, 1994.

Saxena NC and Macdonald RL. Properties of putative cerebellar gamma-aminobutyric acid A receptor isoforms. *Mol Pharmacol* 49: 567–579, 1996.

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

Shen W, Mennerick S, Covey DF, and Zorumski CF. Pregnenolone sulfate modulates inhibitory synaptic transmission by enhancing $GABA_A$ receptor desensitization. *J Neurosci* 20: 3571–3579, 2000.

Steriade M, McCormick DA, and Sejnowski TJ. Thalamocortical oscillations in the sleeping and aroused brain. *Science* 262: 679–685, 1993.

Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, and McKernan RM. Preferential coassembly of $\alpha4$ and δ subunits of the γ-aminobutyric acid A receptor in rat thalamus. *Mol Pharmacol* 56: 110–115, 1999.

Teschemacher A, Kasparov S, Kravitz EA, and Rahamimoff R. Presynaptic action of the neurosteroid pregnenolone sulfate on inhibitory transmitter release in cultured hippocampal neurons. *Brain Res* 772: 226–232, 1997.

Vallee M, Mayo W, Darnaudery M, Corpechot C, Young J, Koehl M, Le Moal M, Baulieu EE, Robel P, and Simon H. Neurosteroids: deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus. *Proc Natl Acad Sci USA* 94: 14865–14870, 1997.

Vicini S, Losi G, and Homanics GE. $GABA_A$ receptor delta subunit deletion prevents neurosteroid modulation of inhibitory synaptic currents in cerebellar neurons. *Neuropharmacology* 43: 646–650, 2002.

Wang MD, Wahlstrom G, and Backstrom T. The regional brain distribution of the neurosteroids pregnenolone and pregnenolone sulfate following intravenous infusion. *J Steroid Biochem Mol Biol* 62: 299–306, 1997.

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

Wohlfarth KM, Bianchi MT, and Macdonald RL. Enhanced neurosteroid potentiation of ternary $GABA_A$ receptors containing the delta subunit. *J Neurosci* 22: 1541–1549, 2002.

Wu FS, Gibbs TT, and Farb DH. Pregnenolone sulfate: a positive allosteric modulator at the *N*-methyl-D-aspartate receptor. *Mol Pharmacol* 40: 333–336, 1991.

Zhu WJ and Vicini S. Neurosteroid prolongs $GABA_A$ channel deactivation by altering kinetics of desensitized states. *J Neurosci* 17: 4022–4031, 1997.

Zhu WJ, Wang JF, Krueger KE, and Vicini S. Delta subunit inhibits neurosteroid modulation of $GABA_A$ receptors. *J Neurosci* 16: 6648–6656, 1996.