THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 288, NO. 35, pp. 25053–25065, August 30, 2013
© 2013 by The American Society for Biochemistry and Molecular Biology, Inc. Published in the U.S.A.

Homeostatic Competition between Phasic and Tonic Inhibition*

Received for publication, June 4, 2013 Published, JBC Papers in Press, July 9, 2013, DOI 10.1074/jbc.M113.491464

Xia Wu[‡1], **Lanting Huang**[‡§1], **Zheng Wu**[‡], **Ce Zhang**[‡], **Dongyun Jiang**[‡], **Yuting Bai**[‡], **Yun Wang**[§], **and Gong Chen**[‡2]

From the [‡]*Department of Biology, The Huck Institutes of Life Sciences, The Pennsylvania State University, University Park, Pennsylvania 16802 and the* [§]*Institutes of Brain Science and State Key Laboratory for Medical Neurobiology, Fudan University, Shanghai 200032, China*

Background: Synaptic and extrasynaptic $GABA_A$ receptors mediate phasic and tonic inhibition, respectively.
Results: Overexpression of extrasynaptic $GABA_A$ receptors decreases synaptic GABAergic transmission.
Conclusion: Synaptic and extrasynaptic $GABA_A$ receptors may have intrinsic competition in single individual neurons.
Significance: Phasic and tonic inhibition interacts to maintain homeostasis of total inhibition.

The $GABA_A$ receptors are the major inhibitory receptors in the brain and are localized at both synaptic and extrasynaptic membranes. Synaptic $GABA_A$ receptors mediate phasic inhibition, whereas extrasynaptic $GABA_A$ receptors mediate tonic inhibition. Both phasic and tonic inhibitions regulate neuronal activity, but whether they regulate each other is not very clear. Here, we investigated the functional interaction between synaptic and extrasynaptic $GABA_A$ receptors through various molecular manipulations. Overexpression of extrasynaptic α6β3δ-$GABA_A$ receptors in mouse hippocampal pyramidal neurons significantly increased tonic currents. Surprisingly, the increase of tonic inhibition was accompanied by a dramatic reduction of the phasic inhibition, suggesting a possible homeostatic regulation of the total inhibition. Overexpressing the α6 subunit alone induced an up-regulation of δ subunit expression and suppressed phasic inhibition similar to overexpressing the α6β3δ subunits. Interestingly, blocking all $GABA_A$ receptors after overexpressing α6β3δ receptors could not restore the synaptic GABAergic transmission, suggesting that receptor activation is not required for the homeostatic interplay. Furthermore, insertion of a gephyrin-binding-site (GBS) into the α6 and δ subunits recruited $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors to postsynaptic sites but failed to rescue synaptic GABAergic transmission. Thus, it is not the positional effect of extrasynaptic α6β3δ receptors that causes the down-regulation of phasic inhibition. Overexpressing α5β3γ2 subunits similarly reduced synaptic GABAergic transmission. We propose a working model that both synaptic and extrasynaptic $GABA_A$ receptors may compete for limited receptor slots on the plasma membrane to maintain a homeostatic range of the total inhibition.

$GABA_A$ receptor-mediated inhibition is critical to maintain normal brain functions. $GABA_A$ receptors are localized at both synaptic and extrasynaptic membranes and mediate phasic and tonic inhibition, respectively (1). Synaptic $GABA_A$ receptors typically contain the γ2 subunit, whereas δ subunit-containing $GABA_A$ receptors often localize at extrasynaptic sites. Our recent work revealed that, in addition to γ2 and δ subunits, α subunits also play a direct role in targeting $GABA_A$ receptors to synaptic and extrasynaptic sites (2). Dysfunction of both synaptic and extrasynaptic $GABA_A$ receptors has been associated with neurological disorders such as anxiety, depression, and epilepsy (1, 3–5). Because synaptic and extrasynaptic $GABA_A$ receptors have distinct spatial localization and mediate distinct temporal inhibition, it is unclear how synaptic and extrasynaptic $GABA_A$ receptors may interact with each other to regulate the total inhibition.

Synaptic $GABA_A$ receptors are mainly composed of $\alpha_{1-3}\beta_{2-3}\gamma_2$ subunits. The γ2 and α1–3 subunits together target the $GABA_A$ receptors to postsynaptic sites, juxtaposing to presynaptic GABAergic terminals to respond rapidly to vesicular GABA release (2, 6, 7). Gene deletion of either the γ2 or α1/α2/α3 subunit will result in a significant decrease of $GABA_A$ receptor puncta in neuronal subcellular regions (8–12). Loss of postsynaptic $GABA_A$ receptor clusters often leads to a reduction of presynaptic GABAergic terminals, possibly because of a retrograde feedback (13–16). The extrasynaptic $GABA_A$ receptors are mainly composed of α4βδ, α6βδ, and α5βγ2 subunits (17, 18). The α5-$GABA_A$ receptors are mainly localized at extrasynaptic membranes but are also found in synaptic sites (19, 20). The δ-containing $GABA_A$ receptors are mostly found in the dentate and cerebellar granule cells, as well as in the thalamic neurons (21–24). In developing cerebellum, the level of tonic inhibition increases significantly in the first postnatal month, but synaptic GABAergic transmission decreases substantially in the same time (25, 26). It has also been reported that in transgenic mice with ectopic expression of the α6 subunit, tonic conductance increases, but synaptic GABA responses decrease simultaneously (27). These studies suggest a possible interplay between synaptic and extrasynaptic $GABA_A$ receptors, but the molecular mechanism underlying such interaction is currently unknown.

In this study, we employed a series of molecular manipulations of extrasynaptic $GABA_A$ receptors to investigate their

* This work was supported, in whole or in part, by National Institute of Health Grants MH083911 and MH092740 (to G. C.). This work was also supported by Natural Science Foundation of China Grant 31129003 (to G. C. and Y. W.).

[1] Both authors contributed equally to this work.

[2] To whom correspondence should be addressed: Department of Biology, Huck Institutes of Life Sciences, The Pennsylvania State University, University Park, PA 16802. Tel.: 814-865-2488; E-mail: gongchen@psu.edu.

functional effects on fast GABAergic synaptic transmission. Overexpression of $\alpha6\beta3\delta$ receptors in cultured hippocampal pyrimidal neurons resulted in a dramatic decrease of synaptic GABAergic events as well as a significant reduction of presynaptic GABAergic terminals. Surprisingly, such a functional outcome did not require the activation of extrasynaptic GABA$_A$ receptors. We further demonstrated that the down-regulation of phasic inhibition is not due to the positional effect of $\alpha6\beta3\delta$ receptors because inserting a gephyrin-binding site (GBS)[3] into the $\alpha6$ and δ subunits targeted $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors to postsynaptic sites but could not rescue phasic inhibition. Interestingly, overexpressing $\alpha5\beta3\gamma2$-receptors also decreased synaptic GABAergic transmission. On the basis of these results, we propose that synaptic and extrasynaptic GABA$_A$ receptors will compete for limited receptor slots on the cell surface so that the total GABA inhibition can be regulated in a homeostatic manner.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Hippocampal and cortical neurons were prepared form newborn C57BL/6 mouse pups (of either sex) as described previously (2). Briefly, the hippocampi or cerebral cortex tissue was cut into 1-mm^3 cubes and digested with 0.05% trypsin-EDTA containing 50 units/ml DNase I. Dissociated single neurons were then plated onto a monolayer of astrocytes on poly-D-lysine-coated coverglass at a density of 1×10^4 cells/cm^2. The neuronal culture medium contained 500 ml minimum Eagle's medium (Invitrogen), 5% fetal bovine serum (HyClone, Logan, UT), 10 ml B-27 supplement (Invitrogen), 100 mg $NaHCO_3$, 0.5 mM L-glutamine, 25 unit/ml penicillin/streptomycin, and 4 μM AraC to suppress the excessive proliferation of astrocytes. Cells were maintained in a 5% CO_2 incubator at 37 °C for 2–3 weeks.

Neurons were transfected at 2–4 days *in vitro* using our modified high-efficiency calcium phosphate transfection protocol (28). About 1 μg of each plasmid was used for the $\alpha6$, $\alpha6\beta3\delta$, or $\alpha2\beta3\gamma2$ transfection. The $\alpha6_{GBS,}$ $\beta3$, and δ_{GBS} subunits were cotransfected at a ratio of 2:1:0.5 μg. About 0.4 μg each of GFP or mCherry was coexpressed to identify the transfected neurons. Neurons at 10–14 days *in vitro* were used for electrophysiological and immunocytochemical analyses.

HEK 293T cells were maintained in high-glucose DMEM (Invitrogen) and supplemented with 10% fetal bovine serum and 25 units/ml penicillin/streptomycin. HEK cells were transfected with PEI (molecular weight 25,000, Polysciences, Inc.). HEK cells were split 1 day ahead and allowed to reach 90% confluence by the time of transfection. For each well in a 24-well plate, 1 μg of total DNA was diluted in 50 μl of Opti-MEM (Invitrogen), mixed with 4 μl of PEI (1 μg/μl), sat for 5 min, and added drop-by-drop to the culture well containing 500 μl of media. After 5 h of incubation, the cells were rinsed with fresh medium to remove the excess reagents. The transfected HEK cells were used for electrophysiological study 1–3 days after transfection.

Constructs—The CMV-based expression vectors for the GABA$_A$ receptor $\alpha2$, $\beta3$, and $^{myc}\gamma2$ subunits were described previously (6). The $\alpha6$ and δ constructs in the pCMVneo backbone were gifts from Dr. R. L. MacDonald (Vanderbilt University Medical Center). The $\alpha5$ subunit was a gift from Dr. Matthias Kneussel (University of Hamburg Medical School). The $\alpha6_{GBS}$ and δ_{GBS} were constructed in our laboratory as described recently (2). The GBS originated from the glycine receptor β subunit (29) was inserted into the intracellular loop of the δ and $\alpha6$ subunits after amino acid 341 and amino acid 340, respectively.

Drugs and Treatment—GABA and tetrodotoxin (TTX) were purchased from Sigma. Bicuculline methobromide (BIC), 6,7-dinitroquinoxaline-2,3-dione (DNQX), and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride (THIP) were purchased from Tocris (Ellisville, MO). DNQX and THIP were dissolved in dimethyl sulfoxide to make concentrated stock solutions. Other drugs were dissolved in deionized water. All drugs were freshly diluted to working concentrations in the bath solution on the day of the experiment. The final dimethyl sulfoxide concentration was lower than 0.1%. For chronic BIC treatment, BIC (20 μM) was added to the neuronal culture medium 2–4 days before electrophysiological recordings.

Immunocytochemistry—Neurons were fixed with 4% paraformaldehyde diluted in PBS for 12 min and permeabilized with 0.01% Triton X-100 in the blocking solution (PBS with 3% normal goat serum and 2% normal donkey serum) for 30 min. All steps were conducted at room temperature unless specified otherwise. Antibodies were diluted in the same blocking solution. The δ/δ_{GBS} subunit on the neuronal surface was labeled before permeabilization with rabbit-anti-δ-Nterm antibody (1:500, PhosphoSolutions, Aurora, CO). Other primary antibodies were applied after Triton X-100 and incubated overnight at 4 °C. The following primary antibodies were used: mouse-anti-GAD65 (1:100, Developmental Studies Hybridoma Bank), mouse-anti-gephyrin mAb7a (1:500, Synaptic Systems, Göttingen, Germany), and chicken-anti-GFP (1:2000, Abcam, Cambridge, MA). The following secondary antibodies were used as appropriate to detect primary antibodies: Alexa Fluor 488 goat-anti-chicken (1:500), Alexa Fluor 488 donkey-anti-rabbit (1:300), and Alexa Fluor 647 goat-anti-mouse (1:300, Molecular Probes, Eugene, OR).

Images for GAD immunoreactivity were captured on an inverted epifluorescent microscope (Nikon Eclipse TE2000S) equipped with a digital charge-coupled device (CCD) camera (Hamamatsu C4742-95). To quantify GABAergic synaptic terminals, images of 60 neurons were taken from each group. For each transfected neuron, two 50-μm segments of major dendrites were randomly chosen on the basis of the GFP image, and GAD-positive puncta were counted in each segment. The total number of GAD puncta along the 100-μm dendrite was compared among neurons transfected with GFP, $\alpha6\beta3\delta$, or $\alpha6$ subunit alone.

To investigate the surface expression of δ or δ_{GBS}, confocal images were acquired from an Olympus FV1000 microscope. Quantification of the δ immunosignal was conducted using

[3] The abbreviations used are: GBS, gephyrin-binding site; TTX, tetrodotoxin; BIC, bicuculline methobromide; DNQX, 6,7-dinitroquinoxaline-2,3-dione; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride; mIPSC, miniature inhibitory postsynaptic current; mEPSC, miniature excitatory postsynaptic current; ANOVA, analysis of variance; GAD, glutamic acid decarboxylase; P7, postnatal day 7.

ImageJ software. The neuronal soma together with two major dendrites of each transfected neuron was traced manually under the mCherry image. The mean fluorescence intensity of δ immunostaining in the region of interest was analyzed. The background signal in the nearby area without any neurons was subtracted during analysis.

Electrophysiology—Whole cell recordings were performed in voltage clamp mode using a Multiclamp 700A amplifier (Molecular Devices) as described before (30). A coverglass with cultured cells was placed in the recording chamber with continuous perfusion of the bath Tyrode's solution (128 mM NaCl, 30 mM glucose, 25 mM HEPES, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ (pH 7.3), osmolarity ~320 mM). Fire-polished borosilicate glass pipettes with a resistance of 3–6 MΩ were used for recording. The internal pipette solution contained 135 mM KCl, 10 mM HEPES, 2 mM EGTA, 10 mM Tris-phosphocreatine, 4 mM MgATP, and 0.5 mM Na_2GTP (pH 7.3), osmolarity ~300 mM). Recordings were conducted at room temperature. The membrane potential was held at −70 mV. Data were acquired using pClamp 9 software (Molecular Devices), sampled at 5 kHz, and filtered at 1 kHz.

Drugs were applied through a six-channel manifold connected to a valve control system (VC-6, Warner Instruments, Hamden, CT) to achieve a fast switch between different solutions. Whole cell GABA/THIP-induced currents were recorded in the presence of TTX (0.5 μM) and DNQX (10 μM) to block voltage-gated sodium channels and AMPA receptors. TTX and DNQX were also added to the bath solution to record miniature inhibitory postsynaptic currents (mIPSCs), whereas mEPSCs were recorded in the presence of TTX and bicuculline (GABA$_A$ receptor antagonist, 20 μM). To examine tonic currents in neurons, GABA (2 μM) was bath-applied, and, after the GABA-induced current reached a plateau (normally after 1 min), 40 μM bicuculline was added to block all GABA$_A$ receptors. The shift in holding current after bicuculline application was quantified as the tonic current. DNQX (10 μM) was applied throughout the entire recording period to block AMPA receptor activation.

To analyze the kinetics of mIPSCs, single events recorded from each cell were identified using MiniAnalysis software (Synaptosoft). The events were aligned at the 50% rise point, and the average traces were used for curve fitting. The decay was fitted using two-exponential equations, and the weighted time constant ($\tau_{weighted}$) was calculated using the following equation: $\tau_{weighted} = (\tau 1 \times A1 + \tau 2 \times A2)/(A1 + A2)$. Pooled data were presented as mean ± S.E., and unpaired Student's *t* test was used for two-group comparison, whereas one-way ANOVA was used for multiple group comparison.

RESULTS

Overexpression of Extrasynaptic GABA$_A$ Receptors Down-regulates Synaptic GABAergic Transmission—To investigate the interrelationship between synaptic and extrasynaptic GABA$_A$ receptors, we overexpressed the α6, β3, and δ subunits in cultured hippocampal neurons to increase tonic inhibition and examined the effect on synaptic GABAergic transmission. Pyramidal neurons with a large apical dendrite and multiple basal dendrites were selected for recordings. THIP (also called Gaboxadol) is a relatively selective agonist for δ-containing GABA$_A$ receptors at low concentrations (31–33). As expected, neurons transfected with α6β3δ subunits (together with GFP) showed significantly larger THIP (5 μM) currents, whereas α2β3γ2 overexpression did not increase THIP currents compared with non-transfected neurons (Fig. 1, *A–C* and *G*; non-TF, 17 ± 5 pA, $n = 22$; α6β3δ, 192 ± 32 pA, $n = 11$; α2β3γ2, 6 ± 3 pA, $n = 10$; $p < 0.001$, one-way ANOVA). Interestingly, overexpression of either α6β3δ or α2β3γ2 receptors did not change the whole-cell GABA (100 μM) currents (Fig. 1, *D–F* and *H*; non-TF, 3861 ± 461 pA, $n = 20$; α6β3δ, 4228 ± 453 pA, $n = 12$; α2β3γ2, 4997 ± 445 pA, $n = 8$; $p > 0.3$, one-way ANOVA). The γ2 subunit was myc-tagged, and immunostaining confirmed that it was expressed after transfection and clustered at postsynaptic sites (2). Thus, overexpression of α6β3δ subunits in hippocampal neurons results in a large tonic current, but the total GABA current remains unchanged. On the other hand, overexpression of α2β3γ2 subunits has no effect on the tonic current nor on the total GABA current, as if the total number of GABA$_A$ receptors on the cell surface had reached the plateau before overexpression.

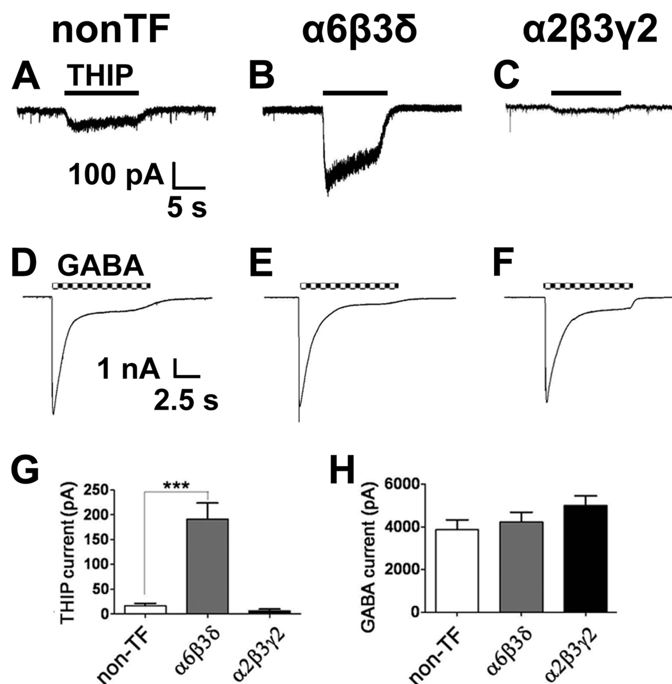

FIGURE 1. **Overexpression of the α6β3δ receptors increased tonic current in cultured hippocampal neurons.** *A–C*, representative traces showing the whole-cell currents induced by 5 μM THIP in non-transfected (*A*), α6β3δ-transfected (B), and α2β3γ2-transfected neurons (*C*). *D–F*, representative traces showing the whole-cell currents induced by 100 μM GABA in non-transfected (*D*), α6β3δ-transfected (*E*), and α2β3γ2-transfected neurons (*F*). *G*, neurons expressing α6β3δ receptors showed significantly larger THIP-induced currents. *Non-TF*, non-transfected. ***, $p < 0.001$. *H*, overexpression of α6β3δ or α2β3γ2 receptors did not change the GABA-induced whole-cell currents.

We next examined whether the increased expression of extrasynaptic GABA$_A$ receptors might affect synaptic GABAergic transmission. mIPSCs were recorded in the presence of TTX (0.5 μM) and DNQX (10 μM) to block action potentials and glutamatergic responses, respectively. The average mIPSC frequency recorded in non-transfected control neu-

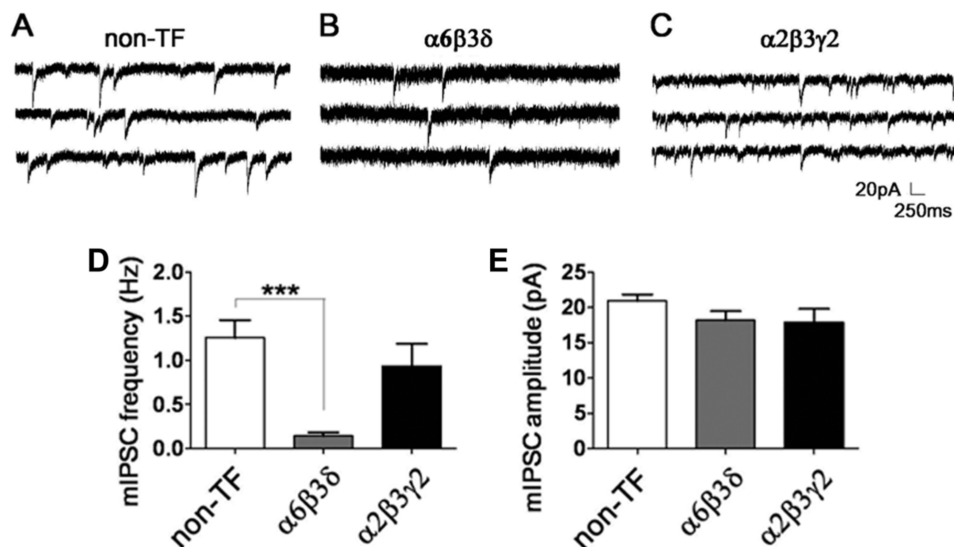

FIGURE 2. **Overexpression of the α6β3δ receptors decreased synaptic GABAergic transmission in cultured hippocampal neurons.** *A–C*, representative traces showing mIPSCs in non-transfected (*A*), α6β3δ-expressing (*B*), and α2β3γ2-expressing neurons (*C*). *D*, the frequency of mIPSCs was reduced significantly after overexpression of α6β3δ receptors, whereas α2β3γ2 overexpression did not change the mIPSC frequency. *Non-TF*, non-transfected. ***, $p < 0.001$. *E*, overexpression of $GABA_A$ receptors did not change the amplitude of mIPSCs.

rons was 1.25 ± 0.20 Hz ($n = 30$, Fig. 2, *A* and *D*). Surprisingly, in neurons overexpressing α6β3δ-receptors, the mIPSC frequency was reduced significantly to 0.14 ± 0.04 Hz (Fig. 2*B*, $n = 9$, $p < 0.001$), a dramatic 9-fold decrease from the control level. We wondered whether such a drastic reduction was caused by transfecting multiple plasmids in neurons. Therefore, we overexpressed α2β3γ2 subunits as a control but found no significant change in the frequency of mIPSCs (Fig. 2, *C* and *D*, 0.94 ± 0.25 Hz, $n = 9$, $p > 0.5$), suggesting that transfection of multiple plasmids did not cause mIPSC reduction. Furthermore, the average mIPSC amplitude was found to be not different among the three groups (Fig. 2*E*, *non-TF*, 20.9 ± 0.9 pA, $n = 30$; α6β3δ, 18.2 ± 1.3 pA, $n = 9$; α2β3γ2, 17.9 ± 1.9 pA, $n = 9$; $p > 0.15$, one-way ANOVA). These experiments revealed that increasing extrasynaptic α6β3δ receptor expression in hippocampal neurons significantly down-regulates synaptic GABAergic transmission.

Overexpression of the α6 Subunit Alone Decreases Synaptic GABAergic Transmission—Our recent studies demonstrated that, in addition to the γ2 and δ subunits, the α subunits also play a direct role in $GABA_A$ receptor targeting (2). Therefore, we tested whether expressing the α6 subunit alone in cultured hippocampal neurons can affect synaptic GABAergic transmission. Similar to α6β3δ cotransfection, we found that transfection with the α6 subunit alone (together with mCherry for identification purposes) significantly decreased the mIPSC frequency (Fig. 3, *A–C*; control, 1.2 ± 0.2 Hz, $n = 19$; α6, 0.27 ± 0.06 Hz, $n = 21$; $p < 0.001$, unpaired Student's *t* test). Despite the remarkable 77% reduction in mIPSC frequency, α6 expression did not change the amplitude of mIPSCs in a significant way (Fig. 3*D*; control, 25.2 ± 2.2 pA, $n = 19$; α6, 21.0 ± 1.0 pA, $n = 19$; $p = 0.09$, unpaired Student's *t* test). We next investigated whether overexpressing the α6 subunit alone resulted in any changes of tonic current. In control neurons (non-transfected or mCherry-transfected), a small tonic GABA current was revealed by the application of the $GABA_A$ receptor antagonist BIC (40 μM) in the continuous presence of GABA (2 μM) and DNQX (10 μM) (Fig. 4, *A* and *B*, 79 ± 20 pA, $n = 13$). In contrast, the α6-transfected neurons displayed much larger tonic GABA currents (Fig. 4, *A* and *B*, 486 ± 86 pA, $n = 8$, $p < 0.001$), a 6-fold increase over the control level. Furthermore, application of THIP (5 μM), a relatively selective agonist for δ-containing $GABA_A$ receptors, also revealed a significantly larger tonic current in α6-transfected neurons than the control (Fig. 4, *C* and *D*; control, 104.8 ± 30.3 pA, $n = 12$; α6, 247.1 ± 38.3 pA, $n = 8$; $p < 0.01$; unpaired Student's *t* test). Therefore, overexpression of the α6 subunit alone increased tonic inhibition and down-regulated synaptic GABAergic transmission at the same time, consistent with previous findings in α6 transgenic mice (27).

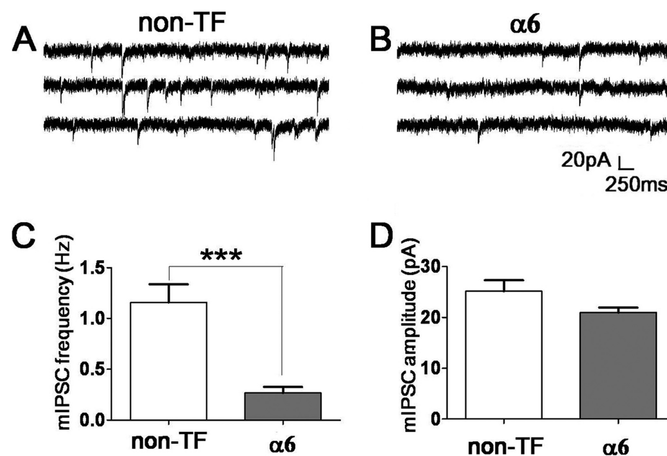

FIGURE 3. **Overexpression of the α6 subunit alone decreased the mIPSC frequency.** *A* and *B*, representative traces showing mIPSCs in non-transfected (*non-TF*) (*A*) and α6-transfected neurons (*B*). *C*, quantitative analysis showed a significant reduction of the mIPSC frequency in α6-transfected neurons. ***, $p < 0.001$. *D*, ectopic expression of the α6 subunit did not change the amplitude of mIPSCs significantly.

Because the α6 subunit can form multiple subtypes of functional receptors, such as α6β, α6βγ2, and α6βδ receptors (22,

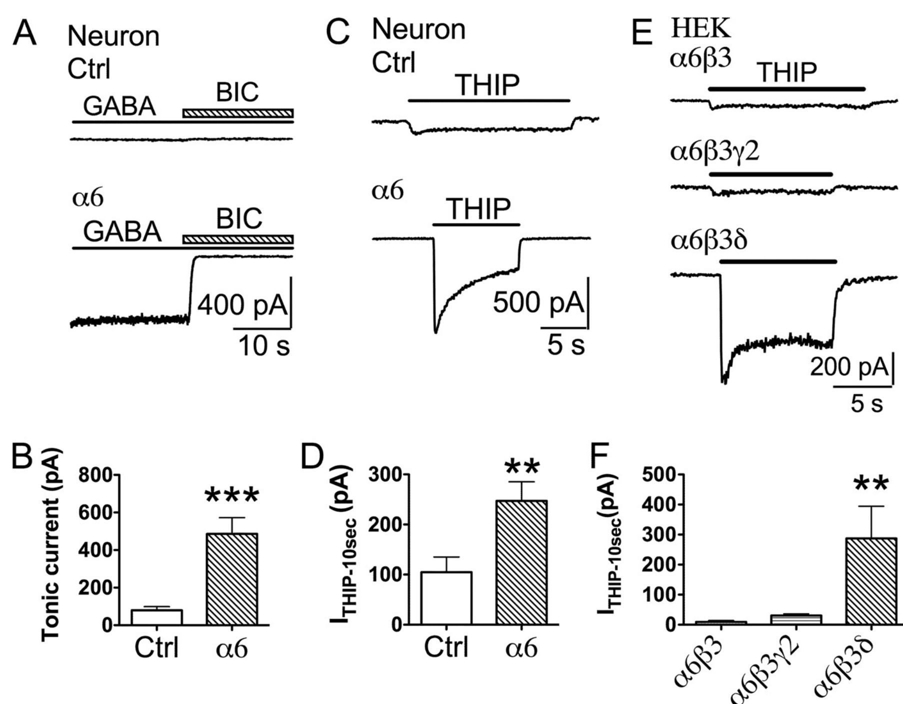

FIGURE 4. **Overexpression of the α6 subunit increased the tonic current in hippocampal pyramidal neurons.** *A*, application of BIC (40 μM) revealed a significantly larger tonic current activated by 2 μM GABA (in the presence of TTX and DNQX) in α6-transfected neurons. *Ctrl*, control. *B*, quantified data illustrating larger tonic currents in α6-transfected neurons. ***, $p < 0.001$, unpaired Student's *t* test. *C* and *D*, representative traces (*C*) and quantified data (*D*) illustrating significantly larger THIP-induced (5 μM) currents in α6-transfected neurons. TTX (0.5 μM) and DNQX (10 μM) were used to block action potentials and glutamate receptors. **, $p < 0.01$. *E*, THIP (5 μM) induced small whole-cell currents in HEK cells expressing α6β3 or α6β3γ2 receptors but activated a large current in HEK cells transfected with α6β3δ receptors. *F*, quantified data illustrating that HEK cells expressing α6β3δ-GABA$_A$ receptors showed a significantly larger whole-cell current induced by 5 μM THIP. **, $p < 0.01$.

34), we wondered what kind of GABA$_A$ receptor subtype was responsible for such a large tonic current in α6-transfected neurons. To answer this question, we overexpressed the α6β3, α6β3γ2, or α6β3δ subunits in HEK293T cells and tested their responses to THIP (5 μM). As shown in Fig. 4*E*, THIP-induced currents in HEK cells transfected with α6β3 or α6β3γ2 subunits were very small, but HEK cells transfected with α6β3δ subunits showed a significantly larger tonic current. Quantitatively, the average sustained current induced by THIP (5 μM) was 9.2 ± 4.8 pA ($n = 6$) for α6β3 receptors and 30.7 ± 5.4 pA ($n = 7$) for α6β3γ2 receptors, respectively (Fig. 4*F*). In comparison, the THIP-induced current in α6β3δ-expressing cells was 287 ± 107 pA (Fig. 4*F*, $n = 5$, $p < 0.01$, one-way ANOVA), a 9-fold increase over that mediated by α6β3γ2 receptors. Because overexpression of α6β3γ2 receptors in HEK cells can only generate a very small THIP current, the large tonic current observed in α6-overexpressing neurons is likely not mediated by α6βγ2 receptors but possibly by THIP-sensitive α6βδ receptors (33). However, most of our recordings were performed in hippocampal pyramidal neurons, which usually have a low level of δ receptor expression. We therefore hypothesized that the δ subunit expression level may be up-regulated in α6-transfected neurons to form α6β3δ receptors.

The α6 Subunit Induces the Expression of the δ Subunit in Pyrimidal Neurons—To test our hypothesis that the δ subunit expression can be induced by overexpressing the α6 subunit in hippocampal pyramidal neurons, we directly examined the expression level of the δ subunit on the cell surface of neurons with or without α6 transfection. Cultured neurons were transfected with mCherry alone or α6 plus mCherry, and the antibodies specific for the δ subunit (PhosphoSolutions) were used to label the neuronal surface expression in the non-permeabilized condition. As expected, the endogenous δ subunit expression level was very low in hippocampal and cortical pyrimidal neurons (Fig. 5, *A* and *B*). Intriguingly, neurons transfected with the α6 subunit alone showed a significantly elevated expression level of the δ subunit on the cell surface of both soma and dendrites (Fig. 5, *C* and *D*). It is noteworthy that the δ immunosignal showed a diffused distribution pattern in α6-transfected neurons, consistent with the pattern of α6β3δ receptors (Fig. 8*B*). Quantitative analysis of the fluorescent intensity showed that the expression level of the δ subunit on the plasma membrane was significantly higher after α6 transfection (Fig. 5*E*, *mCherry*, 12.1 ± 1.0 arbitrary units, $n = 24$; α6, 40.3 ± 3.7 arbitrary units, $n = 24$; $p < 0.001$, unpaired Student's *t* test). Therefore, overexpression of the α6 subunit alone in pyramidal neurons can enhance tonic inhibition through the induction of the δ subunit expression, suggesting that tonic inhibition in pyramidal neurons is much more plastic than we thought before.

Overexpressing Extrasynaptic GABA$_A$ Receptors Reduces GABAergic Synapses—The substantial reduction of the mIPSC frequency after transfection of the α6 or α6β3δ subunits in pyramidal cells prompted us to further examine the changes in GABAergic synapses using an immunostaining method. As shown in Fig. 6*A*, control neurons transfected with GFP alone received a normal level of GABAergic innervation, as indicated by the GAD-positive presynaptic puncta (*red*) apposing the

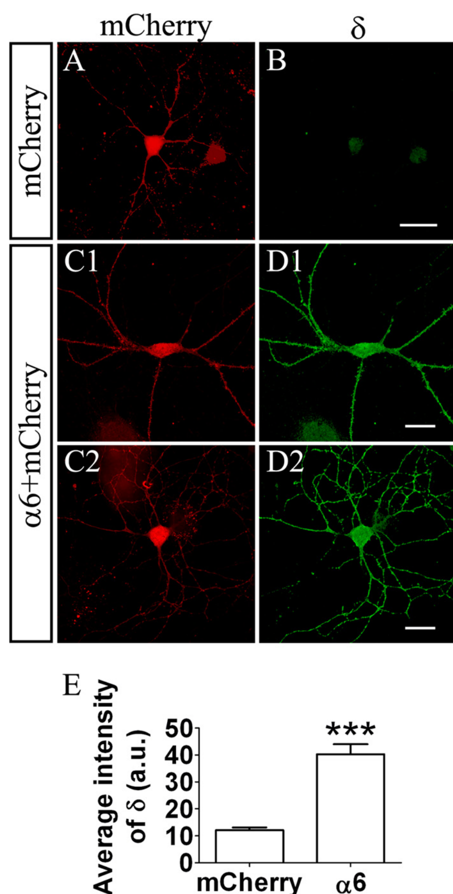

FIGURE 5. **Ectopic expression of α6 subunit-induced δ subunit expression in pyramidal neurons.** *A* and *B*, a control neuron transfected with mCherry (*red*) showed a very low level of δ immunofluorescence signal (*green*). *C* and *D*, two representative neurons transfected with the α6 subunit and mCherry showed increased an δ immunofluorescence signal on the soma and dendrites. *Scale bars* = 20 μm. *E*, quantified fluorescence intensity showing elevated δ expression in α6-transfected neurons ($n = 24$ in both groups). ***, $p < 0.001$, unpaired Student's *t* test. *a.u.*, arbitrary units.

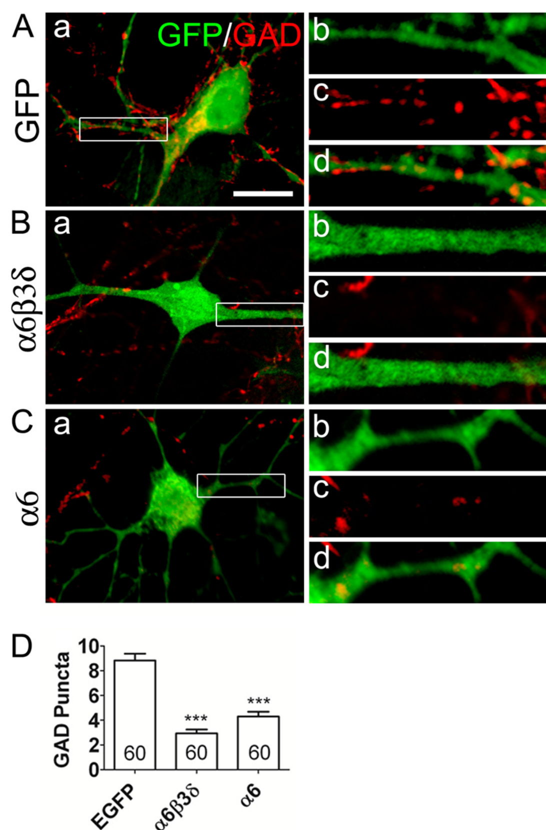

FIGURE 6. **Overexpression of extrasynaptic α6β3δ-$GABA_A$ receptors decreased GABAergic innervation.** *A*, GAD-positive presynaptic puncta (*red*) along the dendrites of a control neuron expressing GFP (*green*). *B*, α6β3δ-transfected neurons showed fewer GAD-positive puncta along the dendrites. *C*, neurons transfected with the α6 subunit also showed fewer GAD puncta. *b*, *c*, and *d* show enlarged views of the areas marked in *a*. *Scale bar* = 10 μm. *D*, quantification of GAD puncta/100 μm dendrites. ***, $p < 0.001$, one-way ANOVA followed by Bonferroni's correction.

dendrites (*green*). However, significantly less GAD puncta were detected on the dendrites of neurons transfected with α6β3δ or α6 subunits (Fig. 6, *B* and *C*). On average, the GABAergic synaptic density on control neurons was 8.8 ± 0.5 puncta/100 μm dendrites (randomly chosen), whereas on α6β3δ- and α6-transfected neurons, the GABAergic density reduced substantially to 2.9 ± 0.3 and 4.3 ± 0.4 puncta/100 μm dendrites, respectively (Fig. 6*D*, $p < 0.001$, one-way ANOVA followed by Bonferroni's correction). No significant difference was found in the GAD puncta density between α6β3δ- and α6-transfected neurons ($p > 0.05$). Therefore, overexpression of extrasynaptic $GABA_A$ receptors resulted in a decrease of presynaptic GABAergic innervation, consistent with the substantial decrease of synaptic GABAergic transmission.

Interaction between Synaptic and Extrasynaptic $GABA_A$ Receptors Is Independent of Receptor Activation—So far we have demonstrated that enhancing tonic inhibition through the overexpression of extrasynaptic $GABA_A$ receptors resulted in a significant decrease of fast synaptic GABAergic transmission. This led to our working hypothesis that, in any given neuron, there is a constant interplay between synaptic and extrasynaptic $GABA_A$ receptors to maintain a normal range of total inhibition. If tonic inhibition is substantially up-regulated, the phasic inhibition will be down-regulated and *vice versa*. Maintaining the total inhibition in each individual neuron may be critical for homeostatic modulation of neural network activity (35, 36). To test our hypothesis, we first investigated whether the activation of synaptic and extrasynaptic $GABA_A$ receptors is required for their interplay. For this purpose, after α6β3δ transfection, neurons were treated with bicuculline (20 μM) for 2–4 days to completely block all $GABA_A$ receptors, including both synaptic and extrasynaptic $GABA_A$ receptors. Global suppression of GABAergic inhibition in non-transfected neurons did result in a significant increase in mIPSC frequency (Fig. 7, *A*, *B*, and *E*; control, 1.04 ± 0.17 Hz, $n = 19$; BIC, 3.24 ± 0.61 Hz, $n = 20$; $p < 0.01$). Similarly, in α6β3δ-transfected neurons, BIC treatment also resulted in an increase in mIPSC frequency (Fig. 7, *C–E*; α6β3δ, 0.08 ± 0.03 Hz, $n = 8$; α6β3δ + BIC, 0.33 ± 0.11 Hz, $n = 16$; $p < 0.05$). The average amplitude of mIPSCs was also increased significantly by BIC treatment in non-transfected neurons (Fig. 7*F*; control, 24.5 ± 1.1 pA, $n = 19$; BIC, 29.9 ± 2.1 pA, $n = 20$; $p < 0.05$). The mIPSC amplitude in α6β3δ-transfected neurons was not changed by chronic BIC treatment (Fig. 7*F*; α6β3δ, 28.1 ± 4.1, $n = 8$; α6β3δ + BIC, 27.6 ± 1.4, $n = 15$; $p > 0.9$). Importantly, after BIC treatment, the mIPSC frequency in α6β3δ-transfected neurons remained

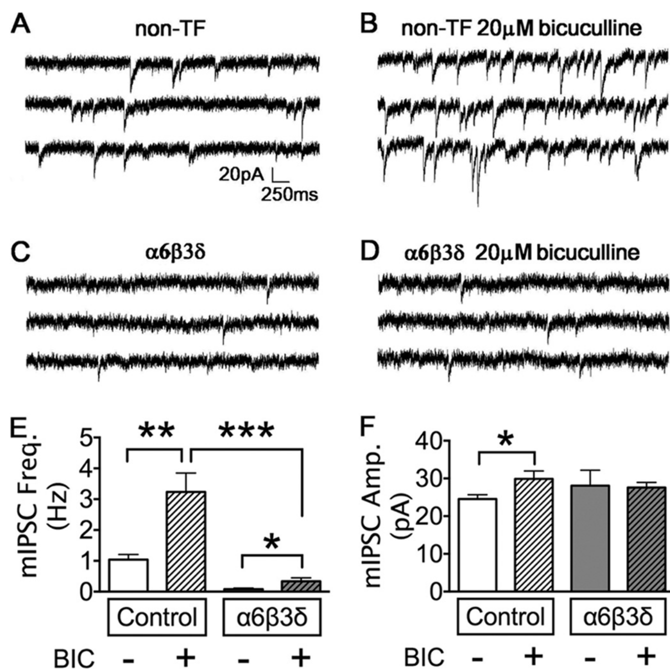

FIGURE 7. **Chronic block of $GABA_A$ receptors increased GABAergic synaptic transmission but did not rescue the $\alpha6\beta3\delta$-induced mIPSC deficit.** *A* and *B*, representative traces showing that chronic BIC treatment increased the mIPSC frequency in non-transfected (*non-TF*) neurons. *C and D*, neurons overexpressing $\alpha6\beta3\delta$ receptors showed a low mIPSC frequency without (*C*) or with bicuculline treatment (*D*). *E*, quantified data showed that chronic BIC treatment significantly increased the mIPSC frequency in control neurons. The mIPSC frequency in $\alpha6\beta3\delta$-transfected neurons remained very low after BIC treatment. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. *F*, chronic BIC treatment increased the mIPSC amplitude in control but not $\alpha6\beta3\delta$-transfected neurons. *, $p < 0.05$.

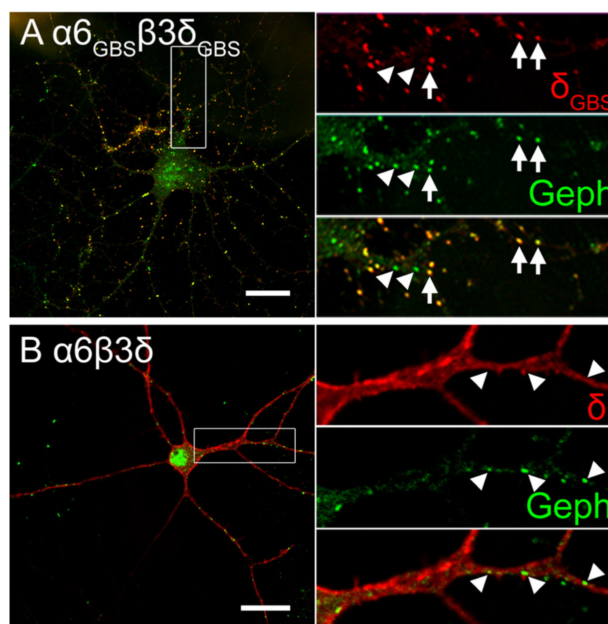

FIGURE 8. **Clustering of $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors at postsynaptic sites.** *A*, the $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors formed clusters and colocalized with gephyrin (*Geph*) at postsynaptic membranes. *Arrows* indicate δ_{GBS} puncta that colocalized with gephyrin puncta. *Arrowheads* indicate some gephyrin puncta devoid of δ_{GBS} receptors. *B*, ectopically expressed $\alpha6\beta3\delta$ receptors were distributed diffusely along the plasma membrane without colocalization with gephyrin puncta. *Arrowheads* indicate gephyrin clusters without corresponding δ-containing receptors. *Scale bar* = 20 μm.

very low compared with non-transfected neurons (Fig. 7, *B*, *D*, and *E*; $p < 0.001$). If increased tonic inhibition is a prerequisite for the down-regulation of phasic inhibition, blocking all $GABA_A$ receptor activity with bicuculline should have abolished the decrease of mIPSC frequency in the $\alpha6\beta3\delta$-transfected neurons. In contrast, our results indicate that overexpression of extrasynaptic $GABA_A$ receptors can down-regulate synaptic GABAergic transmission regardless of whether the overexpressed receptors are activated or not.

Down-regulation of Phasic Inhibition Is Not Caused by the Positional Effect of Extrasynaptic Localization of $\alpha6\beta3\delta$ Receptors—Because $\alpha6\beta3\delta$ receptors are mainly localized on the extrasynaptic membranes, we wondered whether their positional effect could be causing the down-regulation of synaptic GABAergic transmission. We reasoned that if it is due to the positional effect, the defect in synaptic GABAergic transmission may be rescued if the $\alpha6\beta3\delta$ receptors could be targeted to GABAergic postsynaptic sites. Indeed, we have recently engineered chimeric subunits by inserting GBSs into the intracellular loop of the $\alpha6$ ($\alpha6_{GBS}$) and δ (δ_{GBS}) subunits so that they can interact with the scaffold protein gephyrin and localize to synaptic sites (2). Neurons were transfected with $\alpha6\beta3\delta$ or $\alpha6_{GBS}\beta3\delta_{GBS}$ subunits and double-immunostained for gephyrin and surface δ expression. We confirmed that $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors formed clusters in 90% of the transfected neurons (18 of 20 cells). Of the 18 cells, 14 cells showed a high level of colocalization between δ_{GBS} and gephyrin clusters (Fig. 8*A*, *arrows*). Some gephyrin-positive puncta did not colocalize with δ_{GBS} clusters (Fig. 8*A*, *arrowheads*), indicating that the $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors were not recruited to all GABAergic synapses. In contrast, in $\alpha6\beta3\delta$-transfected neurons ($n = 9$), δ immunoreactivity (*red*) was distributed diffusely throughout the soma and dendrites and not colocalized with the gephyrin clusters (Fig. 8*B*, *green*). Thus, $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors are efficiently recruited to GABAergic postsynaptic sites, distinct from the extrasynaptic localization of $\alpha6\beta3\delta$ receptors.

After changing their localization, we next examined the effect of $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors on the fast GABAergic synaptic transmission in pyramidal neurons. Surprisingly, the mIPSC frequency recorded in $\alpha6_{GBS}\beta3\delta_{GBS}$-transfected neurons still showed a significant reduction compared with the control neurons (Fig. 9, *A* and *C*; control, 3.0 ± 0.6 Hz, $n = 12$; $\alpha6_{GBS}\beta3\delta_{GBS}$, 1.0 ± 0.4 Hz, $n = 14$; $p < 0.05$). Unlike the overexpression of $\alpha6\beta3\delta$ receptors, which only decreased mIPSC frequency, the amplitude of mIPSCs in the $\alpha6_{GBS}\beta3\delta_{GBS}$-transfected neurons was also reduced significantly compared with the control cells (Fig. 9, *A* and *D*; control, 28.7 ± 2.8 pA, $n = 12$; $\alpha6_{GBS}\beta3\delta_{GBS}$, 16.8 ± 1.5 pA, $n = 14$; $p < 0.001$), likely because of lower conductance or opening probability of $\alpha6\beta3\delta$-receptors (37, 38). No changes were found in mEPSC frequency (Fig. 9, *B* and *C*; control, 3.0 ± 1.2 Hz, $n = 9$; $\alpha6_{GBS}\beta3\delta_{GBS}$, 5.4 ± 2.1 Hz, $n = 13$; $p > 0.3$) or mEPSC amplitude (*B* and *D*; control, 11.9 ± 1.8 pA, $n = 9$; $\alpha6_{GBS}\beta3\delta_{GBS}$, 13.6 ± 1.6 pA, $n = 13$; $p > 0.4$). These results suggest that although $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors are now localized at synaptic sites, they still down-regulate GABAergic synaptic transmission.

We further analyzed the mIPSCs in $\alpha6_{GBS}\beta3\delta_{GBS}$-transfected neurons. The δ-containing $GABA_A$ receptors are known

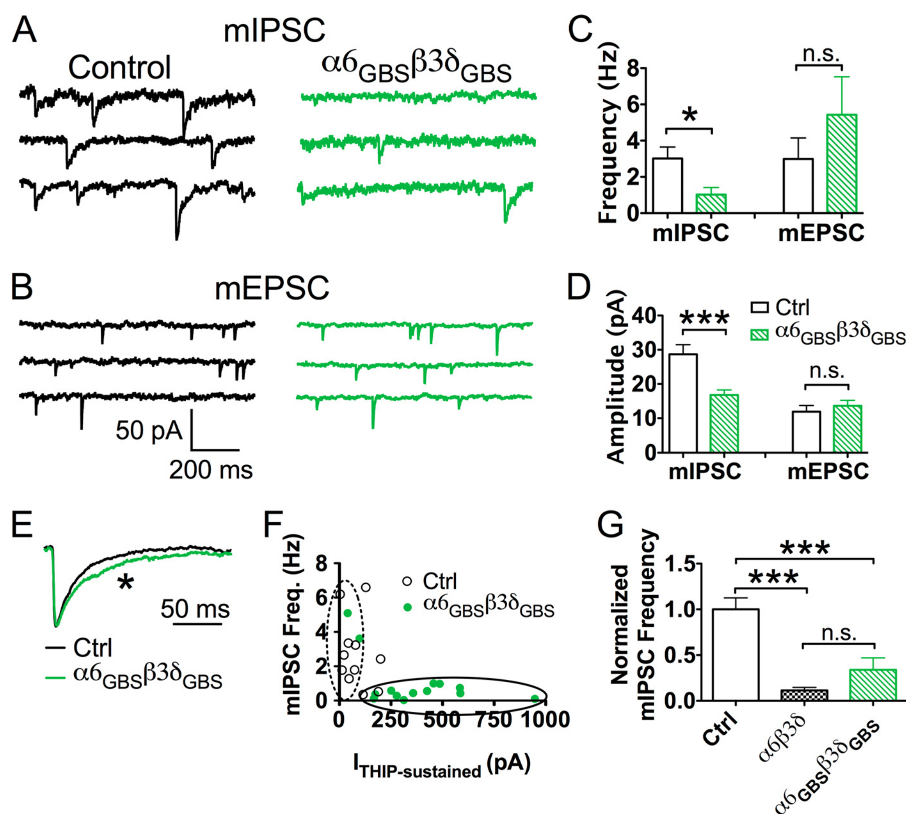

FIGURE 9. **Overexpression of $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors decreased both the frequency and amplitude of mIPSCs.** *A*, representative mIPSC traces in control (*black*) and $\alpha6_{GBS}\beta3\delta_{GBS}$-transfected neurons (*green*). *B*, mEPSC traces in control (*black*) and $\alpha6_{GBS}\beta3\delta_{GBS}$-transfected neurons (*green*). *C*, neurons expressing $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors had a significantly lower frequency of mIPSCs but no change in mEPSCs. *, $p < 0.05$; *n.s.*, not significant. *D*, expression of $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors significantly reduced mIPSC amplitude, but mEPSCs remained unaffected. *Ctrl*, control. ***, $p < 0.001$. *E*, scaled overlay showing a slower decay phase of mIPSCs in $\alpha6_{GBS}\beta3\delta_{GBS}$-expressing neurons. *, $p < 0.05$. *F*, inverse relationship between the mIPSC frequency and whole-cell THIP current. The $\alpha6_{GBS}\beta3\delta_{GBS}$-transfected neurons showed a large THIP (5 μM) current but low mIPSC frequency, whereas control neurons showed a small THIP current but a large mIPSC frequency. *G*, normalized mIPSC frequency showing a similar reduction of mIPSC frequency in neurons transfected with $\alpha6_{GBS}\beta3\delta_{GBS}$ or $\alpha6\beta3\delta$ receptors. ***, $p < 0.001$.

to have slower desensitization compared with many other receptor subtypes, such as $\gamma2$-containing receptors (39, 40). When examining the kinetics of the mIPSC events in the $\alpha6_{GBS}\beta3\delta_{GBS}$-expressing neurons, we found that these events decayed much slower than those in control neurons (Fig. 9*E*; control, $\tau_{weighted} = 37.3 \pm 4.1$ ms, $n = 12$; $\alpha6_{GBS}\beta3\delta_{GBS}$, $\tau_{weighted} = 52.3 \pm 5.3$ ms, $n = 14$; $p < 0.05$; unpaired Student's *t* test). The 10–90% rise time did not differ significantly between control and $\alpha6_{GBS}\beta3\delta_{GBS}$-expressing neurons (control, 2.2 ± 0.2 ms, $n = 12$; $\alpha6_{GBS}\beta3\delta_{GBS}$, 2.8 ± 0.3 ms, $n = 14$; $p > 0.1$, unpaired Student's *t* test). The slower decay in $\alpha6_{GBS}\beta3\delta_{GBS}$-expressing neurons indicated that the $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors were indeed incorporated into postsynaptic receptor clusters and contributed to the mIPSC events. Interestingly, we also observed a negative correlation between the mIPSC frequency and THIP-induced current in $\alpha6_{GBS}\beta3\delta_{GBS}$-transfected neurons (Fig. 9*F*, Pearson's test, $r = -0.6$, $p < 0.01$). 10 of 14 neurons transfected with $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors showed a THIP-induced current greater than 200 pA, and the mIPSC frequency in these 10 neurons were all lower than 1 Hz (Fig. 9*F*, *green dots circled by the solid line*). On the other hand, THIP currents in 7 of 11 control neurons were smaller than 100 pA, and the mIPSC frequency in these neurons was much higher, up to 6.2 Hz (Fig. 9*F*, *white dots circled by the dashed line*). Lastly, we compared the relative reduction in mIPSC frequency caused by $\alpha6\beta3\delta$ or $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors. As shown in Fig. 9*G*, normalized mIPSC frequency showed that overexpressing $\alpha6\beta3\delta$ receptors reduced the mIPSC frequency to 11.4 ± 3.2% of the control level ($n = 10$), whereas the $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors down-regulated the mIPSC frequency to 33.4 ± 13.0% of the control level ($n = 14$). Nevertheless, there was no statistical difference between the relative mIPSC frequency in $\alpha6\beta3\delta$- and $\alpha6_{GBS}\beta3\delta_{GBS}$-expressing neurons (Fig. 9*G*, $\alpha6\beta3\delta$ *versus* $\alpha6_{GBS}\beta3\delta_{GBS}$, $p > 0.2$, one-way ANOVA followed by Bonferroni's correction). In conclusion, the clustering of $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors at postsynaptic sites cannot rescue the defect in fast GABAergic synaptic transmission. Therefore, the interplay between synaptic and extrasynaptic $GABA_A$ receptors cannot be attributed to the positional effect of $\alpha6\beta3\delta$ receptors.

Overexpressing $\alpha5\beta3\gamma2$ Receptors Also Decreased Synaptic GABAergic Transmission—Besides δ subunit-containing extrasynaptic $GABA_A$ receptors, $\alpha5$ subunit-containing receptors are also largely localized at extrasynaptic membranes, although some synaptic puncta were also reported (19, 20). We therefore investigated whether overexpressing a distinctly different subtype of extrasynaptic $GABA_A$ receptors will affect synaptic GABA transmission. Immunocytochemistry experiments confirmed that the HA-$\alpha5$ subunit was significantly expressed in cultured hippocampal neurons (Fig. 10, *A*–*F*). Our recent study also demonstrated that overexpressing HA-$\alpha5\beta3\gamma2$ subunits

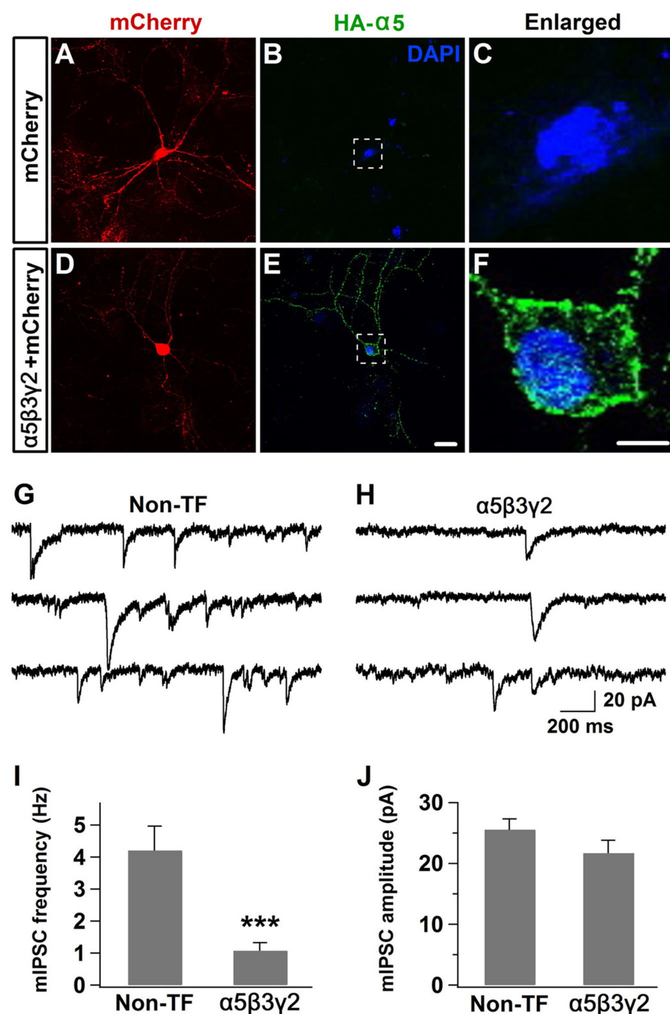

FIGURE 10. **Overexpression of α5-$GABA_A$ receptors also decreased synaptic GABAergic transmission.** *A–C*, control hippocampal neurons overexpressing mCherry showed no HA staining. *D–F*, hippocampal neurons overexpressing the HA-$\alpha5\beta3\gamma2$ subunits showed a substantial HA staining signal throughout the soma and dendrites. *G* and *H*, representative traces showing reduced mIPSC events in hippocampal neurons overexpressing α5-$GABA_A$ receptors. *Non-TF*, non-transfected. *I* and *J*, quantitative data showing the reduction of mIPSC frequency but not the amplitude after overexpressing α5-$GABA_A$ receptors (n = 10–11). ***, $p < 0.001$, Student's *t* test. *Scale bars* = 20 μm for *A*, *B*, *D*, and *E* and 5 μm for *C* and *F*.

significantly increased the tonic current in cultured hippocampal neurons (41). Interestingly, we found that after overexpressing $\alpha5\beta3\gamma2$ receptors, the frequency of mIPSCs was reduced significantly compared with non-transfected controls (Fig. 10, *G* and *H*). Quantified data showed that the frequency, but not the amplitude, of mIPSCs was decreased in $\alpha5\beta3\gamma2$-transfected neurons (Fig. 10, *I* and *J*). Therefore, similar to the overexpression of $\alpha6\beta3\delta$ receptors, increasing the expression level of $\alpha5\beta3\gamma2$ receptors also leads to a reduction of synaptic GABA transmission.

DISCUSSION

About half of the $GABA_A$ receptors are at synaptic sites, and the other half are on extrasynaptic membranes. How synaptic and extrasynaptic $GABA_A$ receptors interact with each other to maintain the homeostasis of GABAergic inhibition has not been well studied. In this work, we demonstrated that overexpression of extrasynaptic $\alpha6\beta3\delta$ receptors in cultured pyramidal neurons resulted in a substantial decrease of the mIPSC frequency and presynaptic GABAergic terminals. Thus, there appears to be a homeostatic interplay between tonic and phasic inhibition. Interestingly, such an interaction between synaptic and extrasynaptic $GABA_A$ receptors does not require the receptor activation, suggesting a novel form of homeostatic plasticity. The extrasynaptic localization of the $\alpha6\beta3\delta$-receptors is also not a precondition for the suppression of synaptic GABAergic transmission because molecularly engineered $\alpha6_{GBS}\beta3\delta_{GBS}$ receptors can cluster at synaptic sites but fail to rescue the mIPSC defects. These results suggest that synaptic and extrasynaptic $GABA_A$ receptors may interact with each other in an unconventional way to maintain a delicate balance between tonic and phasic inhibition.

Homeostatic Regulation of GABAergic Inhibition—Homeostatic plasticity in the central nervous system refers to the capability of neural networks to maintain their activity level within a normal range in response to a variety of stimulations. One type of homeostatic plasticity, synaptic scaling, was first demonstrated in excitatory synapses, and alteration of network activity by TTX or BIC may uniformly change the synaptic strength in all synapses (35). The mechanism of such homeostatic scaling has been studied extensively in excitatory synapses (42–44). In contrast, homeostatic plasticity in inhibitory synapses is much less studied (45). Global inhibition of network activity by TTX decreased the mIPSC amplitude as well as the number of GAD-positive puncta (46). Interestingly, homeostatic synaptic scaling at inhibitory synapses only occurred after inhibiting global activity with TTX, whereas hyperpolarizing individual neurons by overexpressing potassium channels did not change the GABAergic synaptic efficacy (47). Conversely, increasing network activity resulted in an increase of mIPSC frequency and amplitude (36, 48). Our bicuculline experiments confirmed that perturbing global activity can induce homeostatic plasticity at inhibitory synapses. However, in individual neurons overexpressing $\alpha6\beta3\delta$-$GABA_A$ receptors, the mIPSC frequency was still decreased drastically in the presence of BIC (Fig. 7). Our results have two significant implications. 1) The interplay between synaptic and extrasynaptic $GABA_A$ receptors does not require the activation of these receptors. 2) The homeostatic down-regulation of synaptic GABAergic transmission after overexpressing extrasynaptic $GABA_A$ receptors is cell-autonomous and not because of global activity changes. Therefore, the interaction between tonic and phasic inhibition may represent a novel form of homeostatic plasticity.

In fact, GABAergic inhibition may have several different types of homeostatic plasticity. One well studied area is the compensatory changes of $GABA_A$ receptors following targeted gene deletion of specific subunits. For example, targeted deletion of the $GABA_A$ receptor $\alpha1$ subunit resulted in a significant increase in $\alpha2$ and $\alpha3$ subunit expression as well as an elevated number of morphological synapses. However, electrophysiological study showed that the amplitude and frequency of mIPSCs were reduced in $\alpha1^{-/-}$ mice, suggesting that the compensation by $\alpha2$ and $\alpha3$ subunits cannot completely rescue the loss of GABAergic transmission because of the deletion of the $\alpha1$ subunit (49). Another type of plasticity is the change of spe-

cific $GABA_A$ receptor subunits induced by pathological conditions such as epileptic seizures (50–56). Moreover, $GABA_A$ receptor-mediated inhibition may be interacting with other channel-mediated inhibition to maintain homeostatic neuronal activity. For example, in $\alpha6^{-/-}$ neurons, $\alpha6\beta\delta$-$GABA_A$ receptor-mediated tonic conductance was reduced greatly, but a continuously active form of potassium conductance emerged to maintain normal neuronal excitability (23). On the other hand, in HCN1 (hyperpolarization-activated cyclic nucleotide-gated channel 1) knockout neurons, the inhibitory function of HCN1 was partially compensated by increased tonic inhibition mediated by $\alpha5$-containing extrasynaptic $GABA_A$ receptors (57). These various forms of homeostatic plasticity suggest that it is pivotal to maintaining neural inhibition in a normal range for proper neural network function.

Interplay between Synaptic and Extrasynaptic $GABA_A$ Receptors—A potential interplay between tonic and phasic inhibition came from observations during normal brain development, particularly in cerebellar granule cells. At postnatal day 7 (P7), spontaneous IPSCs contribute to most of the total charge transfer mediated by $GABA_A$ receptors (25). As the animals grow older (P21), both the frequency and amplitude of IPSCs decrease substantially, whereas a tonic form of inhibition takes over to control the granule cell firing rate as well as the information flow through the cerebellar cortex (25, 26, 58, 59). Such a developmental shift from phasic to tonic inhibition correlates very well with the up-regulation of $\alpha6$ and δ subunit expression in the cerebellum, with the tonic current contributing to the majority of the total charge transfer by P21 (25, 59–61). The more direct evidence suggesting a possible homeostatic interplay between synaptic and extrasynaptic $GABA_A$ receptors came from transgenic mice overexpressing the $GABA_A$ receptor $\alpha6$ subunit (27). Ectopic expression of the $\alpha6$ subunit in hippocampal pyramidal neurons increased the tonic current but decreased the spontaneous IPSC frequency. An immunoelectron microscopic assay revealed an extrasynaptic localization of the $\alpha6$-containing receptors (27). However, the authors suggested that $\alpha6$ was assembled into receptors composed of the $\alpha6\beta\gamma2$, $\alpha1\alpha6\beta\gamma2$, and $\alpha3\alpha6\beta\gamma2$ subunits. In this study, overexpressing the $\alpha6$ subunit alone is mimicking the expression of $\alpha6\beta3\delta$ subunits in increasing tonic inhibition and decreasing phasic inhibition, which is also reminiscent of the finding in $\alpha6$ transgenic mice. However, we demonstrated that $\alpha6\beta\gamma2$ receptors cannot generate a large tonic current (Fig. 4). More importantly, our immunostaining experiments directly demonstrated that $\alpha6$ overexpression can induce an up-regulation of δ subunit expression (Fig. 5). Therefore, the overexpressed $\alpha6$ subunit is more likely assembled with the up-regulated δ subunit to form $\alpha6\beta\delta$ receptors, which will mediate the enhanced tonic inhibition.

Such an intimate association between the $\alpha6$ and δ subunits is not surprising. A previous study has demonstrated that the inactivation of $\alpha6$ subunit expression suppresses δ subunit expression (62). Similarly, in $\alpha6^{-/-}$ mice, δ subunit expression is almost abolished in cerebellar granule cells (23). Our study suggests that the opposite may also be true. In hippocampal pyramidal neurons where the δ subunit expression level is typically very low, overexpression of the $\alpha6$ subunit can strongly up-regulate δ subunit expression. Thus, tonic inhibition mediated by δ-containing receptors may be quite plastic, particularly in brain regions with a low expression level of the δ subunit under normal physiological conditions. In support of this notion, it has been reported that the mRNA encoding the δ subunit was present (at a low level) in the CA1-CA3 region of the adult hippocampus (63) and that the δ subunit gene promoter may be active in a subpopulation of pyramidal cells (64). It is unclear at this time whether the δ subunit in pyramidal neurons is constantly translated but fails to assemble into functional receptors because of the lack of endogenous $\alpha6/\alpha4$ partners or whether the translation of the δ subunit itself requires the $\alpha6/\alpha4$ partners. In any case, the low level of δ subunit expression at basal conditions may leave ample room for the up-regulation of tonic inhibition in pyramidal cells.

The $\alpha5$ subunit is initially expressed widely in the developing brain but gradually restricted to the hippocampus and olfactory bulb (60). The decrease of the $\alpha5$ subunit is accompanied with an increase of the $\alpha1$ subunit during brain development (60). Thus, it is easier to understand a potential competition between the $\alpha5$ and $\alpha1$ subunits. The overexpression of the $\alpha5$ subunit may displace some $\alpha1$ subunit and, hence, decrease synaptic GABAergic inhibition. Our recent work demonstrated that overexpression of both the $\alpha6\beta3\delta$ and $\alpha5\beta3\gamma2$ receptors significantly inhibited the formation of epileptiform activity in hippocampal neurons (41). This work suggests that synaptic GABA inhibition is actually reduced after enhancing tonic inhibition. Therefore, it is pivotal to look at both synaptic and extrasynaptic $GABA_A$ receptors when assessing the net effect of GABA inhibition on neural network activity. In conclusion, tonic inhibition mediated by extrasynaptic $GABA_A$ receptors is highly dynamic, and the interaction between synaptic and extrasynaptic $GABA_A$ receptors plays an important role in controlling the homeostatic level of the total inhibition.

A Working Model. Synaptic and Extrasynaptic $GABA_A$ Receptors Compete for Limited Receptor Slots on the Plasma Membrane—The easiest explanation for the decreased phasic inhibition after an increased tonic inhibition is that neurons have to maintain an optimal level of total inhibition to control overall neuronal excitability and global network activity. The difficult question is how neurons sense the increase of tonic inhibition and how they send the signal to decrease phasic inhibition. We thought initially that, after overexpressing extrasynaptic $GABA_A$ receptors, the enhanced tonic inhibition reduced neuronal firing in the transfected neurons and that the reduced neuronal activity down-regulates synaptic IPSCs through the homeostatic scaling mechanism. However, we discovered that chronic blockage of $GABA_A$ receptor activity by bicuculline did not restore the GABAergic synaptic transmission in $\alpha6\beta3\delta$-overexpressed neurons. Under bicuculline treatment, neurons cannot sense the enhanced tonic inhibition, suggesting that the down-regulation of phasic inhibition cannot be attributed to the elevated tonic inhibition. Because both synaptic and extrasynaptic $GABA_A$ receptors are blocked by bicuculline, we suspected that there might be a "physical competition" between these receptors.

Although there may be several possible models to explain all of the data observed here, we propose a working model that

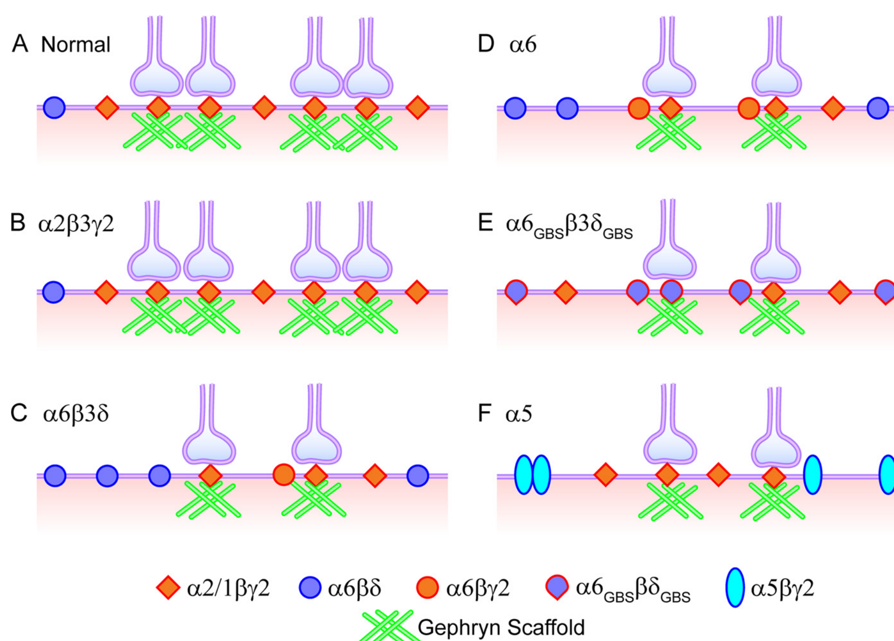

FIGURE 11. **A working model illustrating the homeostatic competition between synaptic and extrasynaptic GABA$_A$ receptors.** There are limited slots for GABA$_A$ receptors on the plasma membrane, with half being synaptic and half extrasynaptic. Overexpression of $\alpha 2\beta 3\gamma 2$ receptors does not further increase the total receptor number or the number of GABAergic synapses. In contrast, overexpression of $\alpha 6\beta 3\delta$ receptors takes some slots that are usually occupied by $\alpha 1$–$3\beta\gamma 2$ receptors, causing the reduction of postsynaptic GABA$_A$ receptor clusters and presynaptic GABAergic terminals. Overexpression of the $\alpha 6$ subunit induces surface expression of $\alpha 6\beta 3\delta$ and $\alpha 6\beta 3\gamma 2$ receptors, also resulting in the down-regulation of synaptic GABAergic transmission. The $\alpha 6_{GBS}\beta 3\delta_{GBS}$ receptors can bind with gephyrin and cluster at synaptic sites, still occupying the receptor slots typically occupied by $\alpha 1$–$3\beta\gamma 2$ receptors, causing a reduction of synaptic GABAergic transmission. Finally, overexpression of $\alpha 5\beta 3\gamma 2$ receptors also reduces the number of $\alpha 1$–$3\beta\gamma 2$ receptors on the plasma membrane, resulting in decreased synaptic GABAergic transmission.

both synaptic and extrasynaptic GABA$_A$ receptors have to compete for the limited receptor slots on the plasma membrane. It is the total number of GABA$_A$ receptors on the cell surface that controls the total inhibition (Fig. 11). Under normal conditions, about half of the GABA$_A$ receptors are at synaptic sites, and half are on extrasynaptic membranes. Some of the $\gamma 2$-containing receptors on the extrasynaptic membranes will diffuse laterally and be stabilized eventually at synaptic sites (65). When additional extrasynaptic GABA$_A$ receptors are overexpressed, they competitively occupy more receptor slots on the cell surface so that the number of slots available for $\gamma 2$-receptors is reduced greatly. As a result, the fast synaptic GABAergic transmission is reduced. Such a competition model does not require the activation of the synaptic or extrasynaptic GABA$_A$ receptors. This model not only explains the results of overexpressing the $\alpha 6$ and $\alpha 6\beta 3\delta$ subunits but also explains the results of overexpressing the $\alpha 2\beta 3\gamma 2$, $\alpha 5\beta 3\gamma 2$, and $\alpha 6_{GBS}\beta 3\delta_{GBS}$ subunits. Overexpressing the $\alpha 2\beta 3\gamma 2$ subunits did not increase the whole-cell GABA current nor change the mIPSC frequency or amplitude, suggesting that $\alpha 2\beta 3\gamma 2$ receptors usually occupy the synaptic receptor slots in neurons and that $\alpha 2\beta 3\gamma 2$ overexpression will not further increase their occupancy. In the case of overexpressing $\alpha 6_{GBS}\beta 3\delta_{GBS}$ subunits, although $\alpha 6_{GBS}\beta 3\delta_{GBS}$ receptors can be recruited to synaptic sites, the total number of $\gamma 2$-receptor clusters at synaptic sites is still reduced because of some receptor slots occupied by $\alpha 6_{GBS}\beta 3\delta_{GBS}$ receptors. Because $\alpha 6_{GBS}\beta 3\delta_{GBS}$ receptors cannot generate large GABA responses to compensate for the loss of $\gamma 2$-receptors (2), the phasic inhibition is reduced significantly. It is worth to point out that besides receptor slot competition, the $\alpha 6$ subunit may compete with the $\alpha 1$–3 subunits for the $\gamma 2$ subunit and that the resulting $\alpha 6\beta\gamma 2$ receptors may likely reside at perisynaptic sites, between synaptic and extrasynaptic sites, as demonstrated by our recent work (2). It is not clear how many $\alpha 6$ subunits will assemble into $\alpha 6\beta 3\gamma 2$ and how many will assemble into $\alpha 6\beta 3\delta$ receptors when overexpressing the $\alpha 6$ or $\alpha 6\beta 3\delta$ subunits in pyramidal neurons. The overexpression of the $\alpha 5$ subunit may result in direct competition with the $\alpha 1$–3 subunits and increase extrasynaptic receptors but, simultaneously, reduce synaptically localized receptors.

Our working model predicts that the total number of receptor slots for GABA$_A$ receptors may be limited on the plasma membrane and that both synaptic and extrasynaptic GABA$_A$ receptors will compete to occupy the same pool of receptor slots on the cell surface. Alternatively, it is also possible that receptor assembly or receptor trafficking may be the rate-limiting step for different receptors to compete. For example, the exocytotic machinery for GABA$_A$ receptors may be limited, so that when more $\alpha 6\beta 3\delta$ receptors are exocytosed to the cell surface, fewer $\gamma 2$-receptors will be exocytosed (7, 17). The limited slot hypothesis has been proposed in the study of calcium channels at nerve terminals, where N-type and P/Q-type Ca^{2+} channels may compete for channel slots to trigger vesicular release (66). Considering the large number of membrane proteins and a limited cell surface, it is possible that only limited membrane slots are allocated for each specific type of receptor or channel and that different subtypes within the same family will have to compete with each other to achieve functional homeostasis.

REFERENCES

1. Brickley, S. G., and Mody, I. (2012) Extrasynaptic $GABA_A$ receptors. Their function in the CNS and implications for disease. *Neuron* **73,** 23–34
2. Wu, X., Wu, Z., Ning, G., Guo, Y., Ali, R., Macdonald, R. L., De Blas, A. L., Luscher, B., and Chen, G. (2012) γ-Aminobutyric acid type A ($GABA_A$) receptor α subunits play a direct role in synaptic versus extrasynaptic targeting. *J. Biol. Chem.* **287,** 27417–27430
3. Macdonald, R. L., Kang, J. Q., and Gallagher, M. J. (2010) Mutations in $GABA_A$ receptor subunits associated with genetic epilepsies. *J. Physiol.* **588,** 1861–1869
4. Fritschy, J. M. (2008) Epilepsy, E/I balance and $GABA_A$ receptor plasticity. *Front. Mol. Neurosci.* **1,** 5
5. Hines, R. M., Davies, P. A., Moss, S. J., and Maguire, J. (2012) Functional regulation of $GABA_A$ receptors in nervous system pathologies. *Curr. Opin. Neurobiol.* **22,** 552–558
6. Alldred, M. J., Mulder-Rosi, J., Lingenfelter, S. E., Chen, G., and Lüscher, B. (2005) Distinct γ2 subunit domains mediate clustering and synaptic function of postsynaptic $GABA_A$ receptors and gephyrin. *J. Neurosci.* **25,** 594–603
7. Luscher, B., Fuchs, T., and Kilpatrick, C. L. (2011) $GABA_A$ receptor trafficking-mediated plasticity of inhibitory synapses. *Neuron* **70,** 385–409
8. Essrich, C., Lorez, M., Benson, J. A., Fritschy, J. M., and Lüscher, B. (1998) Postsynaptic clustering of major $GABA_A$ receptor subtypes requires the γ 2 subunit and gephyrin. *Nat. Neurosci.* **1,** 563–571
9. Fritschy, J. M., Panzanelli, P., Kralic, J. E., Vogt, K. E., and Sassoè-Pognetto, M. (2006) Differential dependence of axo-dendritic and axo-somatic GABAergic synapses on $GABA_A$ receptors containing the α1 subunit in Purkinje cells. *J. Neurosci.* **26,** 3245–3255
10. Kralic, J. E., Sidler, C., Parpan, F., Homanics, G. E., Morrow, A. L., and Fritschy, J. M. (2006) Compensatory alteration of inhibitory synaptic circuits in cerebellum and thalamus of γ-aminobutyric acid type A receptor α1 subunit knockout mice. *J. Comp. Neurol.* **495,** 408–421
11. Studer, R., von Boehmer, L., Haenggi, T., Schweizer, C., Benke, D., Rudolph, U., and Fritschy, J. M. (2006) Alteration of GABAergic synapses and gephyrin clusters in the thalamic reticular nucleus of $GABA_A$ receptor α3 subunit-null mice. *Eur. J. Neurosci.* **24,** 1307–1315
12. Panzanelli, P., Gunn, B. G., Schlatter, M. C., Benke, D., Tyagarajan, S. K., Scheiffele, P., Belelli, D., Lambert, J. J., Rudolph, U., and Fritschy, J. M. (2011) Distinct mechanisms regulate $GABA_A$ receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells. *J. Physiol.* **589,** 4959–4980
13. Li, R. W., Yu, W., Christie, S., Miralles, C. P., Bai, J., Loturco, J. J., and De Blas, A. L. (2005) Disruption of postsynaptic GABA receptor clusters leads to decreased GABAergic innervation of pyramidal neurons. *J. Neurochem.* **95,** 756–770
14. Fang, C., Deng, L., Keller, C. A., Fukata, M., Fukata, Y., Chen, G., and Lüscher, B. (2006) GODZ-mediated palmitoylation of $GABA_A$ receptors is required for normal assembly and function of GABAergic inhibitory synapses. *J. Neurosci.* **26,** 12758–12768
15. Yuan, X., Yao, J., Norris, D., Tran, D. D., Bram, R. J., Chen, G., and Luscher, B. (2008) Calcium-modulating cyclophilin ligand regulates membrane trafficking of postsynaptic $GABA_A$ receptors. *Mol. Cell Neurosci.* **38,** 277–289
16. Yu, W., Jiang, M., Miralles, C. P., Li, R. W., Chen, G., and de Blas, A. L. (2007) Gephyrin clustering is required for the stability of GABAergic synapses. *Mol. Cell Neurosci.* **36,** 484–500
17. Jacob, T. C., Moss, S. J., and Jurd, R. (2008) $GABA_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat. Rev. Neurosci.* **9,** 331–343
18. Farrant, M., and Nusser, Z. (2005) Variations on an inhibitory theme. Phasic and tonic activation of $GABA_A$ receptors. *Nat. Rev. Neurosci.* **6,** 215–229
19. Brünig, I., Scotti, E., Sidler, C., and Fritschy, J. M. (2002) Intact sorting, targeting, and clustering of γ-aminobutyric acid A receptor subtypes in hippocampal neurons *in vitro*. *J. Comp. Neurol.* **443,** 43–55
20. Christie, S. B., and de Blas, A. L. (2002) α5 Subunit-containing $GABA_A$ receptors form clusters at GABAergic synapses in hippocampal cultures. *Neuroreport* **13,** 2355–2358
21. Nusser, Z., Sieghart, W., and Somogyi, P. (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J. Neurosci.* **18,** 1693–1703
22. Nusser, Z., Ahmad, Z., Tretter, V., Fuchs, K., Wisden, W., Sieghart, W., and Somogyi, P. (1999) Alterations in the expression of $GABA_A$ receptor subunits in cerebellar granule cells after the disruption of the α6 subunit gene. *Eur. J. Neurosci.* **11,** 1685–1697
23. Brickley, S. G., Revilla, V., Cull-Candy, S. G., Wisden, W., and Farrant, M. (2001) Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature* **409,** 88–92
24. Chandra, D., Jia, F., Liang, J., Peng, Z., Suryanarayanan, A., Werner, D. F., Spigelman, I., Houser, C. R., Olsen, R. W., Harrison, N. L., and Homanics, G. E. (2006) $GABA_A$ receptor α 4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proc. Natl. Acad. Sci. U.S.A.* **103,** 15230–15235
25. Brickley, S. G., Cull-Candy, S. G., and Farrant, M. (1996) 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
26. Wall, M. J., and Usowicz, M. M. (1997) Development of action potential-dependent and independent spontaneous $GABA_A$ receptor-mediated currents in granule cells of postnatal rat cerebellum. *Eur. J. Neurosci.* **9,** 533–548
27. Wisden, W., Cope, D., Klausberger, T., Hauer, B., Sinkkonen, S. T., Tretter, V., Lujan, R., Jones, A., Korpi, E. R., Mody, I., Sieghart, W., and Somogyi, P. (2002) Ectopic expression of the $GABA_A$ receptor α6 subunit in hippocampal pyramidal neurons produces extrasynaptic receptors and an increased tonic inhibition. *Neuropharmacology* **43,** 530–549
28. Jiang, M., and Chen, G. (2006) High Ca^{2+}-phosphate transfection efficiency in low-density neuronal cultures. *Nat. Protoc.* **1,** 695–700
29. Meyer, G., Kirsch, J., Betz, H., and Langosch, D. (1995) Identification of a gephyrin binding motif on the glycine receptor beta subunit. *Neuron* **15,** 563–572
30. Deng, L., Yao, J., Fang, C., Dong, N., Luscher, B., and Chen, G. (2007) Sequential postsynaptic maturation governs the temporal order of GABAergic and glutamatergic synaptogenesis in rat embryonic cultures. *J. Neurosci.* **27,** 10860–10869
31. Krogsgaard-Larsen, P., Frølund, B., Liljefors, T., and Ebert, B. (2004) $GABA_A$ agonists and partial agonists. THIP (Gaboxadol) as a non-opioid analgesic and a novel type of hypnotic. *Biochem. Pharmacol.* **68,** 1573–1580
32. Brown, N., Kerby, J., Bonnert, T. P., Whiting, P. J., and Wafford, K. A. (2002) Pharmacological characterization of a novel cell line expressing human $\alpha(4)\beta(3)\delta$ $GABA_A$ receptors. *Br. J. Pharmacol.* **136,** 965–974
33. Meera, P., Wallner, M., and Otis, T. S. (2011) Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic $GABA_A$ receptors. *J. Neurophysiol.* **106,** 2057–2064
34. Jechlinger, M., Pelz, R., Tretter, V., Klausberger, T., and Sieghart, W. (1998) Subunit composition and quantitative importance of hetero-oligomeric receptors. $GABA_A$ receptors containing α6 subunits. *J. Neurosci.* **18,** 2449–2457
35. Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C., and Nelson, S. B. (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* **391,** 892–896
36. Peng, Y. R., Zeng, S. Y., Song, H. L., Li, M. Y., Yamada, M. K., and Yu, X. (2010) Postsynaptic spiking homeostatically induces cell-autonomous regulation of inhibitory inputs via retrograde signaling. *J. Neurosci.* **30,** 16220–16231
37. Fisher, J. L., and Macdonald, R. L. (1997) Single channel properties of recombinant $GABA_A$ receptors containing γ 2 or δ subtypes expressed with α 1 and β 3 subtypes in mouse L929 cells. *J. Physiol.* **505,** 283–297
38. Keramidas, A., and Harrison, N. L. (2008) Agonist-dependent single channel current and gating in $\alpha4\beta2\delta$ and $\alpha1\beta2\gamma2S$ $GABA_A$ receptors. *J. Gen. Physiol.* **131,** 163–181
39. Mody, I., and Pearce, R. A. (2004) Diversity of inhibitory neurotransmission through $GABA_A$ receptors. *Trends Neurosci.* **27,** 569–575
40. Bianchi, M. T., Haas, K. F., and Macdonald, R. L. (2001) Structural determinants of fast desensitization and desensitization-deactivation coupling

in $GABA_A$ receptors. *J. Neurosci.* **21,** 1127–1136

41. Sun, Y., Wu, Z., Kong, S., Jiang, D., Pitre, A., Wang, Y., and Chen, G. (2013) Regulation of epileptiform activity by two distinct subtypes of extrasynaptic $GABA_A$ receptors. *Mol. Brain* **6,** 21
42. Turrigiano, G. G., and Nelson, S. B. (2004) Homeostatic plasticity in the developing nervous system. *Nat. Rev. Neurosci.* **5,** 97–107
43. Turrigiano, G. G. (2008) The self-tuning neuron. Synaptic scaling of excitatory synapses. *Cell* **135,** 422–435
44. Yu, L. M., and Goda, Y. (2009) Dendritic signalling and homeostatic adaptation. *Curr. Opin. Neurobiol.* **19,** 327–335
45. Mody, I. (2005) Aspects of the homeostatic plasticity of $GABA_A$ receptor-mediated inhibition. *J. Physiol.* **562,** 37–46
46. Kilman, V., van Rossum, M. C., and Turrigiano, G. G. (2002) Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic $GABA_A$ receptors clustered at neocortical synapses. *J. Neurosci.* **22,** 1328–1337
47. Hartman, K. N., Pal, S. K., Burrone, J., and Murthy, V. N. (2006) Activity-dependent regulation of inhibitory synaptic transmission in hippocampal neurons. *Nat. Neurosci.* **9,** 642–649
48. Rannals, M. D., and Kapur, J. (2011) Homeostatic strengthening of inhibitory synapses is mediated by the accumulation of $GABA_A$ receptors. *J. Neurosci.* **31,** 17701–17712
49. Schneider Gasser, E. M., Duveau, V., Prenosil, G. A., and Fritschy, J. M. (2007) Reorganization of GABAergic circuits maintains $GABA_A$ receptor-mediated transmission onto CA1 interneurons in α1-subunit-null mice. *Eur. J. Neurosci.* **25,** 3287–3304
50. Brooks-Kayal, A. R., Shumate, M. D., Jin, H., Rikhter, T. Y., and Coulter, D. A. (1998) Selective changes in single cell $GABA_A$ receptor subunit expression and function in temporal lobe epilepsy. *Nat. Med.* **4,** 1166–1172
51. Goodkin, H. P., Joshi, S., Mtchedlishvili, Z., Brar, J., and Kapur, J. (2008) Subunit-specific trafficking of $GABA_A$ receptors during status epilepticus. *J. Neurosci.* **28,** 2527–2538
52. Scimemi, A., Semyanov, A., Sperk, G., Kullmann, D. M., and Walker, M. C. (2005) Multiple and plastic receptors mediate tonic $GABA_A$ receptor currents in the hippocampus. *J. Neurosci.* **25,** 10016–10024
53. Naylor, D. E., Liu, H., and Wasterlain, C. G. (2005) Trafficking of $GABA_A$ receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J. Neurosci.* **25,** 7724–7733
54. Peng, Z., Huang, C. S., Stell, B. M., Mody, I., and Houser, C. R. (2004) Altered expression of the δ subunit of the $GABA_A$ receptor in a mouse model of temporal lobe epilepsy. *J. Neurosci.* **24,** 8629–8639
55. González, M. I., and Brooks-Kayal, A. (2011) Altered $GABA_A$ receptor expression during epileptogenesis. *Neurosci. Lett.* **497,** 218–222
56. Sun, C., Mtchedlishvili, Z., Erisir, A., and Kapur, J. (2007) Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the α4 subunit of $GABA_A$ receptors in an animal model of epilepsy. *J. Neurosci.* **27,** 12641–12650
57. Chen, X., Shu, S., Schwartz, L. C., Sun, C., Kapur, J., and Bayliss, D. A. (2010) Homeostatic regulation of synaptic excitability: tonic $GABA_A$ receptor currents replace I (h) in cortical pyramidal neurons of HCN1 knock-out mice. *J. Neurosci.* **30,** 2611–2622
58. Hamann, M., Rossi, D. J., and Attwell, D. (2002) Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. *Neuron* **33,** 625–633
59. Tia, S., Wang, J. F., Kotchabhakdi, N., and Vicini, S. (1996) Developmental changes of inhibitory synaptic currents in cerebellar granule neurons. Role of $GABA_A$ receptor α 6 subunit. *J. Neurosci.* **16,** 3630–3640
60. Laurie, D. J., Wisden, W., and Seeburg, P. H. (1992) The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J. Neurosci.* **12,** 4151–4172
61. Zheng, T., Santi, M. R., Bovolin, P., Marlier, L. N., and Grayson, D. R. (1993) Developmental expression of the α 6 $GABA_A$ receptor subunit mRNA occurs only after cerebellar granule cell migration. *Brain Res. Dev. Brain Res.* **75,** 91–103
62. Jones, A., Korpi, E. R., McKernan, R. M., Pelz, R., Nusser, Z., Mäkelä, R., Mellor, J. R., Pollard, S., Bahn, S., Stephenson, F. A., Randall, A. D., Sieghart, W., Somogyi, P., Smith, A. J., and Wisden, W. (1997) Ligand-gated ion channel subunit partnerships. $GABA_A$ receptor α6 subunit gene inactivation inhibits δ subunit expression. *J. Neurosci.* **17,** 1350–1362
63. Wisden, W., Laurie, D. J., Monyer, H., and Seeburg, P. H. (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J. Neurosci.* **12,** 1040–1062
64. Lüscher, B., Häuselmann, R., Leitgeb, S., Rülicke, T., and Fritschy, J. M. (1997) Neuronal subtype-specific expression directed by the $GABA_A$ receptor δ subunit gene promoter/upstream region in transgenic mice and in cultured cells. *Brain Res. Mol. Brain Res.* **51,** 197–211
65. Bogdanov, Y., Michels, G., Armstrong-Gold, C., Haydon, P. G., Lindstrom, J., Pangalos, M., and Moss, S. J. (2006) Synaptic $GABA_A$ receptors are directly recruited from their extrasynaptic counterparts. *EMBO J.* **25,** 4381–4389
66. Cao, Y. Q., Piedras-Rentería, E. S., Smith, G. B., Chen, G., Harata, N. C., and Tsien, R. W. (2004) Presynaptic Ca^{2+} channels compete for channel type-preferring slots in altered neurotransmission arising from Ca^{2+} channelopathy. *Neuron* **43,** 387–400