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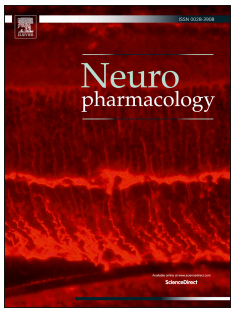

In vitro gamma oscillations following partial and complete ablation of δ subunit-containing $GABA_A$ receptors from parvalbumin interneurons

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Abstract

Perisynaptic and extrasynaptic δ subunit-containing $GABA_A$ receptors (δ-$GABA_A$Rs) mediate tonic conductances in many neurons. On principal cells of the neocortex and hippocampus they comprise α4 subunits, whereas they usually contain α1 on various interneurons. Specific characteristics of δ-$GABA_A$Rs are their pharmacology and high plasticity. In particular δ-$GABA_A$Rs are sensitive to low concentrations of neurosteroids (NS) and during times of altered NS production (stress, puberty, ovarian cycle and pregnancy) δ-$GABA_A$Rs expression varies in many neurons regardless of the α subunits they contain, with direct consequences for neuronal excitability and network synchrony. For example δ-$GABA_A$Rs plasticity on INs underlies modifications in hippocampal γ oscillations during pregnancy or over the ovarian cycle.

Most δ-$GABA_A$R-expressing INs in CA3 stratum pyramidale (SP) are parvalbumin (PV)+INs, whose fundamental role in γ oscillations generation and control has been extensively investigated. In this study we reduced or deleted δ-subunits in PV+INs, with the use of a PV/*Cre-Gabrd*/floxed genetic system. We find that *in vitro* CA3 γ oscillations of both PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice are characterized by higher frequencies than WT controls. The increased frequencies could be lowered to control levels in PV-*Gabrd*$^{+/-}$ by the NS allopregnanolone (3α,5α-tetrahydroprogesterone, 100 nM) but not the synthetic δ-$GABA_A$R positive allosteric modulator 4-Chloro-N-[2-(2-thienyl)imidazo[1,2-a]pyridin-3-yl] benzamide (DS-2, 10 μM). This is consistent with the idea that DS-2, in contrast to ALLO, selectively targets α4/δ-$GABA_A$Rs but not the α1/δ-$GABA_A$Rs found on INs. Therefore, development of drugs selective for IN-specific α1/δ-$GABA_A$Rs may be useful in neurological and psychiatric conditions correlated with altered PV+IN function and aberrant γ oscillations.

Highlights

1) In the CA3 network γ frequency modulation is possible through NS potentiation of δ-$GABA_A$Rs on PV+INs.
2) Increase in *in vitro* CA3 γ oscillation frequencies in both PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$.
3) Endogenous NS ALLO decreases γ oscillation frequencies in PV-*Gabrd*$^{+/-}$ mice.
4) DS-2 does not potentiate the α1/δ-$GABA_A$Rs of PV+INs.
5) Opens the possibility for the development of α1/δ-$GABA_A$Rs-specifc drugs for disorders comprising IN dysfunction.

In vitro gamma oscillations following partial and complete ablation of δ subunit-containing $GABA_A$ receptors from parvalbumin interneurons.

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Abbreviated Title:

Key Words: Gamma oscillations, tonic inhibition, $GABA_A$ receptors pharmacology, delta subunit, CA3 interneurons, parvalbumin, neurosteroids, DS-2.

Abstract

Perisynaptic and extrasynaptic δ subunit-containing $GABA_A$ receptors (δ-$GABA_A$Rs) mediate tonic conductances in many principal cells and interneurons (INs) of the central nervous system. δ-$GABA_A$Rs comprise α4 subunits on principal cells of the neocortex and hippocampus, whereas they usually contain α1 on various INs. δ-$GABA_A$Rs are highly plastic and exhibit a unique pharmacology, being insensitive to benzodiazepines, but sensitive to low concentrations of neurosteroids (NS). The neuroactive forms of several steroid hormones, NS act on δ-$GABA_A$Rs as potent positive allosteric modulators leading to changes in neuronal excitability and network synchrony. In fact, δ-$GABA_A$Rs on INs have been shown to control the dynamics of γ oscillations.

During times of altered NS production, including stress, puberty, ovarian cycle and pregnancy, δ-$GABA_A$R expression varies in different neurons regardless of the α subunits they contain. This plasticity has direct consequences for network functioning. In particular, plasticity of these receptors in pregnancy and the postpartum period affects CA3 γ oscillation frequencies (recorded *in vitro*), and during the ovarian cycle they underlie fluctuations in the amplitude of CA1 γ oscillations (recorded *in vivo*).

Most δ-$GABA_A$R-expressing INs in CA3 stratum pyramidale (SP) are parvalbumin (PV)+INs, whose fundamental role in γ oscillation generation and control has been extensively investigated. In this study we reduced or deleted δ-subunits in PV+INs, with the use of a PV/*Cre-Gabrd*/floxed genetic system. By means of immunohistochemistry we show that PV expression in PV-$Gabrd^{-/-}$ and PV-$Gabrd^{+/-}$ mice remains unaltered. Additionally, we confirm the absence of δ-$GABA_A$Rs specifically from PV+INs. We find that in both PV-$Gabrd^{-/-}$ and PV-$Gabrd^{+/-}$ mice, CA3 γ oscillation frequencies measured *in vitro* were increased compared to $Cre^{-/-}$ controls. The increased frequencies could be lowered to control levels in PV-$Gabrd^{+/-}$ by the NS allopregnanolone (3α,5α-tetrahydroprogesterone, ALLO, 100 nM) but not by the synthetic δ-$GABA_A$R positive allosteric modulator 4-Chloro-N-[2-(2-thienyl)imidazo[1,2-a]pyridin-3-yl] benzamide (DS-2, 10 μM). This is consistent with the idea that DS-2, in contrast to ALLO, selectively targets α4/δ-$GABA_A$Rs but not the α1/δ-$GABA_A$Rs found on INs. Therefore, development of drugs selective for IN-specific α1/δ-$GABA_A$Rs may be useful in neurological and psychiatric conditions correlated with altered PV+IN function and aberrant γ oscillations.

1. Introduction

Tonic inihibition in the cortex is predominantly mediated by extrasynaptic or perisynaptic $GABA_A$ receptors containing either δ or α5 subunits depending on the cell type and the brain region (Sperk et al., 1997; Brickley and Mody, 2012). The physiology and pharmacology of the tonic GABA conductances are of particular interest as these actions of GABA exercise powerful constrains on neuronal excitability and gain (Mody and Pearce, 2004; Farrant and Nusser, 2005). δ-$GABA_A$Rs are highly plastic receptors with a unique pharmacology. They are insensitive to benzodiazepines and sensitive to low concentrations of NS, which act as potent allosteric modulators (Belelli and Lambert, 2005). δ-$GABA_A$Rs are expressed on cortical principal cells, where they selectively contain α4 subunits (Chandra et al., 2006), and on several INs, where the α4 subunits are replaced by α1 subunits (Glykys et al., 2007). An increasing evidence points at δ-$GABA_A$Rs on different neuron types as an important factor in neurological and psychiatric disorders. For example, δ-$GABA_A$Rs plasticity on dentate gyrus granule cells and INs has been described in models of temporal lobe epilepsy (Ferando and Mody, 2012). Polimorphisms in the *GABRD* gene have been found to correlate with major depression and certain epilepsies (Dibbens et al., 2004; Feng et al., 2006; 2010). Homeostatic δ-$GABA_A$Rs plasticity on hippocampal principal cells during pregnancy and the ovarian cycle can modify network excitability and disruptions of this homeostasis has been proposed as a cofactor in PMS, PMDD and postpartum depression (Griffiths and Lovick, 2005; Lovick et al., 2005; Maguire et al., 2005; Lovick, 2006; Brack and Lovick, 2007; Lovick, 2008; Maguire and Mody, 2008; Maguire et al., 2009; Shen et al., 2010; Smith, 2013). Moreover, we have previously reported δ-$GABA_A$Rs plasticity on hippocampal cornu ammonis (CA) INs during pregnancy and over the ovarian cycle, with direct consequences on frequency or amplitude of γ oscillations (Ferando and Mody, 2013); (Barth, Ferando, and Mody, unpublished observations). Gamma oscillations (30-120 Hz), a synchronized network activity thought to be involved in learning and memory (Buzsáki and Wang, 2012), are controlled by δ-$GABA_A$Rs expressed by unidentified INs of CA3 SP (Mann and Mody, 2010). Of note, γ oscillations are heavily dysregulated in schizophrenia, a finding attributed to pathological modifications in PV+INs (Lewis et al., 2005; Uhlhaas and Singer, 2012). Lastly, pregnancy, the postpartum, and the ovarian cycle may be sensitive periods during

which pathological alterations in γ oscillations are facilitated. Ideally, therapeutical strategies would target δ-$GABA_A$Rs on principal cells or INs, so that symptoms produced by alterations of δ-$GABA_A$R-mediated tonic inhibition on one or the other cell type may be addressed separately. To this regard, the differential inclusion of α4 or α1 subunits in δ-$GABA_A$Rs in excitatory vs. inhibitory neurons is a promising tool to pharmacologically distinguish between the two cell types. In this paper we address this question by examining the effects of allosteric modulators of δ-$GABA_A$Rs on kainic acid (KA)-induced *in vitro* γ oscillations in CA3 SP of WT, heterozygous (PV-*Gabrd*$^{+/-}$) or knock out (PV-*Gabrd*$^{-/-}$) mice for the δ subunit on PV+INs.

2. Materials and Methods

2.1 Animal Handling

In this study we used adult (9–15 weeks of age) male mice, either WT littermate controls (referred to as WT) or lacking one or both copies of *Gabrd* gene specifically on PV-INs (PV-*Gabrd*$^{+/-}$ or PV-*Gabrd*$^{-/-}$). These mice were generated by crossing PV-*Cre* (PV-IRES-Cre line; JAX Stock # 008069) and *Gabrd*-flox mice (generous gift of Dr. Jamie Maguire, Tufts University) (Lee and Maguire, 2013). All animal experiments were carried out in accordance with the National Institute of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Mice were housed under the care of the division of Laboratory Animal Medicine of the University of California, Los Angeles. Mice were maintained on a light/dark cycle of 12h with *ad libitum* access to food and water, and all experiments were done during the light cycle. Particular care was taken to minimize stress in the test subjects. In particular, mice were moved to the experimental area in their home cage at least 2 hrs prior to the experiment. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques, if available. Genotyping was performed by Transnetyx.

2.2 Immunohistochemistry and Microscopy

Brains were perfused and tissues processed as previously described (Maguire et al., 2009; Ferando and Mody, 2013). Briefly, deeply anesthetized mice (inhalational isoflurane and sodium pentobarbital, i.p. 100 mg/kg) were transcardially perfused with 50 ml of 4% paraformaldehyde in 0.12 M phosphate buffer, pH 7.3. Brains were cryoprotected in 30% sucrose solution in Millonigs modified PBS, frozen and later sectioned (coronal, 35 µm) at −16°C with use of a cryostat. For the same staining all slices were stained in parallel. For diaminobenzidine (DAB) δ-$GABA_A$Rs stain: quenching of endogenous peroxidases, 3% H_2O_2 in methanol (30 min); blocking, 10% normal goat serum (2 h); primary antibody, δ-$GABA_A$R-specific polyclonal antibody raised in rabbit (1:500 in 10% NGS, overnight; generous gift from Dr. Werner Sieghart, Medizinische Universität, Wien, Austria). Specificity of the δ-$GABA_A$R antibody used in this study has been previously proven by different groups, including us, by showing a complete lack of staining in *Gabrd*$^{-/-}$ mice, and lack of staining in principal cells in *Gabra4*$^{-/-}$ mice, in which expression of δ-$GABA_A$R is suppressed (Peng et al., 2002; Glykys et al.,

2007; Ferando and Mody, 2013; Milenkovic et al., 2013). Secondary antibody: biotinylated goat anti-rabbit antibody (1:200 in 10% NGS, 4 h; Vector Laboratories). Signal amplification: HRP-conjugated avidin enzyme complex (30 min, ABC Elite; Vector Laboratories). Signal development: DAB (Vector Lab). For DAB PV stain: quenching of endogenous peroxidases, 1.5% H_2O_2 in TBS; blocking, 10% normal horse serum (2 h); primary antibody, PV-specific polyclonal antibody raised in mouse (1:5000 in 50% NHS, overnight (monoclonal, Swant). Secondary antibody: biotinylated horse anti-mouse antibody (1:200 in 10% NHS, 4 h; Vector Laboratories). Signal amplification and development was done as above. PV DAB staining was used for PV+INs number comparisons between the three genotypes. For each mouse 3 to 4 coronal slices 3 slices apart (thickness 35 µm) were taken from the mid hippocampal region and stained in parallel. For δ-$GABA_A$Rs-PV double labeling: antigen retrieval, 70 min at 90◦C in a citrate buffer solution (0.05 M sodium citrate in 1% NaCl, pH 8.6); blocking, 10% NGS and 0.3% Triton X-100 (2 h); primary antibodies, rabbit anti-δ-$GABA_A$R and mouse anti-PV (monoclonal, Swant) (1:100; 1:5000 respectively, 0.1% NaN3 in TBS, 4 days); secondary antibodies, Alexa Fluor-647 and Alexa Fluor-488 goat anti-rabbit and anti-mouse antibodies (1:750; Millipore, 1 h). Slices were mounted on Superfrost Plus slides (Fisher Scientific), and coverslipped with DPX Mountant for Histology (DAB stains, Sigma-Aldrich) or Fluoromount G (fluorescence stains, Southern Biotechnology).

For bright field microscopy, digital images were taken with an Axioskop 2 Microscope and an AxioCam digital camera system and AxioVision 4.8 software (Zeiss). For the same magnification and the same staining images were taken under identical conditions of brightness and exposure time. For optical density measurements, random regions of interest (ROIs) were selected in each slice and analyzed in ImageJ. For fluorescent microscopy, images were collected using a confocal microscope (Leica TCS SP2) equipped with Hex PL APO 100x/1.40-0.7 OIL CS objective using LAS AF software (Leica Microsystems). Digital projection images of 35 µm *z-stacks* were assembled and using NIH ImageJ software. All images were captured under the same light intensity and exposure limits.

2.3 Electrophysiology

Hippocampal slices were cut as previously reported (Ferando and Mody, 2013). Briefly mice were anesthetized with isoflurane and decapitated following UCLA Chancellor's Animal Research Committee protocol. Horizontal 350µm slices were cut on a Leica VT1000S Vibratome in ice-cold N-Methyl-D-Glucamine (NMDG)-based HEPES-buffered solution, containing in mM: 135 NMDG, 10 D-glucose, 4 $MgCl_2$, 0.5 $CaCl_2$, 1 KCl, 1.2 KH_2PO_4, 26 HEPES, (bubbled with 100% O_2, pH 7.4, 290 –300 mOsm/L). Slices were incubated at 32°C in an interface chamber in a reduced sodium artificial CSF (aCSF), containing in mM: NaCl 85, D-glucose 25, sucrose 55, KCl 2.5, NaH_2PO_4 1.25, $CaCl_2$ 0.5, $MgCl_2$ 4, $NaHCO_3$ 26, pH 7.3–7.4 when bubbled with 95% O_2, 5% CO_2. After 20 min low sodium aCSF was substituted for normal aCSF at 32°C, containing in mM: NaCl 126, D-glucose 10, $MgCl_2$ 2, $CaCl_2$ 2, KCl 2.5, NaH_2PO_4 1.25, Na Pyruvate 1.5, L-Glutamine 1, $NaHCO_3$ 26, pH 7.3-7.4 when bubbled with 95% O_2, 5% CO_2. Recordings were done in an interface chamber at 35°C perfused with normal aCSF and 50 nM kainic acid - KA (Tocris). Oscillatory network activity was recorded in CA3 SP with the use of a patch pipette (3-5 MΩ resistance) filled with nACSF connected to the headstage of an amplifier (A-M Systems Inc., model 3000) where it was band-pass filtered between 0.1 and 1000 Hz. The signal was fed through an instrumentation amplifier (Brownlee BP Precision, model 210A) and sampled at 4096 s^{-1} with a National Instruments A/D board. Field potentials were recorded using EVAN (custom-designed LabView-based software from Thotec) and analyzed with a custom written procedure (Wavemetrics, IGOR Pro 6.22A). Peak frequencies, power at peak frequency and total power were obtained from the corresponding power spectral densities (psd), calculated from 180 s period averages. ALLO and DS-2 were purchased from Tocris, and were dissolved in DMSO (final vehicle concentration 0.01%). Morlet wavelet transform was used to illustrate the power of γ-frequency band in time as previously described (Mann and Mody, 2010). Evoked field potentials were recorded in the dentate gyrus molecular layer. The perforant path was stimulated every 30 s with a paired pulse 50 ms apart. Average stimulus intensity was 60 µA, 50 µs. Slope was calculated by fitting a straight line to the steepest part of the fEPSP (EVAN). Evoked fEPSP slopes used for determining statistical significance were calculated as mean slope over 5 minutes immediately preceding and 10-15 minutes after drug application.

2.4 Data Analysis

All data shown are expressed as mean ± SEM. Normality of the data set was determined by D'Agostino and Pearson omnibus normality test. In case of normally distributed data sets parametric tests were used. Data not normally distributed were compared using non-parametric tests. Specific statistical tests used to determine statistical significance are indicated in each section. Statistical significance was defined as $p<0.05$.

3. Results

3.1 Expression of δ-GABA$_A$Rs in PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice in PV+INs and PV-INs.

Our previous findings show how δ-GABA$_A$Rs downregulation on CA3 INs, such as found during pregnancy, is sufficient to alter KA-induced CA3 γ oscillations (Ferando and Mody, 2013). Interestingly, concentrations of ALLO relevant to pregnancy could normalize γ oscillations in slices of pregnant mice (Ferando and Mody, 2013), raising the question whether a moderate alteration in IN-δ-GABA$_A$Rs expression, in the absence of the large NS alterations typical of pregnancy may alter γ oscillations dynamics. In order to address this question, we generated a mouse where the δ subunit is knocked down or knocked out specifically from PV+INs, by crossing PV-Cre and *Gabrd*-flox mice. We chose this cell type as i) PV+INs (and in particular PV+ basket cells) are responsible for the generation and maintenance of γ oscillations (Traub et al., 2000; Fuchs et al., 2007; Cardin et al., 2009; Sohal et al., 2009; Wulff et al., 2009; Gulyás et al., 2010; Korotkova et al., 2010; Zheng et al., 2011; Carlén et al., 2012; Lasztoczi and Klausberger, 2014), ii) the large majority of PV+INs located in the principal cell layer of the various hippocampal areas also express δ-GABA$_A$Rs, and δ-GABA$_A$Rs expressed in CA3 SP are almost exclusively confined to PV+INs (Ferando and Mody, 2013; Milenkovic et al., 2013; Yu et al., 2013), iii) δ-GABA$_A$Rs on CA3 INs control the frequency of γ oscillations (Mann and Mody, 2010). Although the Cre-lox system is widely used and well established in mouse genetic manipulations (Sauer, 1998), it is important to show the specificity of the ablation of a target gene, as different floxed genes may react incongruously with the same Cre-expressing system. With the use of δ-GABA$_A$R-spefic antisera in brain sections of PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice, we show a diminished or fully ablated δ-GABA$_A$Rs expression in INs located within 30 µm of CA1 and CA3 SP (**Fig. 1**). This is shown more clearly in the CA3 SP (**Fig. 1B**) where the lack of δ-GABA$_A$Rs expression in CA3 pyramidal cells allows for a clearer visualization of interneuronal staining. As expected from the specificity of the PV-Cre system, in PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice δ-GABA$_A$Rs expression remains at WT levels in cells that do not normally express PV. For example, δ-GABA$_A$Rs expression is unaltered in CA1 pyramidal cells and dentate gyrus granule cells, as reflected by the lack of variation in the δ-GABA$_A$R specific staining of CA1 SO and SR, and the molecular layer of the dentate gyrus (DGML), where the dendritic domain of these cells

lays and where δ-GABA$_A$Rs are expressed. Optical densities (in arbitrary units) for WT, PV-*Gabrd*$^{+/-}$, and PV-*Gabrd*$^{-/-}$ were, respectively: 132.1 ± 2.2, 132.5 ± 1.9, 130.3 ± 1.3 for the DGML; and 75.3 ± 1.1, 74.5 ± 2.1, 74.3 ± 1.2 for the CA1 SP. ($p > 0.05$, one-way ANOVA, n = 20 ROIs in 2 sections, see Materials and Methods). Since PV+INs are not the only IN types expressing δ-GABA$_A$R, but other INs such as neurogliaform cells (NGCs) of the neocortex and the hippocampus also heavily express δ-GABA$_A$Rs (Oláh et al., 2009; Faragó et al., 2013), we exmined δ-GABA$_A$Rs distribution in areas where few PV+INs are present. **Fig. 1C** shows INs in CA1 lacunosum moleculare, an area where NGCs are abundant, displaying comparable δ-GABA$_A$R staining in all three experimental groups. The expression of PV in PV+INs is unaffected following pregnancy-induced δ-GABA$_A$R plasticity in these cells (Ferando and Mody, 2013). Here we also show that the pattern of PV expression and PV+INs localization in CA3 SP remains unaltered following δ-GABA$_A$R ablation from these cells (**Fig 2A-B**). The average number of PV+INs in one hippocampal CA3 SP and 30 μm towards SO and SR was also similar between the three genetic groups (for WT, PV-*Gabrd*$^{+/-}$, and PV-*Gabrd*$^{-/-}$ = 31 ± 2, 29 ± 2 and 28 ± 1, respectively. *n* = 16, 14, 16 hippocampi, 2 mice per group. $p > 0.05$, one-way ANOVA. See Materials and Methods for staining procedures). Moreover, a double-staining experiment with antisera specific for PV and δ-GABA$_A$R confirmed that in PV-*Gabrd*$^{-/-}$ mice PV+INs in CA3 SP do not express δ-GABA$_A$R, whereas in WT mice the overlap of the two antigens is nearly complete (**Fig. 2B**), as previously described (Ferando and Mody, 2013). In PV-*Gabrd*$^{-/-}$ mice INs expressing δ-GABA$_A$R but not PV could be found in other hippocampal areas, such as the CA3 and CA1 str. lacunosum-moleculare (**Fig. 2C**). These anatomical data demonstrate the specificity of the δ-GABA$_A$R ablation from PV+INs in our mouse model.

3.2 Frequency of *in vitro* CA3 γ oscillations following reduction or ablation of δ-GABA$_A$Rs from PV-INs.

We next recorded KA-induced γ oscillations in CA3 SP in all three experimental groups. We have previously shown how the frequency of carbachol-induced and KA-induced CA3 γ oscillations is increased in *Gabrd*$^{-/-}$ mice as a consequence of disinhibition of CA3 SP INs (Mann and Mody, 2010). In the model we proposed δ-GABA$_A$Rs on INs control γ oscillations dynamics through

modulation of IN excitability. Here we show how in both PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice CA3 γ oscillation frequency is increased compared to WT littermate controls, with no effect of oscillations power. Peak frequencies (Hz) were: WT 46.0 ± 0.8; PV-*Gabrd*$^{+/-}$ 52.2 ± 0.8; PV-*Gabrd*$^{-/-}$ 52 ± 0.7. $p < 0.0001$. $F_{(2,77)} = 15.74$. . Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparisons test. There were no differences in power at peak frequency or total power (measured between 30-120 Hz). Powers at peak frequency (×E-11 V^2s^{-1}) were: WT 1.8 ± 0.3; PV-*Gabrd*$^{+/-}$ 5.3 ± 1.6; PV-*Gabrd*$^{-/-}$ 3.1 ± 0.6. $p > 0.05$. Total powers (×E-10 V^2) were: WT 4.9 ± 0.7; PV-*Gabrd*$^{+/-}$ 11.1 ± 3.3; PV-*Gabrd*$^{-/-}$ 7.0 ± 1.0. $p > 0.05$. *n* (mice, slices) = WT 5, 25; PV-*Gabrd*$^{+/-}$ 7, 33; PV-*Gabrd*$^{-/-}$ 4, 22. Statistical significance was determined by Kruskal-Wallis test (**Fig. 3)**. These findings are consistent with the idea that the observed increased frequency of γ oscillations in both *Gabrd*$^{-/-}$ and PV-*Gabrd*$^{+/-}$ mice are solely due to the reduced expression of δ-$GABA_A$Rs in PV+INs, and that a down-regulation of δ-$GABA_A$Rs in these cells, as it happens during pregnancy in WT mice (Ferando and Mody, 2013), is sufficient to modify network activity.

3.3 Differential modulation of γ oscillation frequency by ALLO and DS-2 in slices of PV-*Gabrd*$^{+/-}$ mice.

The increased frequency in γ oscillations found in *Gabrd*$^{-/-}$ mice with global ablation of δ-$GABA_A$Rs is normalized to control levels by selectively decreasing NMDAR-mediated excitation in PV+INs with the NMDA-R antagonists D-AP5 (25 µM) or PPDA (1 µM) (Mann and Mody, 2010). In pregnant WT mice, with a diminished expression of δ-$GABA_A$Rs on CA3 INs, increasing the activity of the remaining δ-$GABA_A$Rs with ALLO (100 nM) also reduces the frequencies to control values (Ferando and Mody, 2013). However, NS potentiate δ-$GABA_A$R-mediated conductances in both INs and principal cells, in the same concentration range (10-100 nM) (Stell et al., 2003; Glykys et al., 2007; Mann and Mody, 2010), indicating that NS do not efficiently differentiate between α1 and α4/δ-$GABA_A$R. Recently a synthetic δ-$GABA_A$R positive allosteric modulator was developed and a thorough investigation was carried out for its selectivity for different $GABA_A$R subunits (Wafford et al., 2009; Jensen et al., 2013). The magnitude of the DS-2-induced potentiation of GABA responses was dependent on the presence of δ subunits and

on the nature of the α but not the β subunits present in δ-$GABA_A$Rs. The compound was most selective for δ-$GABA_A$Rs containing α4 or α6 subunits (Jensen et al., 2013). Moreover, in a recent study done in mice, DS-2 was much less effective in potentiating tonic conductances in all dentate gyrus INs (including PV+INs) compared to dentate gyrus granule cells (DGGCs) (Wei et al., 2013). These findings have interesting pharmacological implications given the anatomical distribution of δ-$GABA_A$Rs containing α1 or α4 subunits. We tested whether ALLO (100 nM) and DS-2 (10 μM, above the EC50 concentration for α4/δ-$GABA_A$Rs) can differentially modify neuronal network activities modulated by δ-$GABA_A$Rs containing α1 or α4 subunits. We first recorded evoked field EPSPs in the molecular layer of the DG, where network excitability is under strong regulatory control of α4/δ-$GABA_A$Rs expressed by DGGCs. Both drugs decreased the slope of evoked fEPSPs by the same amount, suggesting that at these concentrations they potentiate α4/δ-$GABA_A$Rs in a similar way (% slope decreases were for DS-2: 27.0 ± 5.9, $p < 0.05$, $n = 5$ slices; for ALLO: 29.0 ± 5.0, $p < 0.01$, $n = 5$ slices. Significance was determined by two-tailed paired *t*-test done on the raw slopes, see Materials and Methods). Lastly we recorded γ oscillations in slices of WT, PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice before and after application of ALLO (100 nM) or DS-2 DS-2 (10 μM). Interestingly, ALLO but not DS-2 decreased the frequency of γ oscillations to control values in slices of PV-*Gabrd*$^{+/-}$ mice, with no effect on the frequency of γ oscillations in slices WT or PV-*Gabrd*$^{-/-}$ mice (**Table 1**). Neither drug had any effects on oscillatory power at peak frequency or total power (between 30-120 Hz). These results confirm that CA3 SP γ oscillation frequencies are controlled by δ-$GABA_A$Rs expressed on PV+INs alone, as selectively potentiating α4/δ-$GABA_A$Rs with DS-2 does not result in appreciable changes in γ oscillation frequency in PV-*Gabrd*$^{+/-}$. Taken together, these results indicate that specific pharmacological modulation of δ-$GABA_A$Rs containing either α4 or α1 subunits may provide control over different network behaviors.

4. Discussion

In this study we show that δ-$GABA_A$Rs expressed on PV+INs modulate *in vitro* γ oscillations dynamics. By crossing the recently generated *Gabrd*-flox mice (Lee and Maguire, 2013) with PV-*Cre* reporter mice we generated PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice, making it possible to investigate the impact of partial or total loss of PV+IN δ-$GABA_A$Rs on γ oscillations. We targeted PV+INs as they have a central role in the local generation of γ oscillations (Traub et al., 2000; Mann et al., 2005; Fuchs et al., 2007; Cardin et al., 2009; Sohal et al., 2009; Wulff et al., 2009; Gulyás et al., 2010; Korotkova et al., 2010; Zheng et al., 2011; Carlén et al., 2012; Lasztoczi and Klausberger, 2014).

The NMDAR-mediated tonic excitation of hippocampal INs becomes facilitated in *Gabrd*$^{-/-}$ mice (Mann and Mody, 2010) as well as in slices of WT pregnant mice, where δ-$GABA_A$Rs become down-regulated on CA3 SP INs (Ferando and Mody, 2013). In our previous studies on the modulation of γ oscillation frequency we could not unequivocally rule out potential contributions of δ-$GABA_A$Rs on other INs and of presynaptic δ-$GABA_A$R expressed on mossy fiber boutons (Trigo et al., 2008; Ruiz et al., 2010). Although the dentate gyrus is unlikely to participate in CA3 γ oscillations recorded *in vitro*, as it needs intact enthorhinal inputs to generate oscillations in the γ frequency (Bragin et al., 1995; Csicsvari et al., 2003), release from mossy fiber boutons may synchronize local networks. We have now addressed this specificity by demonstrating a clear increase in CA3 γ oscillation frequency in both PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice compared to WT littermate controls. Moreover we show how ALLO (100 nM) but not the synthetic positive allosteric modulator DS-2 (10 μM) can lower the frequency of γ oscillations in slices of PV-*Gabrd*$^{+/-}$ mice. This is consistent with previous reports of DS-2 being selective for α4/δ-$GABA_A$Rs (Jensen et al., 2013; Wei et al., 2013).

As shown following partial reduction of δ-$GABA_A$Rs expressed by PV+INs during pregnancy (Ferando and Mody, 2013), γ oscillation frequency was increased in slices of PV-*Gabrd*$^{+/-}$ mice. However, no further increase in frequency was noted in PV-*Gabrd*$^{-/-}$ slices where δ-$GABA_A$Rs are altogether missing from PV+INs. A possible explanation for this finding may have to do with the necessity of limiting the frequencies of locally generated γ oscillations. In contrast to γ oscillations recorded *in vitro* which do not usually surpass frequencies beyond 60

Hz, hippocampal γ oscillations recorded *in vivo* have a broader spectrum and have thus been categorized as slow and fast γ depending on their frequency (usually 60-100 Hz for fast γ, although consensus lacks on the cutoff frequency) (Belluscio et al., 2012). The γ oscillations generated locally are slow and are tightly controlled not to interfere with long distance inputs (Lasztoczi and Klausberger, 2014). The fast γ oscillations in the hippocampus are thought to be generated by entorhinal inputs (Brun et al., 2002); (Colgin et al., 2009), and the two types of γ oscillations are involved in different aspects of memory formation and retrieval (Bieri et al., 2014; Yamamoto et al., 2014). Allowing an overlap between locally generated slow and distal input fast γ frequencies may disrupt these processes. Therefore, it is plausible that the highest frequency of the slow γ oscillations locally generated by PV+INs is limited by their tonic excitation. Accordingly, small decreases in δ-$GABA_A$Rs-mediated tonic inhibition would fully release the NMDAR-mediated tonic excitation of PV+INs, such that greater decreases in tonic inhibition will not allow a further increase in γ frequency. Conversely, potentiating the δ-$GABA_A$Rs-mediated tonic inhibition of PV+INs has little effect on reducing γ frequencies, as in WT slices NMDA antagonists have no effect on γ oscillations recorded *in vitro* (Mann and Mody, 2010; Ferando and Mody, 2013). Therefore, the tonic inhibition of PV+INs under control conditions must be sufficient to prevent all NMDAR activation such that a further increase in tonic inhibition is without effect. This hypothesis is fully supported by our previous findings showing that the enhancement of δ-$GABA_A$Rs-mediated tonic inhibition in WT slices could decrease γ frequency only in a low Mg^{2+} modified aCSF when NMDA receptor activity was increased (Mann and Mody, 2010).

We have previously shown how CA3 INs in global *Gabrd*$^{-/-}$ mice lose their tonic conductances with no apparent upregulation of other $GABA_A$Rs subunits that mediate tonic inhibition (Mann and Mody, 2010). Although the recording of tonic GABA conductances in CA3 PV+INs was beyond the scope of this study, based on our previous evidence it is reasonable assume that PV+INs of PV-*Gabrd*$^{-/-}$ mice had no such conductance. Even if PV+INs in these mice compensated for the loss of δ-$GABA_A$Rs by expressing other tonically active $GABA_A$Rs, a lower GABA sensitivity of the newly expressed receptors may explain their inability to prevent an increase in γ oscillation frequency.

Future studies will be needed to determine the effects of reduced or missing δ-$GABA_A$Rs in PV+INs on *in vivo* network dynamics. Based on our present findings θ oscillations should not be affected, as deleting δ-$GABA_A$Rs in PV+INs does not affect these receptors in other INs (NGCs and Ivy Cells) that are involved in these lower frequency oscillations (Fuentealba et al., 2008; Capogna and Pearce, 2011). However, as these other INs also express δ-$GABA_A$Rs most likely with α1 subunits, it is important to keep in mind that compounds designed to modify γ oscillations by acting on α1/δ-$GABA_A$Rs receptors of PV+INs will also alter network activities controlled by other INs. Alterations in θ-γ coupling may be expected in PV-*Gabrd*$^{-/-}$ mice, based on previous work in mice with the $GABA_A$R γ2 subunit selectively deleted from PV+INs. In these mice phasic inhibition of PV+INs was necessary for θ-γ coupling *in vivo*, but the properties of γ oscillations remained unaltered (Wulff et al., 2009).

In this study we specifically focused on γ oscillations recorded in the CA3 region, where δ-$GABA_A$Rs are exclusively found on INs. In other brain areas where both α4-δ and α1-δ/$GABA_A$R-expressing neurons are present, non-selective drugs like ALLO would potentiate tonic inhibition on both cell types. Potentiating tonic inhibition on principal cells may decrease the power of γ oscillations, based on the higher γ power found in *Gabra5*$^{-/-}$ mice where the α5-$GABA_A$Rs specific to principal cells are missing (Glykys et al., 2008). Here we found no differences across genotypes in the power of γ oscillations. The relationship between γ oscillations power and frequency is not easily predicted (Jia and Kohn, 2011), as they can be influenced by independent variables such as network volume and single cell properties (Cardin et al., 2009; Mann and Mody, 2010; Buzsáki and Wang, 2012; Pavlov et al., 2014).

Our *in vitro* model shows the ability of the CA3 network to modulate γ frequency through NS potentiation of δ-$GABA_A$Rs on PV+INs. This may be an important tool to allow fast responses to instantaneous behavioral needs, and may be a mechanism which facilitates optimization of information processing (Lu et al., 2011; Uhlhaas et al., 2011; Bieri et al., 2014). Moreover, mounting evidence showing a pivotal role of δ-$GABA_A$Rs and their plasticity in many neurological and psychiatric disorders such as epilepsy, anxiety, and postpartum depression may involve the mechanism of γ frequency modulation described in this study. For example, in models of temporal lobe epilepsy, δ-$GABA_A$Rs plasticity has been reported in both principal cells

(downregulation) and INs (upregulation) (Peng et al., 2004; Ferando and Mody, 2012; Yu et al., 2013). Moreover, δ-$GABA_A$Rs expression is homeostatically modulated during times of altered NS production in hippocampal principal cells and INs (Lovick et al., 2005; Maguire et al., 2005; Lovick, 2006; Maguire and Mody, 2007; Maguire et al., 2009; Ferando and Mody, 2013), with consequences on network excitability and dynamics. Dysregulations in the control of δ-$GABA_A$Rs plasticity on principal cells or INs have been proposed as contributing factors in some symptoms of postpartum depression, PMS and PMDD and catamenial epilepsy (Maguire et al., 2005; Maguire and Mody, 2008; Maguire et al., 2009; Ferando and Mody, 2013) (Barth, Ferando, and Mody, unpublished observations). Moreover disruption of PV+IN function and resulting network dysfunction are well documented in schizophrenia (Brickley and Mody, 2012; Curley and Lewis, 2012; Lewis et al., 2012; Uhlhaas and Singer, 2012; Lewis, 2014).

The selective cell-type dependent localization of δ-$GABA_A$Rs containing α1 or α4 subunits has intriguing implications as it may allow for differential pharmacological targeting of various neurons. We have previoulsy shown that α1/δ-$GABA_A$Rs on dentate ML INs were slightly more sensitive to ethanol than the α4/δ-$GABA_A$Rs of DGGCs (Glykys et al., 2007). As α1 and α4/δ-$GABA_A$Rs appear to be equally sensitive to NS (Bianchi and Macdonald, 2003; Meera et al., 2009), these chemicals would be poor candidates for differential therapy. Although to date there are no compounds selective for α1/δ-$GABA_A$Rs, future development of such drugs may have critical clinical applications for the many neurological and psychiatric disorders that involve IN dysfunction and altered γ oscillations.

5. Conflict of Interest

None.

6. Acknowledgments

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Figure Legends

Fig. 1. δ-GABA$_A$Rs expression in the hippocampal formation of WT, PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice. Representative bright field images δ-GABA$_A$Rs staining. **(A).** Whole hippocampal images show δ-GABA$_A$Rs expression is similar between the three experimental groups in CA1 SO and SR and DGML. (**B).** High magnification images show hippocampal area CA3. δ-GABA$_A$Rs is exclusively expressed in INs. In PV-*Gabrd*$^{+/-}$ mice there's a partial loss in the δ-GABA$_A$Rs staining of CA3 SP INs, while in PV-*Gabrd*$^{-/-}$ mice very few δ-GABA$_A$Rs+ INs can be found. **(C).** δ-GABA$_A$Rs staining of interneurons that do not normally express PV remains unchanged in all three experimental groups. Representative image of CA1 LM INs that stain for δ-GABA$_A$Rs in WT, PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice. These INs are putative neurogliaform cells, with a radial dentritic tree around a large circular soma. Scale bars = 200 µm, 50 µm.

Fig. 2. δ-GABA$_A$Rs and PV espression in WT and PV-*Gabrd*$^{-/-}$ mice in two different types of IN. δ-GABA$_A$Rs and PV do not colocalize in PV-*Gabrd*$^{-/-}$ mice. Green: PV, red: δ-GABA$_A$Rs, yellow: colocalization. **(A, B)**. Representative confocal z-stack projections of hippocampal area CA3 in WT and PV-*Gabrd*$^{-/-}$ mice. In WT mice PV/δ-GABA$_A$Rs colocalization is almost complete in CA3 stratum pyramidale (thick dotted lines) and peri-stratum pyramidale area (30 µm – thin dotted lines), as previously reported (Ferando and Mody, 2013; Milenkovic et al., 2013; Yu et al., 2013) **(A)**. In PV-*Gabrd*$^{-/-}$ mice no δ-GABA$_A$Rs and PV colocalization could be found **(B)**. **(C, D)**. Representative confocal z-stack projections of of CA1 lacunosum moleculare. Interneurons positive for δ-GABA$_A$Rs do not express PV. Putative neurogliaform cells in WT and PV-*Gabrd*$^{-/-}$ mice express δ-GABA$_A$Rs but not PV. Scale bars = 20 µm.

Fig. 3. Frequency of CA3 SP γ oscillations is increased in slices of PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice compared to WT controls.

(A). KA induced γ oscillations recorded extracellularly in CA3 SP show increased frequency in PV-*Gabrd*$^{+/-}$ and PV-*Gabrd*$^{-/-}$ mice. Upper traces, representative 1 s local field potentials band-pass filtered between 15 and 120 Hz. Lower panels, corresponding Morlet Wavelet transforms showing instantaneous LFP oscillatory activity. Warmer colors represent higher power. **(B).**

Power spectral densities calculated over a 180 s period corresponding to the same recordings as in **(A)**. **(C).** Box plots showing peak frequencies for the three experimental groups. Peak frequency of each slice calculated as the frequency with the highest power in a 180 s period power spectral density as in **(B)**. Median, Mean (+), whiskers represent largest and smallest value, boxes represent 25th and 75th percentile. Significance established by One-Way ANOVA followed by Tukey's multiple comparisons test. ***p<0.0001. *n*'s in mice/slices.

Table 1. ALLO but not DS-2 reverts the frequency of γ oscillations in slices of PV-*Gabrd*$^{+/-}$ mice to levels found in WT slices. Values represent the mean with SEM in parentheses. The numbers below indicate the number of slices; mice. * indicates significance. Power at peak frequency and total power (calculated at 30-120 Hz) are also shown. Two-way ANOVA (drug and genotype) showed significant drug ($p < 0.05$, $F_{(2, 71)} = 3.547$), genotype ($p < 0.0001$, $F_{(2, 71)} = 12.5$), and interaction ($p < 0.05$, $F_{(4, 71)} = 3.247$) effects on peak frequencies. Tukey's post-hoc test showed a significant difference in peak frequencies of PV-*Gabrd*$^{+/-}$ mice before and after ALLO treatment, $p < 0.001$, and between ALLO and DS-2 treatments $p < 0.01$. For the two-way ANOVA test the no-drug values were grouped from both ALLO and DS-2 experiments. A two-tailed paired *t*-test on values before and after drug treatments showed a significant effect of ALLO $p < 0.05$ but not of DS-2 on reducing γ oscillation frequencies in slices of PV-*Gabrd*$^{+/-}$ mice. A two-tailed Wilcoxon signed rank test on values before and after drug treatments showed no significant effects for power at peak frequency or total power for any of the experimental groups ($p>0.05$). For across genetic groups comparisons and statistical tests see Results.

Figure 1

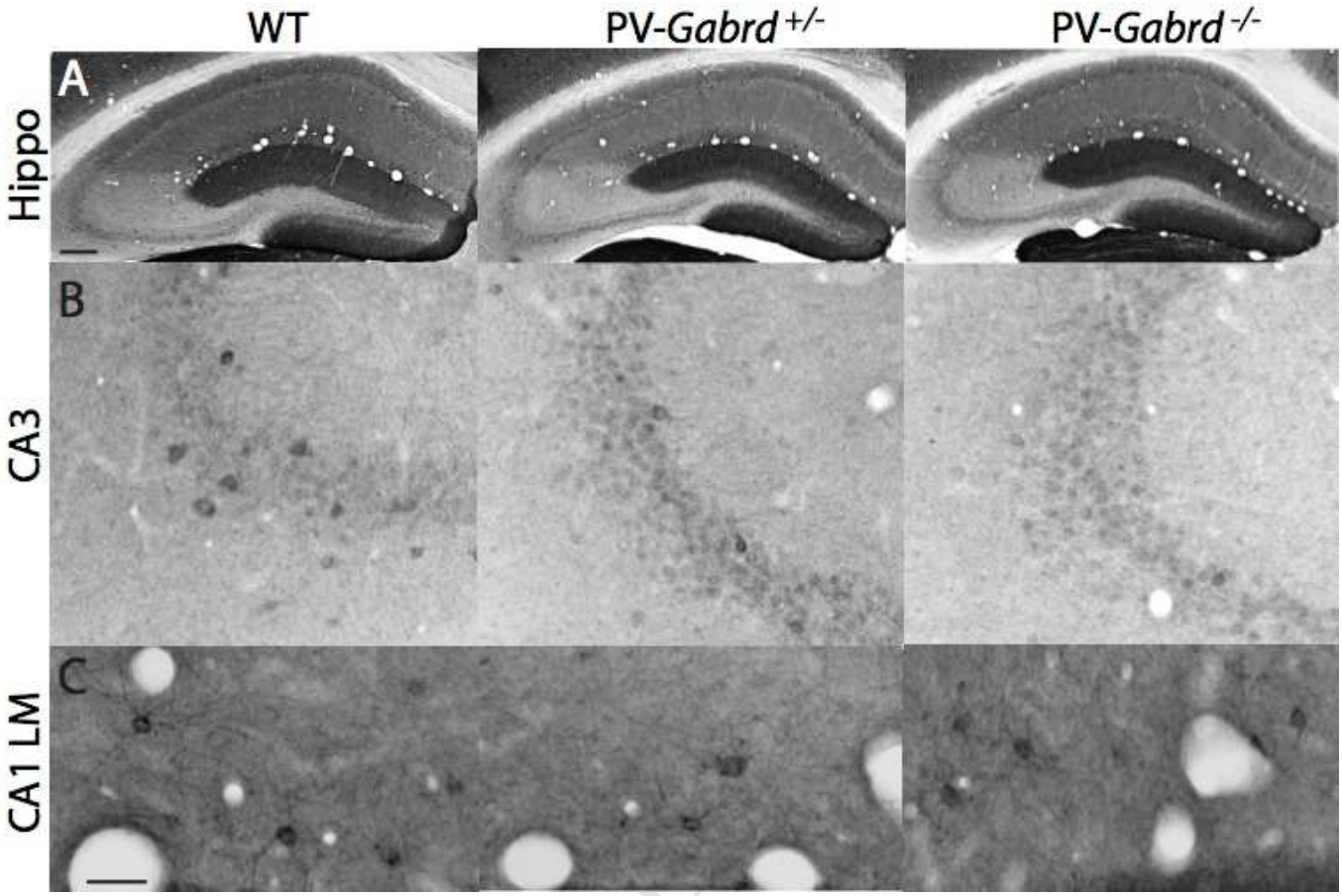

Figure 2

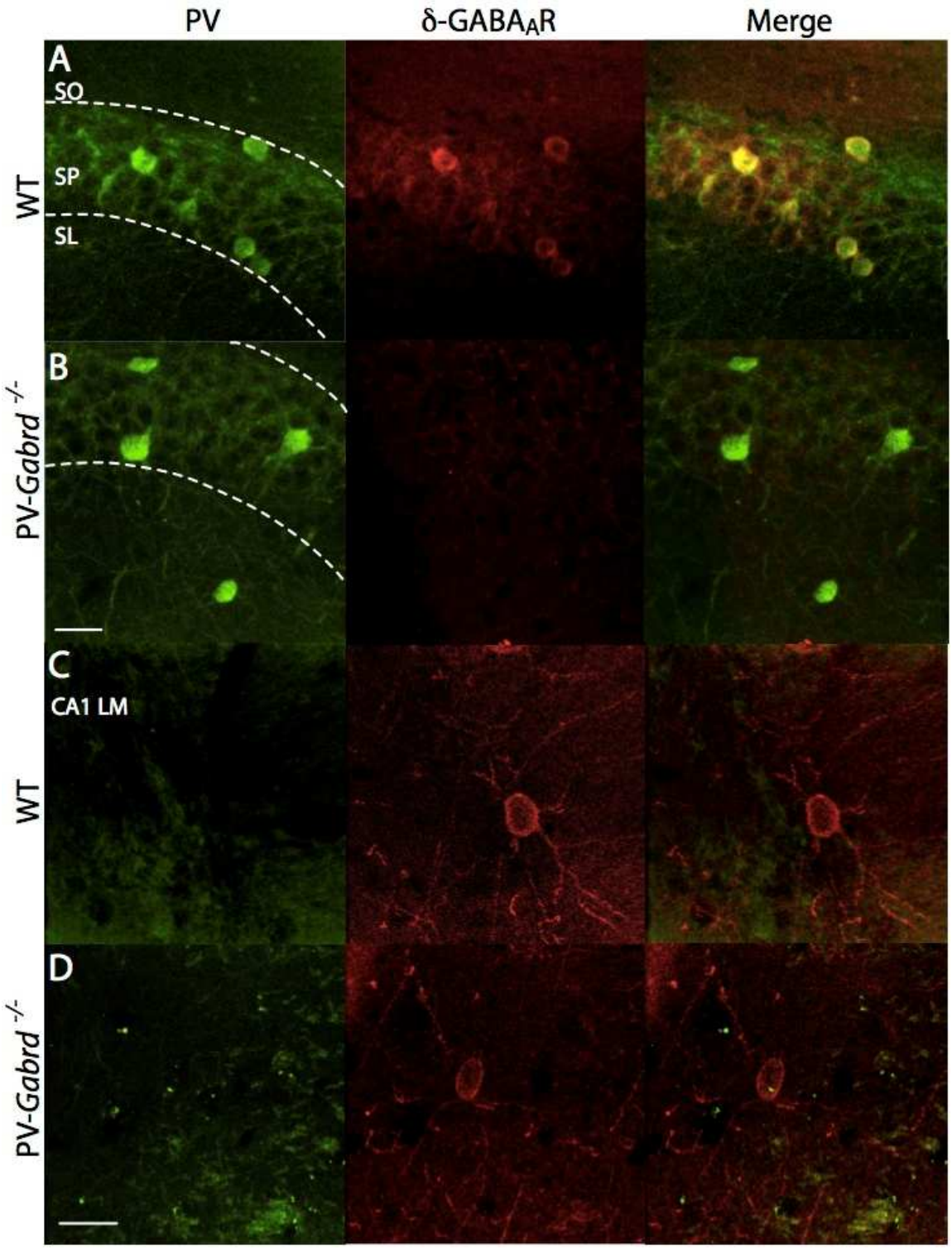

Figure 3

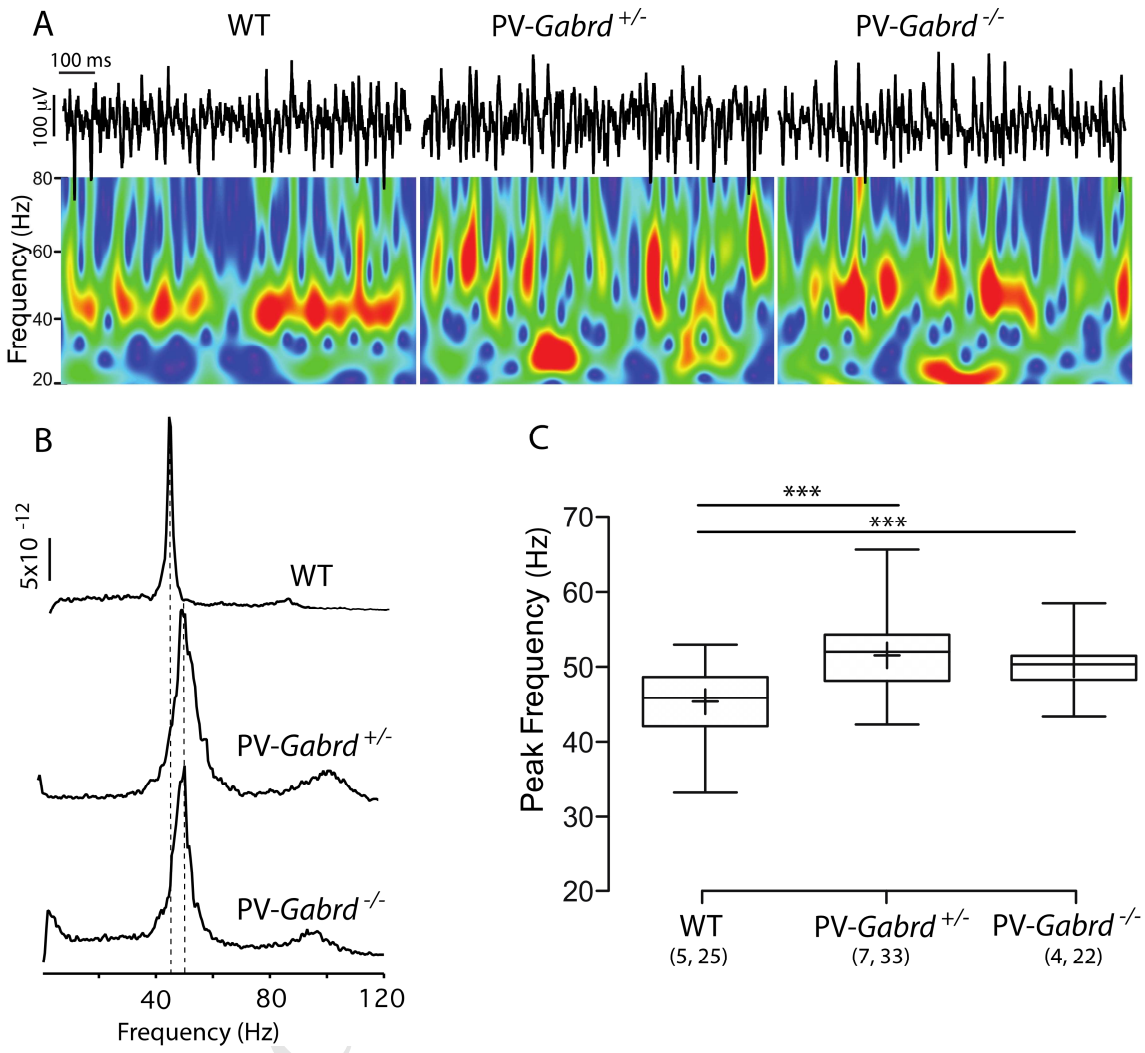
A
WT
PV-Gabrd +/-
PV-Gabrd -/-
100 ms
100 µV
Frequency (Hz)
80
60
40
20
B
5x10 -12
WT
PV-Gabrd +/-
PV-Gabrd -/-
40
80
120
Frequency (Hz)
C
***
***
Peak Frequency (Hz)
70
60
50
40
30
20
WT
(5, 25)
PV-Gabrd +/-
(7, 33)
PV-Gabrd -/-
(4, 22)

Table 1

	Peak frequency (Hz)			Power at peak frequency (×E-11 V^2s^{-1})			Total power 30-120 Hz (×E-10 V^2)		
	WT	PV-*Gabrd*$^{+/-}$	PV-*Gabrd*$^{-/-}$	WT	PV-*Gabrd*$^{+/-}$	PV-*Gabrd*$^{-/-}$	WT	PV-*Gabrd*$^{+/-}$	PV-*Gabrd*$^{-/-}$
No drug	47.8 (0.8) 14; 3	53.4 (1.0) 12; 3	50.8 (0.4) 14; 2	2.4 (0.4) 14; 3	4.2 (1.6) 12; 3	4.1 (0.8) 14; 2	6.6 (0.8) 14; 3	10.7 (4.0) 12; 3	8.5 (1.2) 14; 2
+ ALLO (100 nM)	48.0 (1.2) 7; 2	***47.8** (1.8) 6; 2	50.9 (0.7) 8; 2	3.6 (0.7) 7; 2	2.5 (1.1) 6; 2	6.1 (1.3) 8; 2	7.37 (1.3) 7; 2	5.2 (1.6) 6; 2	13.9 (3.1) 8; 2
+ DS-2 (10 µM)	47.9 (1.2) 7; 3	54.2 (1.1) 6; 3	51.6 (0.7) 6; 2	2.5 (0.6) 7; 3	7.7 (3.5) 6; 3	2.9 (0.9) 6; 2	6.8 (1.5) 7; 3	16.2 (4.9) 6; 3	7.3 (1.8) 6; 2

References

Belelli D, Lambert JJ (2005) Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 6:565–575.

Belluscio MA, Mizuseki K, Schmidt R, Kempter R, Buzsáki G (2012) Cross-frequency phase-phase coupling between θ and γ oscillations in the hippocampus. Journal of Neuroscience 32:423–435.

Bianchi MT, Macdonald RL (2003) Neurosteroids shift partial agonist activation of GABA(A) receptor channels from low- to high-efficacy gating patterns. Journal of Neuroscience 23:10934–10943.

Bieri KW, Bieri KW, Bobbitt KN, Bobbitt KN, Colgin LL, Colgin LL (2014) Slow and fast gamma rhythms coordinate different spatial coding modes in hippocampal place cells. Neuron 82:670–681.

Brack KE, Lovick TA (2007) Neuronal excitability in the periaqueductal grey matter during the estrous cycle in female Wistar rats. Neuroscience 144:325–335.

Bragin A, Jando G, Nadasdy Z, Hetke J, Wise K, Buzsaki G (1995) Gamma (40-100 Hz) oscillation in the hippocampus of the behaving rat. J Neurosci 15:47–60.

Brickley SG, Mody I (2012) Extrasynaptic GABA(A) Receptors: Their Function in the CNS and Implications for Disease. Neuron 73:23–34.

Brun VH, Otnass MK, Molden S, Steffenach H-A, Witter MP, Moser M-B, Moser EI (2002) Place cells and place recognition maintained by direct entorhinal-hippocampal circuitry. Science 296:2243–2246.

Buzsáki G, Wang X-J (2012) Mechanisms of gamma oscillations. Annu Rev Neurosci 35:203–225.

Capogna M, Pearce RA (2011) GABA(A,slow): causes and consequences. Trends Neurosci 34:101–112.

Cardin JA, Carlén M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai L-H, Moore CI (2009) Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature 459:663–667.

Carlén M, Meletis K, Siegle JH, Cardin JA, Futai K, Vierling-Claassen D, Rühlmann C, Jones SR, Deisseroth K, Sheng M, Moore CI, Tsai L-H (2012) A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. Mol Psychiatry 17:537–548.

Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, Houser CR, Olsen RW, Harrison NL, Homanics GE (2006) GABAA receptor alpha 4 subunits mediate

extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. Proc Natl Acad Sci USA 103:15230–15235.

Colgin LL, Denninger T, Fyhn M, Hafting T, Bonnevie T, Jensen O, Moser M-B, Moser EI (2009) Frequency of gamma oscillations routes flow of information in the hippocampus. Nature 462:353–357.

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

Curley AA, Lewis DA (2012) Cortical basket cell dysfunction in schizophrenia. J Physiol (Lond) 590:715–724.

Dibbens LM, Feng H-J, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, Berkovic SF, Macdonald RL, Mulley JC (2004) GABRD encoding a protein for extra- or peri-synaptic GABAA receptors is a susceptibility locus for generalized epilepsies. Hum Mol Genet 13:1315–1319.

Faragó N, Kocsis ÁK, Lovas S, Molnár G, Boldog E, Rózsa M, Szemenyei V, Vámos E, Nagy LI, Tamás G, Puskás LG (2013) Digital PCR to determine the number of transcripts from single neurons after patch-clamp recording. BioTechniques 54:327–336.

Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 6:215–229.

Feng H-J, Kang J-Q, Song L, Dibbens L, Mulley J, Macdonald RL (2006) Delta subunit susceptibility variants E177A and R220H associated with complex epilepsy alter channel gating and surface expression of alpha4beta2delta GABAA receptors. J Neurosci 26:1499–1506.

Feng Y, Kapornai K, Kiss E, Tamás Z, Mayer L, Baji I, Daróczi G, Benák I, Kothencné VO, Dombovári E, Kaczvinszk E, Besnyo M, Gádoros J, Székely J, Kovacs M, Vetró A, Kennedy JL, Barr CL (2010) Association of the GABRD gene and childhood-onset mood disorders. Genes Brain Behav 9:668–672.

Ferando I, Mody I (2012) GABAA receptor modulation by neurosteroids in models of temporal lobe epilepsies. Epilepsia 53 Suppl 9:89–101.

Ferando I, Mody I (2013) Altered gamma oscillations during pregnancy through loss of δ subunit-containing GABA(A) receptors on parvalbumin interneurons. Front Neural Circuits 7:144.

Fuchs EC, Zivkovic AR, Cunningham MO, Middleton S, Lebeau FEN, Bannerman DM, Rozov A, Whittington MA, Traub RD, Rawlins JNP, Monyer H (2007) Recruitment of parvalbumin-positive interneurons determines hippocampal function and associated behavior. Neuron 53:591–604.

Fuentealba P, Begum R, Capogna M, Jinno S, Marton LF, Csicsvari J, Thomson A, Somogyi P, Klausberger T (2008) Ivy cells: a population of nitric-oxide-producing, slow-spiking GABAergic neurons and their involvement in hippocampal network activity. Neuron 57:917–929.

Glykys J, Mann EO, Mody I (2008) Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? Journal of Neuroscience 28:1421–1426.

Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I (2007) A new naturally occurring GABA(A) receptor subunit partnership with high sensitivity to ethanol. Nat Neurosci 10:40–48.

Griffiths J, Lovick T (2005) Withdrawal from progesterone increases expression of alpha4, beta1, and delta GABA(A) receptor subunits in neurons in the periaqueductal gray matter in female Wistar rats. J Comp Neurol 486:89–97.

Gulyás AI, Szabó GG, Ulbert I, Holderith N, Monyer H, Erdélyi F, Szabó G, Freund TF, Hájos N (2010) Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. Journal of Neuroscience 30:15134–15145.

Jensen ML, Wafford KA, Brown AR, Belelli D, Lambert JJ, Mirza NR (2013) A study of subunit selectivity, mechanism and site of action of the delta selective compound 2 (DS2) at human recombinant and rodent native GABA(A) receptors. Br J Pharmacol 168:1118–1132.

Jia X, Kohn A (2011) Gamma rhythms in the brain. PLoS Biol 9:e1001045.

Korotkova T, Fuchs EC, Ponomarenko A, Engelhardt von J, Monyer H (2010) NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. Neuron 68:557–569.

Lasztoczi B, Klausberger T (2014) Layer-Specific GABAergic Control of Distinct Gamma Oscillations in the CA1 Hippocampus. Neuron 81:1126–1139.

Lee V, Maguire J (2013) Impact of inhibitory constraint of interneurons on neuronal excitability. J Neurophysiol 110:2520–2535.

Lewis DA (2014) ScienceDirectInhibitory neurons in human cortical circuits: substrate for cognitive dysfunction in schizophrenia. Curr Opin Neurobiol 26:22–26.

Lewis DA, Curley AA, Glausier JR, Volk DW (2012) Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci 35:57–67.

Lewis DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6:312–324.

Lovick TA (2006) Plasticity of GABAA receptor subunit expression during the oestrous cycle of the rat: implications for premenstrual syndrome in women. Exp Physiol 91:655–660.

Lovick TA (2008) GABA in the female brain -- oestrous cycle-related changes in GABAergic function in the periaqueductal grey matter. Pharmacol Biochem Behav 90:43–50.

Lovick TA, Griffiths JL, Dunn SMJ, Martin IL (2005) Changes in GABA(A) receptor subunit expression in the midbrain during the oestrous cycle in Wistar rats. Neuroscience 131:397–405.

Lu CB, Jefferys JGR, Toescu EC, Vreugdenhil M (2011) In vitro hippocampal gamma oscillation power as an index of in vivo CA3 gamma oscillation strength and spatial reference memory. NEUROBIOLOGY OF LEARNING AND MEMORY 95:221–230.

Maguire J, Ferando I, Simonsen C, Mody I (2009) Excitability changes related to GABAA receptor plasticity during pregnancy. Journal of Neuroscience 29:9592–9601.

Maguire J, Mody I (2007) Neurosteroid synthesis-mediated regulation of GABA(A) receptors: relevance to the ovarian cycle and stress. Journal of Neuroscience 27:2155–2162.

Maguire J, Mody I (2008) GABA(A)R plasticity during pregnancy: relevance to postpartum depression. Neuron 59:207–213.

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

Mann EO, Mody I (2010) Control of hippocampal gamma oscillation frequency by tonic inhibition and excitation of interneurons. Nat Neurosci 13:205–212.

Mann EO, Suckling JM, Hajos N, Greenfield SA, Paulsen O (2005) Perisomatic feedback inhibition underlies cholinergically induced fast network oscillations in the rat hippocampus in vitro. Neuron 45:105–117.

Meera P, Olsen RW, Otis TS, Wallner M (2009) Etomidate, propofol and the neurosteroid THDOC increase the GABA efficacy of recombinant alpha4beta3delta and alpha4beta3 GABA A receptors expressed in HEK cells. Neuropharmacology 56:155–160.

Milenkovic I, Vasiljevic M, Maurer D, Höger H, Klausberger T, Sieghart W (2013) The parvalbumin-positive interneurons in the mouse dentate gyrus express GABAA receptor subunits alpha1, beta2, and delta along their extrasynaptic cell membrane. Neuroscience 254C:80–96.

Mody I, Pearce RA (2004) Diversity of inhibitory neurotransmission through GABA(A) receptors. Trends Neurosci 27:569–575.

Oláh S, Füle M, Komlósi G, Varga C, Báldi R, Barzó P, Tamás G (2009) Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. Nature 461:1278–1281.

Pavlov I, Savtchenko LP, Song I, Koo J, Pimashkin A, Rusakov DA, Semyanov A (2014) Tonic GABAA conductance bidirectionally controls interneuron firing pattern and synchronization in the CA3 hippocampal network. Proc Natl Acad Sci USA 111:504–509.

Peng Z, Hauer B, Mihalek RM, Homanics GE, Sieghart W, Olsen RW, Houser CR (2002) 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.

Peng Z, Huang CS, Stell BM, Mody I, Houser CR (2004) Altered expression of the delta subunit of the GABAA receptor in a mouse model of temporal lobe epilepsy. Journal of Neuroscience 24:8629–8639.

Ruiz A, Campanac E, Scott RS, Rusakov DA, Kullmann DM (2010) Presynaptic GABAA receptors enhance transmission and LTP induction at hippocampal mossy fiber synapses. Nat Neurosci 13:431–438.

Sauer B (1998) Inducible gene targeting in mice using the Cre/lox system. Methods 14:381–392.

Shen H, Sabaliauskas N, Sherpa A, Fenton AA, Stelzer A, Aoki C, Smith SS (2010) A critical role for alpha4betadelta GABAA receptors in shaping learning deficits at puberty in mice. Science 327:1515–1518.

Smith SS (2013) α4βδ GABAA receptors and tonic inhibitory current during adolescence: effects on mood and synaptic plasticity. Front Neural Circuits 7:135.

Sohal VS, Zhang F, Yizhar O, Deisseroth K (2009) Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 459:698–702.

Sperk G, Schwarzer C, Tsunashima K, Fuchs K, Sieghart W (1997) GABA(A) receptor subunits in the rat hippocampus I: immunocytochemical distribution of 13 subunits. Neuroscience 80:987–1000.

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

Traub RD, Bibbig A, Fisahn A, LeBeau FE, Whittington MA, Buhl EH (2000) A model of gamma-frequency network oscillations induced in the rat CA3 region by carbachol in vitro. Eur J Neurosci 12:4093–4106.

Trigo FF, Marty A, Stell BM (2008) Axonal GABAA receptors. Eur J Neurosci 28:841–848.

Uhlhaas PJ, Pipa G, Neuenschwander S, Wibral M, Singer W (2011) A new look at gamma? High-

(>60 Hz) γ-band activity in cortical networks: function, mechanisms and impairment. Prog Biophys Mol Biol 105:14–28.

Uhlhaas PJ, Singer W (2012) Neuronal dynamics and neuropsychiatric disorders: toward a translational paradigm for dysfunctional large-scale networks. Neuron 75:963–980.

Wafford KA, van Niel MB, Ma QP, Horridge E, Herd MB, Peden DR, Belelli D, Lambert JJ (2009) Novel compounds selectively enhance delta subunit containing GABA A receptors and increase tonic currents in thalamus. Neuropharmacology 56:182–189.

Wei W, Peng Z, Mody I, Houser CR (2013) GABA(A) receptor mediated tonic currents in five types of neurons of the mouse dentate gyrus

. Neuroscience Meeting Planner Society for Neuroscience:1–2.

Wulff P, Ponomarenko AA, Bartos M, Korotkova TM, Fuchs EC, Bähner F, Both M, Tort ABL, Kopell NJ, Wisden W, Monyer H (2009) Hippocampal theta rhythm and its coupling with gamma oscillations require fast inhibition onto parvalbumin-positive interneurons. Proc Natl Acad Sci USA 106:3561–3566.

Yamamoto J, Suh J, Takeuchi D, Tonegawa S (2014) Successful execution of working memory linked to synchronized high-frequency gamma oscillations. Cell 157:845–857.

Yu J, Proddutur A, Elgammal FS, Ito T, Santhakumar V (2013) Status epilepticus enhances tonic GABA currents and depolarizes GABA reversal potential in dentate fast-spiking basket cells. J Neurophysiol 109:1746–1763.

Zheng K, An JJ, Yang F, Xu W, Xu Z-QD, Wu J, Hökfelt TGM, Fisahn A, Xu B, Lu B (2011) TrkB signaling in parvalbumin-positive interneurons is critical for gamma-band network synchronization in hippocampus. Proc Natl Acad Sci USA 108:17201–17206.