Altered GABA<sub>A,slow</sub> Inhibition and Network Oscillations in Mice Lacking the GABA<sub>A</sub> Receptor β<sub>3</sub> Subunit

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INTRODUCTION

Neuronal ensemble activity in the hippocampus of exploring rodents is dominated by network oscillations in the theta (5–12 Hz) and gamma (40–90 Hz) range. This activity hinges on the characteristics of inhibitory interneurons, many of which discharge during specific phases of theta and gamma oscillations (Fuentetalla et al. 2008; Klausberger et al. 2003; Penttonen et al. 1998; Tukker et al. 2007), inhibiting other interneurons and pyramidal neurons for periods ranging from a few milliseconds up to several hundred milliseconds (Banks et al. 2000; Chapman and Lacaille 1999; Cobb et al. 1995). By virtue of this phase-locked inhibition, interneurons orchestrate the interwoven hippocampal rhythms, including “nesting,” the amplitude modulation of gamma oscillations at theta frequency (Bragin et al. 1995; Penttonen et al. 1998; White et al. 2000).

A specific GABAergic conductance in hippocampal CA1, termed GABA<sub>A,slow</sub> (Banks et al. 1998; Pearce 1993), has a decay time of 30–70 ms and is able, in vitro, to silence interneurons producing fast inhibitory postsynaptic currents (IPSCs; GABA<sub>A,fast</sub>) for approximately one theta period (Banks et al. 2000). It has therefore been postulated to contribute to the nested coordination of theta and gamma rhythms (Banks et al. 2000; White et al. 2000). There is accumulating evidence that GABA<sub>A,slow</sub>, which also occurs in neocortex and subiculum, is generated by distal dendrite-targeting interneurons: neurogliaform neurons (Price et al. 2005, 2008; Szabadics et al. 2007), LM neurons (Ouardouz and Lacaille 1997), and OLM neurons (Pouille and Scanziani 2004), and ivy cells (Fuentetalla et al. 2008).

We hypothesized that the different kinetics of GABA<sub>A,fast</sub> and GABA<sub>A,slow</sub> would reflect different subunit compositions of their postsynaptic GABA<sub>A</sub> receptors. Of particular interest is the β<sub>3</sub> subunit, which is prominent in dendritic regions of hippocampus and dentate gyrus (Sperk et al. 1997). In other brain regions, it has been associated with slow GABAergic current kinetics: its absence in GABA<sub>A</sub> receptor β<sub>3</sub>-deficient (β<sub>3</sub><sup>−/−</sup>) mice confers fast decay kinetics on IPSCs in thalamic reticular (Huntsman et al. 1999) and neocortical neurons (Ramadan et al. 2003). Importantly, the β<sub>3</sub> subunit plays a crucial role in the coordination of network rhythms as has been shown in thalamus (Huntsman et al. 1999) and olfactory bulb (Nusser et al. 2001) of β<sub>3</sub><sup>−/−</sup> mice. These findings suggest that in addition to controlling hyperexcitability and preventing seizures, GABA<sub>A</sub> receptors with the β<sub>3</sub> subunit may occupy strategic positions in neuronal networks generating GABA<sub>A</sub> receptor-dependent rhythms. We used β<sub>3</sub><sup>−/−</sup> mice to determine the role these receptors play at GABA<sub>A,slow</sub> synapses and their contribution to the generation of hippocampal rhythms.

We show that GABA<sub>A,slow</sub> synapses containing β<sub>3</sub> subunits provide inhibitory input into pyramidal cells and that the absence of these currents in β<sub>3</sub><sup>−/−</sup> mice translates into weaker and less regular theta oscillations as well as weaker and faster gamma oscillations. By contrast, slow inhibitory control of...
GABA_{A,fast}-generating interneurons and amplitude modulation of gamma oscillations at theta frequency is maintained in the mutant mice. These results support a model in which the interaction between relatively independent inhibitory subcircuits oscillating at different frequencies generates the nested rhythms observed in hippocampus and other cortical structures.

**METHODS**

All experimental protocols conformed to American Physiological Society/National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee of the University of Wisconsin and the Institutional Animal Care and Use Committee of the University of Pittsburgh.

**Generation of β₃ knockouts**

Global β₃ knockouts and wild-type littermate controls were produced from heterozygous breeding pairs and genotyped by Southern blot analysis as described (Homanics et al. 1997). Mice were of a mixed C57BL/6J × Strain 129X1/S1 genetic background of the F10-14 generations.

**Preparation of hippocampal slices**

Mice aged 30–35 days (120–180 days for field recordings) were decapitated under deep halothane or isoflurane and ketamine anesthesia, and transverse hippocampal slices (400 μm thick) were prepared according to standard procedures (Banks et al. 1998). They were allowed to recover in dissection buffer at room temperature (field potential recordings) or 35°C (IPSC recordings) for ≥1 h before transfer to the recording chamber, which was perfused at 3 ml/min with artificial cerebrospinal fluid [ACSF, composition (in mM): 127 NaCl, 1.2 KCl, 26 NaHCO₃, 2.2 CaCl₂, 1.4 MgSO₄, and 10 glucose] saturated with 95% O2-5% CO2. Dissection buffer was identical to ACSF except for the addition of ascorbic acid (2.5 mM) and kynurenic acid (5 mM) in the case of field potential recordings.

**Whole cell recordings of IPSCs**

Putative pyramidal cells in stratum pyramidale (SP) of CA1 were visualized using a video camera (VE-1000; DAGE MTI, Michigan City, IN) connected to an upright microscope (BX-50WI; Olympus America, Melville, NY) equipped with an infrared band-pass filter (775 ± 75 nm), a long working-distance water-immersion objective (×40, NA 0.7) and differential interference contrast optics. The microscope and recording pipette were under remote control using an integrated motorized control system (Luigs and Neumann, Ratingen, Germany).

Whole cell recordings were obtained at room temperature (22–24°C) using a MultiClamp 700A (Axon Instruments, Union City, CA) patch-clamp amplifier. All data were collected using pClamp software (Axon Instruments). Data were filtered at 2–5 kHz and digitized at 5–10 kHz (Diigidata 1200, Axon Instruments). Patch pipettes were fabricated from borosilicate glass (K3-33, 1.7 mm OD, 1.1 mm ID; Garner Glass, Claremont, CA) using a Flaming-Brown two-stage puller (P-87; Sutter Instruments, Novato, CA), fire polished and coated with silicone elastomer (Sylgard, Dow Corning) to reduce electrode capacitance. Patch pipettes had open-tip resistances of 2–4 MΩ when filled with the recording solution (composition, in mM: 140 CsCl, 10 Na-HPEPS, 10 EGTA, 2 MgATP, and 5 QX-314, pH 7.3). Access resistances were typically ≥10–20 MΩ and were then compensated 60–80%. Cells were held at ~60 mV. GABA_A,fast IPSCs were isolated by bath application of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 40 μM d,l-2-amino-5-phosphonovaleric acid (d,l-APV) to block AMPA and N-methyl-D-aspartate (NMDA)-mediated currents and by the inclusion of CsCl and QX-314 in the patch pipette, both of which block, among other potassium currents, GABA_B receptor-mediated currents (Nathan et al. 1990).

Evoked IPSCs were elicited by applying stimuli (1–100 μA) to SP and s. lacunosum-moleculare (SLM) using patch electrodes filled with ACSF. SP stimulating electrodes were placed as close to the recorded cell body as possible, and stimuli were applied at a rate of 0.2 Hz. For SLM stimuli, a maximum stimulation rate of 0.05 Hz was used to minimize the previously observed rundown of GABA_A,slow over time (Pearce 1993). SLM stimulating electrodes were consistently placed at ~50 μm on the SLM side of the hippocampal fissure (HF), ~100 μm deep in the tissue and at the same mediolateral position in CA1 as the apical dendrite of the cell being recorded. In those cells in which no evoked GABA_A,slow IPSC was observed, the electrode was repositioned within SLM until a response could be elicited or (more typically) a region ~100 × 100 μm in SLM had been searched to no avail. In all cases, maximal currents were determined by varying stimulus intensity until the response amplitude no longer increased.

**IPSC analysis**

Spontaneous GABAergic IPSCs were detected using an automated event detection algorithm based on a “pseudo-differentiation” as described previously (Banks et al. 2000). GABA_A,slow IPSCs were well fit with the sum of single rising and decaying exponential components. Although GABA_A,fast IPSCs decayed biexponentially (Banks et al. 1998), the decay was characterized using the weighted sum of these two exponential components (τ_{Decay, wi}). Spontaneous GABA_A,slow IPSCs were defined as those events having 10–90% rise times >4 ms and decay times >40 ms. Individual spontaneous IPSCs were selected for averaging and exponential curve fitting when no other detected events occurred within ±100 ms (GABA_A,fast) or ±250 ms (GABA_A,slow) of the peak.

Stimulation in SLM inhibits interneurons producing GABA_A,fast IPSCs for several hundred milliseconds poststimulus (Banks et al. 2000). We quantified this suppression of fast inhibition (SFI) in terms of changes in the frequency of GABA_A,fast IPSCs recorded in pyramidal neurons. As action-potential-independent miniature IPSCs persist during SFI (Banks et al. 2000), the frequency of IPSCs underestimates the extent of SFI. Therefore we also quantified the average “instantaneous” poststimulus current in pyramidal neurons carried by GABA_A,fast IPSCs. To this end, the amplitudes of all detected GABA_A,fast IPSCs were determined by subtracting from the peak IPSC amplitude the mean of the current trace in an interval of [−2.5 to −1.5] ms relative to the peak. Note that this procedure was robust to changes in the base line or to the presence of GABA_A,slow IPSCs but underestimated the amplitudes of IPSCs preceded by other GABA_A,fast IPSCs. Each peristimulus current trace was reconstructed by adding artificial IPSCs of assessed amplitudes and uniform decay times (16 ms for wild-type animals, 14 ms for β₃−/− animals, see Fig. 2) to a zero baseline. All sweeps of one experiment were averaged and then normalized to the average current in the recorded prestimulus interval, which was either [−400 to 0] or [−800 to 0] ms. The extent of SFI was quantified by the average normalized current in a poststimulus interval of 50–300 ms. Due to the long duration of SFI, the method was insensitive to the exact value of the decay time: variations of this parameter by ±2 ms (>10%) resulted in <1% change of the average normalized poststimulus current.

**Conditioned depression and field recordings**

Experiments were performed at room temperature (22–24°C). Pipettes were similar to those used for whole cell recordings but were filled with ACSF (resistance: 2–4 MΩ) to record field potentials. Bipolar stimulating electrodes were fabricated from tungsten (Micro-electrodes Tungsten, World Precision Instruments, Sarasota, FL). For the conditioned depression paradigm (Fig. 3A) (Benkwitz et al. 2007; Pouille and Scanziani 2004), the recording electrode was placed in the
CA1 pyramidal layer. One stimulating electrode was placed in alveus, lateral of the recording electrode, to activate recurrent (feedback) inhibition (conditioning pulse). The second electrode was placed in s. radiatum (SR) to activate Schaffer collateral inputs and thus evoke population responses (population spike) in pyramidal neurons. Conditioned responses were obtained at interstimulus intervals ranging from 5 to 2,000 ms and compared with the unconditioned response, which was the population spike evoked without prior alveus stimulus. Current pulses (0.1 ms duration) were delivered via constant current stimulus isolators (Model A365D, World Precision Instruments) at a stimulus rate of 0.05 Hz and were adjusted throughout the course of the experiment such that SR stimulation elicited half-maximal responses. Alveus stimuli were always supramaximal and thus most likely recruited, directly or via CA1 axon collaterals, a substantial number of “late persistent” (putative GABA$_{A,slow}$-generating) (Maccalferri et al. 2000) O-LM interneurons in addition to the more excitable “onset transient” (putative GABA$_{A,fast}$-generating) interneurons (Pouille and Scanziani 2004).

All recordings were obtained in current-clamp mode using an Axopatch 200B patch clamp amplifier (Axon Instruments, Union City, CA) and pClamp 8.0 software (Axon Instruments). Field potentials were low-pass filtered at 5 kHz and digitized at 10 kHz (Digidata 1200, Axon Instruments). For data analysis ClampFit 8.0 (Axon Instruments), Origin 6.1 (MicroCal, Northampton, MA), MS Excel (Microsoft, Redmond, WA), Prism 4.0 (GraphPad, San Diego, CA) and custom-written Matlab 6.5.1 (The MathWorks, Natick, MA) routines were used.

In vivo surgery and electrophysiology

Animals used for in vivo recordings were aged 10–56 wk ($\beta_3^{+/-}$) and 32–57 wk ($\beta_3^{-/-}$).

Field potentials from area CA1 of the dorsal hippocampus were recorded as described previously (Hentschke et al. 2007). Briefly, under isoflurane anesthesia, the animals were chronically implanted with 16-channel linear microwire arrays (Jellemza and Weijnen 1991). With 16 electrode sites separated by 100 $\mu$m, the electrodes spanned the hippocampus from the granule cell layer of the ventral leaf of dentate gyrus to the alveus in CA1.

The animals were allowed to recover for 1 wk before their first recording session. They were placed in an open plastic tray (20 x 30 cm) and allowed to move freely throughout the experiment. An observer classified the animal’s behavior as “exploring,” “immobile,” and “grooming.” Local field potentials were recorded at a bandwidth of 1–300 Hz using a small headstage preamplifier (HS-16, Neuralynx, Tucson, AZ) and two 8-channel amplifiers (Lynx-8, Neuralynx). A screw in the occipital bone served as the electrical reference (“animal ground”). Data were digitized at 1 kHz (Digidata 1322A, Molecular Devices, Union City, CA) and allowed to move freely throughout the experiment. Alveus stimuli were always supramaximal and thus most likely recruited, directly or via CA1 axon collaterals, a substantial number of “late persistent” (putative GABA$_{A,slow}$-generating) (Maccalferri et al. 2000) O-LM interneurons in addition to the more excitable “onset transient” (putative GABA$_{A,fast}$-generating) interneurons (Pouille and Scanziani 2004).

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In vivo data analysis

All data analysis was performed with custom-written routines in Matlab v7.2 (Mathworks) as previously described (Hentschke et al. 2007). Briefly, in a preprocessing step, the raw data were passed through digital band-pass filters designed for the extraction of signals in different frequency bands. The –3 dB (corner) frequencies were 5–12 Hz for theta and 40–90 Hz for gamma. Gamma signals were additionally passed through a bandstop (notch) filter (corner frequencies: 59 and 61 Hz) to eliminate A/C line frequency noise. The raw and filtered field potential data were divided into variably sized chunks according to the animal’s behavior. These chunks were subdivided into segments of 4,096 points each, corresponding to 4,096 ms, overlapping by a third (1,365 points). Remaining data segments shorter than this duration were discarded. All spectral and cross-correlation parameters were computed for each segment, averaged within each animal, and then averaged across the population. The gamma centroid (Fig. 5D), a measure of the average gamma frequency, was obtained by weighting frequency bins in the range [40–90 Hz] by their power and calculating the mean.

Statistical analysis

For statistical evaluation of the differences between genotypes in in vitro experiments, we used unpaired, two-tailed t-test or analyses of variance with Bonferroni-corrected post hoc tests. Statistical analyses of in vivo data were based on analytical descriptions of the depth profiles of the various parameters analyzed (Hentschke et al. 2007). For each parameter, we chose a function (see following text) with recording depth as the independent variable. The functions have no theoretical underpinnings related to hippocampal physiology but were chosen to fit the data well with a minimum of free parameters. $P$ values <0.05 computed from an $F$-test were interpreted to indicate a difference between the complete depth profiles of the genotypes. In cases of significant effects, we employed Bonferroni-corrected t-test for unpaired data to identify individual recording sites with significant differences between genotypes.

The functions fitted to the depth profiles were as follows [x, the recording depth, ranged from 0 mm (hippocampal fissure) to 6.6 mm (alveus)]: $a + be^{-cx}$; theta and gamma power (Fig. 5, C and D); $a + bx + cx^2 + dx^3$; gamma centroid (Fig. 5D); $a + be^{-cx^{1.5}}$; CV$_{amplitude}$ and CV of the gamma envelope (Fig. 6A) and CV$_{ei}$ of the gamma envelope (Fig. 7E); $a + bx + cx^2$; CV$_{amplitude}$ of the gamma envelope (Fig. 7E); $a/(1 + be^{-cx})$; lags of theta cross-correlation (Fig. 6C); $1 - a + bx^2 + ae^{-cx^2}$; peaks of theta cross-correlation (Fig. 6C); $a + bx + [c/(1 + e^{-dx^2})]$; lags of C$_{theta,yam}$ (Fig. 8C); and $a + bx + cx^2$; peaks of C$_{theta,yam}$ (Fig. 8C) and $|\gamma_{theta,theta}(\beta_3^{-/-})|/|\gamma_{theta,theta}(\beta_3^{+/-})|$ (Fig. 7D).

Additionally, to obtain a statistical measure of the difference between genotypes independent of this analytical approach and hypothesis testing, we also report an effect statistic, Hedges’ $d$ (denoted $d$), which is a standardized difference between two means, including 95% confidence intervals (CIs) (Nakagawa and Cuthill 2007) for all recording sites and parameters. $d$ can attain any value. The higher the absolute value of $d$, the stronger the effect, and in cases in which the 95% CI do not encompass zero the effect can be considered significant ($P < 0.05$) within the framework of hypothesis testing (Nakagawa and Cuthill 2007).

All results except for $d$ are presented as means ± SD.

RESULTS

GABA$_{A,slow}$ is largely absent from CA1 pyramidal neurons of $\beta_3^{-/-}$ mice

Spontaneous IPSCs were recorded from CA1 pyramidal neurons in hippocampal slices of wild-type and $\beta_3^{-/-}$ mice at room temperature. In wild-type animals, IPSCs with fast decay kinetics (~15 ms, here termed GABA$_{A,fast}$) were interspersed with events of much slower decay, corresponding to GABA$_{A,slow}$ (Pearce 1993) (Fig. 1A). As has been described before, these events were three to four orders of magnitude rarer than fast IPSCs but could be readily identified by their slower kinetics (Banks et al. 1998) (Fig. 1, B and C). Current traces from pyramidal neurons in $\beta_3^{-/-}$ slices also contained numerous fast IPSCs but, in contrast to traces from wild-type animals, were largely devoid of slow IPSCs (Fig. 1, A–C). Analysis of current traces from 11 wild-type and 14 $\beta_3^{-/-}$ cells confirmed that spontaneous GABA$_{A,slow}$ IPSCs were ex-
extremely rare in the latter (Fig. 2A). The few spontaneous events that were detected did not differ significantly from those in wild-type animals in amplitude or kinetics (Fig. 2A).

The paucity of spontaneous GABA<sub>slow</sub> IPSCs has been attributed to very low spontaneous firing rates of the underlying interneuron classes (Banks et al. 1998). The even lower incidence of GABA<sub>slow</sub> sIPSCs in <sup>−/−</sup> mice might be attributed to accordingly lower firing rates of the underlying interneurons in this genotype. Therefore to assess the prevalence of GABA<sub>slow</sub> independently of spontaneous activity, we recorded evoked GABA<sub>slow</sub> IPSCs by electrically stimulating in SLM. In slices from mutant mice, GABA<sub>slow</sub> currents thus evoked had less than a third of the amplitude of the wild-type counterparts (Fig. 2A), which was consistent with a substantial reduction of the number of postsynaptic receptors mediating GABA<sub>slow</sub> currents in <sup>−/−</sup> mice.

Our findings thus far demonstrate that elimination of the β<sub>3</sub> subunit in fact severely diminished GABA<sub>slow</sub> in pyramidal neurons but did not completely abolish it. Interestingly, differences between genotypes were not restricted to GABA<sub>slow</sub>-GABA<sub>fast</sub> IPSCs decayed slightly faster in mutant mice than in wild-type animals (Fig. 2B) as can also be seen in the example traces in Fig. 1. Other IPSC parameters were not significantly different between the genotypes.

Divergent roles of β<sub>3</sub>-containing GABA<sub>fast</sub> receptors in recurrent inhibition and suppression of fast inhibition

GABA<sub>slow</sub> IPSCs exert a powerful and long-lasting (up to hundreds of milliseconds) inhibition of interneurons and pyramidal neurons when evoked by electrical stimulation (Banks et al. 2000). Therefore alterations of this current should affect the excitability of both neuron types within the duration of the evoked IPSC. We tested this hypothesis with two different sets of experiments. First, to assess the inhibitory impact of GABA<sub>slow</sub> on pyramidal neurons, we applied the condition-
ing pulse paradigm (Fig. 3A) (Pearce 1996). One stimulation electrode in the alveus delivered the first (conditioning) pulse. The pulse excited CA1 pyramidal axons antidromically and always produced a population spike at a very short latency (Fig. 3B). Strong electrical stimuli, employed here, also produced a field excitatory postsynaptic potential (EPSP) and, in pyramidal neurons, long-lasting inhibition, which is largely independent of GABAA,fast (Pearce 1993). With a variable time delay, the second stimulation electrode, positioned in SR, excited Schaffer collateral/commissural input and gave rise to a second, orthodromically evoked population spike. The long-lasting inhibition of pyramidal neurons due to the first (conditioning) pulse caused the population spike evoked by the second pulse to be reduced in amplitude compared with an unconditioned response, evoked by the same stimulus in SR but without prior conditioning stimulus (Fig. 3B, left). This effect has been attributed to GABAA,slow (Pearce 1993; Pearce et al. 1995). In wild-type animals, the conditioned response recovered with time in an almost logarithmic fashion, approaching amplitudes of the unconditioned pulse with interstimulus intervals of 500 ms (Fig. 3C). In \( \beta_3^{-/-} \) hippocampus,

\[ \text{FIG. 3. Conditioned depression is weaker in } \beta_3^{-/-} \text{ mice.} \]

- **A**: simplified schematic of CA1 circuitry and illustration of the approximate locations of the recording electrode in stratum pyramidale (SP) and stimulation sites in alveus (StimALV, conditioning stimulus) and stratum radiatum (StimSR). Also shown are pyramidal cells (PYR), interneurons (IN), and the major putative axonal pathways activated by the stimuli.
- **B**: field potential responses elicited with a single stimulus in SR (left) and paired stimuli (conditioning stimulus in ALV followed by a single stimulus in SR) with different inter stimulus intervals (ISIs; 3 separate sweeps overlaid; stimuli in SR marked by arrows on top and population spikes marked by thin arrows at the bottom). Note the antidromically evoked, short-latency population spike (PS) following the conditioning stimulus in alveus. C: plots of the amplitude of the conditioned population spike, normalized to the amplitude as obtained with a stimulus in SR without prior conditioning pulse. Interstimulus intervals ranged from 5 to 2,000 ms. Stars indicate \( P < 0.05 \) as computed by a 2-way ANOVA followed by Bonferroni-corrected post hoc tests comparing wild-type animals \((n = 7)\) and mutants \((n = 4)\).
by contrast, conditioned responses were stronger already at the shortest interstimulus interval tested (5 ms). At an interstimulus interval of 20 ms responses reached a plateau of ~80% recovery, and from 200 ms onward roughly corresponded to those seen in wild-type animals. Statistically significant differences between the genotypes were found for interstimulus intervals of 10–40 ms (2-way ANOVA followed by Bonferroni-corrected post hoc tests), a range that is compatible with both dendritic shunting and somatic hyperpolarization of pyramidal cells by GABA_A,slow currents. Over these intervals, the conditioned response in β_3−/− mice was about twice that of the wild-type animals. We conclude that in β_3−/− mice, stimulus-evoked, long-lasting inhibition in pyramidal neurons is severely impaired, and that the substantial reduction of GABA_A,slow currents (Fig. 2A) is the likely cause.

To assess the inhibitory impact of GABA_A,slow on GABA_A,fast–producing interneurons, an electrical stimulus was delivered in SLM and the ensuing temporary suppression of fast inhibition (SFI) quantified (Banks et al. 2000) (Fig. 4). In the recording from a wild-type hippocampus shown in Fig. 4, A–C, fast IPSCs were diminished in frequency and amplitude for a period of several hundred milliseconds poststimulus as has been reported previously (Banks et al. 2000). The resultant drop in the “instantaneous” average current (Fig. 4C, see METHODS) was quantified in a poststimulus time window of 50–300 ms. Surprisingly, fast IPSCs were suppressed to similar levels in both genotypes: in wild-type animals to 42 ± 20% and in β_3−/− animals to 31 ± 17% of the prestimulus level (t-test, P = 0.32; Fig. 4D). There was only a weak negative correlation (correlation coefficient, −0.26) between spontaneous IPSC frequency and the degree of SFI (Fig. 4E), indicating only a weak contribution of (nonsuppressible) miniature IPSCs to the net inhibition of pyramidal cells.

In summary, these in vitro experiments established that there is a substantial loss of GABA_A,slow/long-lasting inhibition in pyramidal cells but not in fast IPSC-generating interneurons, in the CA1 region in the β_3−/− genotype.

![Image](https://example.com/image1.png)

**FIG. 4.** Suppression of fast inhibition. A: 10 current sweeps with stimulation in s. lacunosum-moleculare (SLM) at t = 0 (arrowhead) recorded from a pyramidal cell of a wild-type animal. Stimulation artifact was clipped. B: peri-stimulus time histogram of GABA_A,fast IPSCs. The amplitude range was clipped to 400 pA to enhance detail in the lower range. Bin widths were 25 ms and 25 pA. C: instantaneous average current, normalized to the average value in [−800 0] ms. Gray shaded area demarcates poststimulus analysis window of [50 300] ms. D: population averages of average current in poststimulus window (β_3+/+, n = 6; β_3−/−, n = 7). E: scatterplot of poststimulus current vs. IPSC frequency.
Absence of the β3 subunit alters hippocampal theta and gamma oscillations but does not interfere with nesting

To investigate how the differences between genotypes in terms of slow inhibition in hippocampal subnetworks translated into differences of rhythms involving the whole hippocampal network, we performed multi-site field potential recordings from CA1 of freely moving β3−/− and wild-type animals. In agreement with previous work (Buzsáki et al. 2003; Hentschke et al. 2007), field potentials in wild-type mice had strong components in the theta (5–12 Hz) and gamma (40–90 Hz) bands that were more prominent when the animals explored their environment than when they were immobile (Fig. 5, A and B). As the behavioral dependence of hippocampal rhythms in mice has been investigated in detail (Hentschke et al. 2007) and due to the occurrence of epileptiform activity during immobility in β3−/− mice (Supplementary Methods and Supplementary Fig. S1), we restricted the following analyses to exploring animals. Theta and gamma oscillations were altered in several respects in the mutant mice. Theta oscillations were significantly

1 The online version of this article contains supplemental data.
weaker and slower than in wild-type animals (Fig. 5C; \( P < 0.01 \) for theta power and \( P < 0.0001 \) for peak frequency. Also note the high values of \( d \) in SR/SLM). Gamma oscillations were also much weaker in \( \beta_3^{-/-} \) mice (Fig. 5D, left, \( P < 0.0001 \)). However, in contrast to theta oscillations, gamma oscillations in \( \beta_3^{-/-} \) animals were faster than in wild-type animals (Fig. 5D, right, \( P < 0.0001 \)), possibly reflecting the faster decay of IPSCs in these animals (Fig. 2B).

Next we investigated the regularity and coordination across laminae of theta rhythms. The regularity of theta oscillations at each recording site was expressed as the coefficient of variation of the time intervals between troughs (CV_{IPI}, Fig. 6A, left) and the coefficient of variation of trough amplitudes (CV_{amplitude}, Fig. 6A, right). In both genotypes, CV_{amplitude} was approximately twice as large as CV_{IPI} (Fig. 6A). The laminar profiles of both parameters had a peak at the border of SP and SR, highlighting the sites of least regular theta rhythms. In \( \beta_3^{-/-} \) mice, theta oscillations were significantly less regular (CV_{IPI} and CV_{amplitude}, \( P < 0.001 \)), particularly in the dendritic laminae.

Cross-correlations of theta signals between the hippocampal fissure (HF), which served as the reference site, and all other sites, revealed the well-known gradual shift of theta phase across laminae reaching half a theta period lag (50 ms) in the alveus (Fig. 6B). This average laminar profile of theta phase lags was indistinguishable between genotypes (Fig. 6C, left, \( P = 0.15 \)). However, \( \beta_3^{-/-} \) mice had significantly lower peak cross-correlation values (Fig. 6C, right, \( P < 0.01 \)). This impaired coordination of theta oscillations at different sites, together with the finding that theta was less powerful and more variable in the apical dendritic region, is consistent with the idea that \( \beta_3^{-/-} \) mice lack an important element shaping theta oscillations.

Next we examined whether the amplitude modulation of gamma at theta frequency (nesting, Fig. 7A) was affected by the elimination of the \( \beta_3 \) subunit. For this purpose, we computed the power of the gamma envelope (\( \gamma_{\text{Env}} \)) in a narrow theta frequency band \( |\gamma_{\text{Env}}(\text{theta})|^2 \) (Fig. 7B) and compared it to gamma power \( |\gamma|^2 \) as quantified in Fig. 5D. Within each genotype, there was an excellent correlation between both parameters at HF (Fig. 7C), SLM, distal SR, SO and alveus (data not shown, \( R^2 \) ranging from 0.66 to 0.98). Furthermore, the ratio of \( |\gamma_{\text{Env}}(\text{theta})|^2 \) and \( |\gamma|^2 \) was not significantly different between genotypes (Fig. 7D, \( P = 0.26 \)). The fact that both parameters scaled linearly and to very similar degrees in both genotypes illustrates that the absence of the \( \beta_3 \) subunit did not impair the amplitude modulation of gamma appreciably despite a strong effect on gamma power. This was further underlined by minor or no differences in the amplitude and timing variability of \( \gamma_{\text{Env}} \), respectively (Fig. 7E): only CV_{IPI} was significantly higher in \( \beta_3^{-/-} \) animals than in wild-type animals (\( P < 0.0001 \)), and the difference between the genotypes was small (maximum: 7.8% at HF).

Finally, we inspected the relation between \( \gamma_{\text{Env}} \) to theta oscillations (Fig. 8). For each recording site, the cross-correlation between the two signals was computed. As \( \gamma_{\text{Env}} \) did not shift across laminae (Fig. 8A), the resulting cross-correlation functions essentially recapitulated the laminar phase shift of theta signals (Fig. 8B) (Hentschke et al. 2007). As in the case of theta, laminar phase profile differences did not differ between the genotypes (Fig. 8C, left, \( P = 0.27 \)). However, the peak cross-correlation, which quantifies the similarity between theta and \( \gamma_{\text{Env}} \), was significantly lower in \( \beta_3^{-/-} \) mice (Fig. 8C, right, \( P < 0.001 \)). This impaired coordination between the two signals likely reflects de-
graded theta oscillations (Fig. 6) because gamma nesting was largely preserved (Fig. 7).

**DISCUSSION**

In the present study, we show that in the hippocampal CA1 region, GABA$_A$ receptors with the $\beta_3$ subunit contribute substantially to the generation of GABA$_A$slow in pyramidal neurons, participate in long-lasting recurrent inhibition in hippocampal CA1 pyramidal neurons, and contribute to the generation or expression of gamma and theta rhythms. By contrast, these receptors are not required for the suppression of GABA$_A$fast-producing interneurons and the nesting of gamma oscillations at theta frequencies. Before expanding on these points we discuss the suitability of the animal model we used.

$\beta_3^{−/−}$ phenotype

The $\beta_3$ subunit is found in the neonatal and postnatal rodent brain (Fritschy et al. 1994; Laurie et al. 1992) and is involved in the activity-dependent expression of GABA$_A$ receptors (Saliba et al. 2007). Its unavailability in $\beta_3^{−/−}$ mice halves the expression of GABA$_A$ receptors in the brain and leads to 90% neonatal mortality and behavioral and neurological deficits in the surviving animals (DeLorey et al. 1998; Homanics et al. 1997; Krasowski et al. 1998). A detailed analysis of receptor composition in neocortex revealed that in $\beta_3^{−/−}$ mice the density of GABA$_A$ receptors containing $\alpha_2$ and $\alpha_3$ subunits was greatly reduced (Ramadan et al. 2003), as these subunits from the occurrence of epileptic activity in the animals, such as selective cell loss (see following text).

GABA$_A$slow and GABA$_A$fast in pyramidal neurons are mediated in part by GABA$_A$ receptors containing the $\beta_3^{−/−}$ subunit

The $\beta_3$ subunit is prominent in dendritic compartments of hippocampal pyramidal cells (Sperk et al. 1997). It has previ-
ously been found to confer slow kinetics on GABA_A receptors in other brain regions (Huntsman et al. 1999; Ramadan et al. 2003). As GABA_A,slow IPSCs originate in the dendrites of pyramidal neurons (Banks et al. 1998; Pearce 1993), we considered receptors containing the β_3 subunit to be likely candidates mediating GABA_A,slow in these neurons. Barring the possibility of a drastic and selective reduction of the number of GABA_A,slow-generating neurons in β_3^{-/-} mice, our results confirm this hypothesis. In addition, the slight but significant acceleration of GABA_A,fast IPSC decay in β_3^{-/-} mice betrayed the presence of the β_3 subunit in (synaptic) GABA_A receptors mediating GABA_A,fast IPSCs.

A likely explanation for the alteration of both GABA_A,slow and GABA_A,fast IPSCs is the presence and prevalence in hippocampus of two GABA_A receptor subtypes containing the β_3 subunit with different kinetics and little spatial overlap: α_5β_3γ_2 and α_5β_3γ_2. As both α_5 (Brüning et al. 2002) and β_3 (Scotti and Reuter 2001) subunits are constituents of peri- and extrasynaptic GABA_A receptors of pyramidal neurons, and given the likely contribution of spillover to GABA_A,slow IPSCs (Banks et al. 2000), it appears likely that receptors incorporating these two subunits contribute substantially to GABA_A,slow. The recent findings that in hippocampus the α_5 subunit contributes to slow phasic currents (Prenosil et al. 2006; Zarnowska et al. 2008) as well as tonic GABAergic currents (Caraiscos et al. 2004; Prenosil et al. 2006) is in accord with this idea.

The β_3 subunit also associates with α_5 subunits (Fritschy and Möhler 1995), forming synaptic GABA_A receptors on pyramidal cells (Brüning et al. 2002; Fritschy et al. 1998). Given the strongly reduced expression of the α_5 subunit in β_3^{-/-} mice (Ramadan et al. 2003), we suggest that the significantly faster IPSC decay of GABA_A,fast in this genotype as compared with wild-type animals stems from a relative shift toward receptors containing α_1 and β_2 subunits (Okada et al. 2000; Vicini et al. 2001). The preserved high frequency and amplitude of fast IPSCs in pyramidal neurons of β_3^{-/-} mice suggest that either the other interneurons (such as parvalbumin-positive basket cells) that utilize other receptor types (Klausberger et al. 2002) increase their firing rate or that subunit replacement occurs.

Slow phasic GABAergic currents meeting most of the characteristics of GABA_A,slow have recently been described in neocortex (Scheniak and MacIver 2008; Szabadics et al. 2007) and subiculum (Prenosil et al. 2006). In the subiculum, in contrast to hippocampus, GABA_A,slow does not depend on the α_5 subunit (Prenosil et al. 2006), demonstrating that GABAergic IPSCs with slow kinetics may be mediated by several different receptor subtypes. Similarly the remaining (albeit very sparse) GABA_A,slow in pyramidal neurons of β_3^{-/-} mice in our study point to the existence of subtypes of the GABA_A receptor that are devoid of β_3 subunits but nonetheless possess slow kinetics, such as the population of small-amplitude GABA_A,slow IPSCs that lack α_5 subunits (and likely β_3 subunits) identified recently in CA1.
cells (Zarnowska et al. 2008). Rather than depending on the slow intrinsic kinetics of their constituent receptors, these synapses may reflect the presence of a specific architecture and/or a higher proportion of perisynaptic versus synaptic receptors that could lead to slower current kinetics independent of subunit composition (Szabadi et al. 2007).

**Inhibition in local CA1 circuitry**

Due to the large charges they transfer, IPSCs with slow kinetics are expected to have a strong and long-lasting impact on their neuronal targets. The conditioning pulse paradigm was designed to assess this inhibitory impact by engaging slow inhibition prior to orthodromic excitation of pyramidal neurons. It was important to choose a stimulation site for the preconditioning pulse that would elicit long-lasting dendritic inhibition and yet minimize (interfering) glutamatergic currents in pyramidal neurons. SLM, optimal for eliciting GABA$_A$$_{slow}$ under conditions of blocked glutamatergic receptors, was less suited as a site for the conditioning pulse due to the presence of entorhinal afferents. Therefore we chose to stimulate in the alveus. Stimulation in alveus excited—directly or via recurrent CA1 axons—various interneuron types, among them basket, bistratified, trilaminar, ivy, and O-LM cells (Blasco-Ibáñez and Freund 1995; Fuentealba et al. 2008; Pouille and Scanziani 2004; Wierenga and Wadman 2003). Dendritically projecting O-LM cells are among the putative presynaptic neuron types mediating GABA$_A$$_{slow}$, and, accordingly, stimulation in alveus has been elicited long-lasting dendritic inhibition (Benkwitz et al. 2007; Maccaferrì et al. 2000; Pouille and Scanziani 2004) (see also METHODS) and to “veto” integration of Schaffer and temporoammonic inputs (Ang et al. 2005). This preconditioning pulse depressed subsequent, orthodromically elicited population spikes less in $\beta_3^{-/-}$ mice than in wild-type animals. This finding demonstrates that inhibition mediated by GABA$_A$ receptors harboring the $\beta_3$ subunit has the potential to curb pyramidal action potential activity for long intervals (on the order of 1 theta period) via local dendritic shunting and/or somatic hyperpolarization. Specifically, in view of the largely reduced prevalence of GABA$_A$$_{slow}$ currents in pyramidal neurons of $\beta_3^{-/-}$ mice, we surmise that GABA$_A$$_{slow}$-generating synapses form part of this powerful inhibition of pyramidal dendrites. The preconditioning pulse in alveus most likely did not activate a subset of dendrite-targeting interneurons, lacunosum-moleculare, and neurogliaform cells, which are confined to distal dendritic laminae (Price et al. 2005). As these are also among the putative interneuron types producing GABA$_A$$_{slow}$, we presume that our results are a conservative estimate of the action potential-depressing impact of GABA$_A$$_{slow}$.

By the same reasoning, electrical stimulation in SLM can be expected to tap the full power of slow GABAergic dendritic inhibition of pyramidal cells as it stimulates the axons of both local and dendritically projecting interneurons. Stimuli in this lamina are also very efficient at inducing long-lasting inhibition of interneurons; if administered at a strength sufficient to induce GABA$_A$$_{slow}$ IPSCs in pyramidal cells, they temporarily interrupt the stream of GABA$_A$$_{fast}$ IPSCs impinging on the same cells. This suppression of fast inhibition (SFI) (Banks et al. 2000) is not mediated by GABA$_B$ receptors; it is best explained by a long-lasting inhibition of interneurons producing GABA$_A$$_{fast}$ IPSCs by GABA$_A$$_{slow}$ currents (Banks et al. 2000; White et al. 2000). Previous modeling studies indicated that pauses $\geq 600$ ms in duration reflect the influence of IPSCs with decay times of $\sim 100$ ms, i.e., GABA$_A$$_{slow}$, as they delay the spontaneous firing of the interneurons that generate GABA$_A$$_{fast}$ IPSCs (Banks et al. 2000). The fact that in wild-type hippocampi, the depression of population spikes was much briefer (Fig. 3), and indeed similar in duration to GABA$_A$$_{slow}$ IPSC decay (Fig. 2), may reflect the local shunting influence of the inhibitory conductance on excitatory current rather than the longer-lasting membrane hyperpolarization that underlies SFI. In addition, activation of different sets of presynaptic elements via alveus versus SLM stimulation may contribute to differences in duration of SFI and suppression of evoked firing.

Surprisingly, SFI was as strong in $\beta_3^{-/-}$ mice as in wild-type animals (Fig. 4). Therefore we conclude that long-lasting inhibition of GABA$_A$$_{fast}$-producing interneurons does not require GABA$_A$ receptors harboring the $\beta_3$ subunit. This type of inhibition, and the remaining slow inhibition in pyramidal neurons, must then utilize $\beta_1$ or $\beta_2$ subunits. Although IPSC duration is thought to reflect primarily intrinsic receptor gating properties at some synapses (Jones and Westbrook 1996; Mozrzymas 2004), and the high affinity and slow kinetics imparted by the $\beta_2$ subunit may indeed serve as the primary determinant of slow IPSC decay in some cases (Burgard et al. 1996; Schofield and Huguenard 2007), other factors, such as the spatiotemporal profile of transmitter and spillover onto perisynaptic receptors, clearly play important roles at others. These include slow inhibitory synapses in dentate gyrus (Wei et al. 2003) and neocortex (Szabadi et al. 2007). As noted in the preceding text, the similarity between the duration of spontaneous GABA$_A$$_{slow}$ IPSCs in wild-type and $\beta_3^{-/-}$ animals would indicate that the decay of some classes of IPSCs in hippocampus largely reflects factors other than intrinsic receptor kinetics.

**In vivo rhythms affected by the absence of the $\beta_3$ subunit**

The absence of the $\beta_3$ subunit leads to dramatically increased oscillatory synchrony and power in olfactory bulb (Nusser et al. 2001) and thalamus (Huntsman et al. 1999). In both brain regions, a single inhibitory neuron type, disinhibited in $\beta_3^{-/-}$ mice, inhibits its targets more powerfully and thus enhances the structures’ propensity to oscillate. By contrast, in hippocampus, the absence of the $\beta_3$ subunit weakened and perturbed rhythms. Theta oscillations were weaker, slower, less regular (particularly in distal dendritic regions), and less coordinated across laminae in the $\beta_3^{-/-}$ genotype than in wild-type animals. As pyramidal neurons are the major substrates of theta field potential-generating current dipoles, these findings argue that pyramidal dendritic slow inhibition via $\beta_3$ subunit-containing GABA$_A$ receptors constitutes one of the several, spatially segregated sources of theta rhythms (Banks et al. 2000; Leung 1984; Montgomery et al. 2009) The spike rate and timing of O-LM neurons, which are among potential presynaptic interneuron types mediating GABA$_A$$_{slow}$ in pyramidal neurons, are in good agreement with this idea (Klausberger et al. 2003). Additionally, neurogliaform and LM cells, likely activated by entorhinal (temporoammonic) input, could contribute to regular slow dendritic inhibition.
Because GABA$_{A_{\text{slow}}}$ IPSCs generated by neurogliaform cells do show strong, GABA$_B$ receptor-sensitive short-term depression (Price et al. 2008), their influence may be partially attenuated during repetitive activation. However, this characteristic would also endow them with the capacity to increase, as well as decrease, their contribution to network oscillations, if this attenuation was itself subject to modulation.

Gamma field activity in CA1 depends on fast inhibitory currents in pyramidal neurons, produced by perisomatic fast-spiking cells that receive rhythmic excitatory drive from CA3 pyramidal neurons (Csicsvari et al. 2003; Fisahn et al. 1998). In dendritic regions of CA1, a large proportion of gamma power appears to be volume-conducted from dentate gyrus (Bragin et al. 1995; Csicsvari et al. 2003). As discussed in the preceding text, the absence of the $\beta_3$ subunit results in a net loss of GABAergic inhibition and likely in a preponderance of GABA$_A$ receptors with faster kinetics. These alterations of fast synaptic inhibition may be particularly dramatic in the molecular layer of dentate gyrus in which both $\alpha_3$ and $\beta_3$ subunits are even more prevalent than in hippocampus proper (Sperk et al. 1997). As the decay time of IPSCs is a major determinant of gamma field oscillations, fewer and faster IPSCs should translate into weaker and faster gamma oscillations in $\beta_3^{-/-}$ mice compared with wild-type animals. This is indeed what we observed. Yet less oscillatory power does not necessarily imply lower firing rates of the contributing neurons; it could also be attenuation was itself subject to modulation.

In addition to changes in the individual theta and gamma rhythms, the coordination between the two oscillations was substantially impaired in $\beta_3^{-/-}$ compared with WT mice (Fig. 8). However, when examined independent of the underlying theta rhythm, the degree of amplitude modulation of the gamma oscillation at theta frequency was unchanged (Fig. 7C), and the regularity of the amplitude variation of gamma oscillations was only slightly reduced (Fig. 7E). These findings suggest that slow inhibitory currents in GABA$_{A_{\text{fast}}}$-producing interneurons that do not utilize the $\beta_3$ subunit—such as those that underlie the suppression of fast inhibition that remains intact in $\beta_3^{-/-}$ mice (Fig. 4)—helped maintain the degree of gamma nesting that we observed. Taken together, these results point to the disruption in the theta rhythm as the primary factor in the impaired theta-gamma coordination. Another contributing factor may have been the depression of GABA$_{A_{\text{slow}}}$ currents in pyramidal cells, and the ensuing repercussion on interneuronal firing precision (Tort et al. 2007); desynchronized spike timing of gamma-generating neurons within the theta cycle would cause gamma bouts to be less precisely coordinated with theta (Fig. 8C). In summary, our observations are consistent with a model in which theta and gamma oscillations are produced by two relatively independent subcircuits that oscillate at different frequencies with the coordination of gamma oscillations at theta frequency resulting in part from the periodic inhibition of gamma oscillators by theta oscillators (White et al. 2000).

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