Hippocampal theta rhythm and its coupling with gamma oscillations require fast inhibition onto parvalbumin-positive interneurons

Peer Wulff a, 1, Alexey A. Ponomarenko b, 1, Marlene Bartos c, Tatiana M. Korotkova b, Elke C. Fuchs b, Florian Bähner b, Martin Both b, Adriano B. L. Tort e, f, Nancy J. Kopelle 2, William Wisden a, 2, and Hannah Monyer a, 2

a Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, United Kingdom; b Department of Clinical Neurobiology, IZN, and c Institute of Physiology and Pathophysiology, Im Neuenheimer Feld 326, University of Heidelberg, 69120 Heidelberg, Germany; d Department of Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS 90035, Brazil; and e Center for BioDynamics and Department of Mathematics, Boston University, Boston, MA 20215

Contributed by Nancy J. Kopell, December 24, 2008 (sent for review November 11, 2008)

Hippocampal theta (5–10 Hz) and gamma (35–85 Hz) oscillations depend on an inhibitory network of GABAergic interneurons. However, the lack of methods for direct and cell-type-specific interference with inhibition has prevented better insights that help link synaptic and cellular properties with network function. Here, we generated genetically modified mice (PV-Δγ2) in which synaptic inhibition was ablated in parvalbumin-positive (PV+) interneurons. Hippocampal local field potential and unit recordings in the CA1 area of freely behaving mice revealed that theta rhythm was strongly reduced in these mice. The characteristic coupling of theta and gamma oscillations was strongly altered in PV-Δγ2 mice more than could be accounted for by the reduction in theta rhythm only. Surprisingly, gamma oscillations were not altered. These data indicate that synaptic inhibition onto PV+ interneurons is indispensable for theta- and its coupling to gamma oscillations but not for rhythmic gamma-activity in the hippocampus. Similar alterations in rhythmic activity were obtained in a computational hippocampal network model mimicking the genetic modification, suggesting that intrahippocampal networks might contribute to these effects.

How is information coded in the brain? Action potential firing by individual neurons is certainly central but might not be sufficient. Oscillations of the extracellular field potential may provide temporal reference signals that allow efficient information processing by spiking neurons (1–5). Multiple layers of information could be encoded if the different rhythms interact. One example is the coupling of hippocampal theta and gamma oscillations during exploratory activity and paradoxical/rapid eye movement sleep (6–9). Here, the power (or amplitude) of the gamma oscillations is systematically modulated (coupled) during each theta cycle. Such cross-frequency coupling may aid the execution of cognitive functions such as working memory (10–15).

A striking feature of the hippocampal circuit is the web of inhibition provided by diverse types of local GABAergic interneurons and externally from the medial septum - diagonal band of Broca (MS) (2, 16–19). Indeed, fast synaptic inhibition shapes both theta and gamma oscillations and could also control cross-frequency coupling (6, 16, 20–22). Because local GABAergic interneuron subtypes fire with distinct phase-related patterns (2, 23–26), each subtype probably has a distinct role in orchestrating and/or responding to the rhythm.

In this article we focus on parvalbumin-positive (PV+) interneurons. In CA1, PV+ cells comprise dendrite targeting bistratified and possibly O-LM cells, and perisomatic targeting basket cells (BCs) and axo-axonic cells (2). The latter 2 types are probably important for generating the synchronous network activity. Each of the perisomatic inhibitory PV+ cells innervates >1000 pyramidal cells (27, 28) and fires phase-locked to theta and gamma oscillations evolving rhythmic perisomatic inhibitory postsynaptic currents (IPSCs) in the pyramidal cell layer (6, 7, 9, 24, 29). Via synaptic GABA_A receptors, PV+ BCs reciprocally inhibit each other and receive inhibition from the MS and other local interneurons (19, 20, 30). To investigate how GABAergic inhibition of PV+ cells contributes to the generation and coupling of theta and gamma oscillations in the hippocampus, we selectively removed fast synaptic GABAergic inhibition from these cells, using mouse transgenic methods.

Results

Selective Removal of the GABA_A Receptor γ2 Subunit from PV+ Neurons. PV+ interneurons in the hippocampus express αβγ2-type GABA_A receptors (31, 32). The γ2 subunit is essential to traffic and to anchor the receptor to the postsynaptic membrane and it confers full single channel conductance to the receptor (33, 34). Thus, removing the γ2 subunit impairs GABA_A receptor function and removes fast synaptic inhibition (35). To investigate the role of fast synaptic inhibition of PV+ interneurons in hippocampal network activity, we selectively ablated the γ2 subunit from these cells (PV-Δγ2 mice, see Materials and Methods for details) and thus disconnected them from the fast inhibitory network.

Deletion of the γ2 subunit was examined by in situ hybridisation with a γ2 subunit-specific probe (36). At the level of X-ray film analysis, the hippocampus and neocortex of PV-Δγ2 mice showed a γ2 mRNA signal comparable to littermate controls. This was expected because PV+ cells are a minority in these areas. However, areas with a high number of PV+ cells, such as the inferior colliculus or cerebellum, showed a large reduction in the γ2-specific mRNA signal. No γ2 mRNA was present in the reticular thalamus of PV-Δγ2 mice, which contains only PV+ cells (Fig. S1). PV-Δγ2 mice appeared normal in the first 3 postnatal weeks, but showed reduced body weight, tremor and ataxia (probably because of the loss of the γ2 subunit in PV-expressing cells in motor areas) at P60. PV-Δγ2 mice had a normal life expectancy. General brain morphology was unaltered in PV-Δγ2 mice (Fig. S2). No difference in number (81.8 ± 4.9 vs. 80.5 ± 8.1 cells per hippocampus; p = 0.8; unpaired student's t test), distribution and morphology of hippocam-
pal PV+ interneurons were detected between PV-Δγ2 mice and littermate controls, using light microscopy. In principle, other γ subunits in the GABAγ receptor gene family could substitute for the synaptic targeting function of γ2 in PV-Δγ2 mice. We thus analyzed the expression of the γ1 and γ3 subunit genes by in situ hybridisation but found no up-regulation of γ1 or γ3 subunit mRNA in brains of PV-Δγ2 mice (Fig. S3).

GABAγ Receptor-Mediated Synaptic Inhibition of Fast-Spiking Interneurons Is Lost in PV-Δγ2 Mice. To confirm the loss of GABAγ receptor-mediated synaptic transmission onto PV+ interneurons, we performed whole-cell patch-clamp recordings in acute hippocampal slice preparations from adult PV-Δγ2 and control mice. IPSCs were recorded in CA1 BCs during extracellular stimulation and peak amplitude and decay time constant of IPSCs were compared with data obtained from recordings in pyramidal cells (PCs) under identical extracellular stimulation conditions (Fig. 1, see Materials and Methods for details).

In control mice both BCs and PCs had IPSCs with comparable peak amplitudes (250.3 ± 24.7 pA vs. 192.8 ± 29.9 pA; p = 0.9; n = 4). The average decay time constant was approximately 2-fold faster in BCs than in PCs (BC: 4.3 ± 0.5 vs. PC: 8.05 ± 1.1 ms; p = 0.021; n = 4; Fig. 1B and C). In contrast, fast IPSCs were absent in 5 of 6 BCs in PV-Δγ2 mice. Instead, currents with small peak amplitudes (25.7 ± 9.8 pA) and slow variable decay time constants (23.9 ± 5.9 ms) were seen (Fig. 1B–E). These currents were blocked by bicuculline (n = 3; data not shown) and may reflect spillover of synaptically released GABA onto low-conductance extrasynaptic αβ subunit-containing GABAγ receptors (33). Accordingly, in PV-Δγ2 mice the peak amplitude of inhibitory responses was markedly smaller in BCs than in PCs (BC 25.7 ± 9.8 pA vs. PC 215.4 ± 45.7 pA, p = 0.0039, n = 6; Fig. 1B and C) and the remaining small inhibitory component was markedly slowed down (decay time constant BCs: 23.9 ± 5.9 ms vs. PC: 10.15 ± 2.06 ms; p = 0.078; n = 6). When peak amplitudes of compound IPSCs from all BCs of PV-Δγ2 and littermate control mice were plotted as a function of their corresponding decay time constants, data distributed into 2 distinct groups (Fig. 1E). IPSCs in the first group were characterized by large peak amplitudes (>50 pA) and fast decay time constants (<10 ms) and included all BC recordings from control and 1 of 6 recordings from PV-Δγ2 BCs. IPSCs in the second group were characterized by a small peak amplitude (<50 pA) and slow decay time constant (≥10 ms) and included 5 of 6 BC recordings from PV-Δγ2 slices.

Theta Oscillations Are Reduced in PV-Δγ2 Mice. Inhibitory control of PV+ interneurons has been suggested to be required for the generation of hippocampal theta rhythm (19, 21, 22, 37). To test this hypothesis we recorded local field potentials (LFP) and unit activity in CA1 stratum pyramidale in behaving PV-Δγ2 and control mice, using wire electrodes during exploration in an open field and sleep in a home cage. Theta oscillations (5–10 Hz) were strongly reduced in PV-Δγ2 mice (Fig. 2A and Fig. S4B). Power spectral density (PSD) in the theta frequency band and its harmonics were several fold reduced in PV-Δγ2 mice (Fig. 2B). Integral PSD in the theta band was significantly different between genotypes (8 control and 8 PV-Δγ2 mice, F1,22 = 26.4, P < 0.0001, 2-way ANOVA). Concurrently LFP amplitudes were markedly reduced (0.29 ± 0.03 vs. 0.14 ± 0.03 mV, F1,22 = 10.9, P < 0.01, ANOVA) and cycle duration was increased (Fig. 2B, Upper Inset). The leading frequency of theta oscillations was lower in PV-Δγ2 mice by ~1.5 Hz (F1,22 = 20.7, P < 0.001, Fig. 2B Lower Inset). The theta rhythm was also less stable in PV-Δγ2 mice. Frequency and amplitude variability of theta oscillation were elevated in the PV-Δγ2 mice (amplitude variance was normalized by the mean amplitude: 0.09 ± 0.01 vs. 0.13 ± 0.01, F1,22 = 16.2, P < 0.001; normalized frequency variance: 0.03 ± 0.01 vs. 0.07 ± 0.01 in control and PV-Δγ2 mice, respectively, F1,22 = 32.2, P < 0.0001). Features of theta oscillations did not differ between paradoxical sleep (PS) and waking in either genotype (p = 0.08, ANOVA). Theta amplitude and frequency were different between genotypes when similar values of the running speed were considered (data recorded in additional 3 control and 3 mutant mice, see SI Results for details). Recordings with silicone probes indicated that the changes in theta rhythm in PV-Δγ2 mice were not restricted to stratum pyramidale. Depth profiles of theta oscillations were qualitatively similar between genotypes (2 animals per genotype).
Theta oscillations are reduced in PV-Δγ2 mice. (A) Representative signal examples recorded in CA1 stratum pyramidale during running in control and PV-Δγ2 mice (mean running speed is indicated for the 2 epochs). (B) Power spectral density of theta oscillations during waking and paradoxical sleep (PS). (Inset) Theta peak triggered average of 35–85 Hz bandwidth filtered signal. (C) Frequency of gamma oscillations during waking and PS. (D) Rhythmic modulation of pyramidal cell discharge during theta- and gamma-associated activation. Discharge probabilities are normalized within groups as in Fig. 2E. Theta rhythm modulated the firing of pyramidal cells in the PV-Δγ2 less than in controls (population modulation coefficients: 0.50, control, vs. 0.37, PV-Δγ2, P < 0.0001, paired t test).

**Gamma Oscillations Are Intact in Behaving PV-Δγ2 Mice.** Fast gamma oscillations (35–85 Hz) were largely unchanged in behaving PV-Δγ2 mice. Integral gamma power and gamma wave amplitude and its variability did not differ between genotypes (p ~ 0.1, ANOVA, Fig. 3A and B and Fig. S5 A and B). The frequency (computed from interpeak interval) of gamma oscillations tended to be slightly but not significantly elevated in PV-Δγ2 mice (F1,22 = 3.2, p ~ 0.09, Fig. 3C). Theta-associated gamma oscillations modulated discharge probability of pyramidal cells in control and PV-Δγ2 mice (Fig. 3D). Pyramidal cells (n = 26, control, and n = 11, PV-Δγ2) fired the lowest number of spikes near the gamma peaks, whereas maximal firing probabilities were broader distributed between ~80° before and ~40° following gamma troughs (not significantly different between genotypes). Pyramidal cells were similarly modulated by gamma oscillations in control and PV-Δγ2 mice (population modulation in pyramidal cells: 0.18 in control, vs. 0.16 in PV-Δγ2, p ~ 0.2, paired t test).

**Coupling of Theta and Gamma Oscillations Is Disrupted in PV-Δγ2 Mice.** In control mice gamma oscillations were strongly modulated within the theta cycle with amplitude of gamma oscillation being highest briefly after the peak of the theta cycle (~20°), as reported in rodents for CA1 stratum pyramidale (6, 8, 38). This coupling between theta and gamma oscillations was severely disrupted in PV-Δγ2 mice (Fig. 4). Amplitude modulation of gamma oscillations was reduced more than 2-fold (F1,22 = 34.5, P < 0.0001, ANOVA, Fig. 4B and D). Near theta peaks, gamma amplitude was reduced, whereas, close to theta troughs, it was markedly elevated. Also, theta-modulation of gamma oscillation frequency was almost 3-fold lower in PV-Δγ2 mice (F1,22 = 15.5, P < 0.0001, ANOVA, Fig. 4B and D). The degree of uncoupling was similar during waking and PS (p ~ 0.1, ANOVA, see also Fig. S6).

To test whether disrupted gamma modulation could simply be caused by the observed alterations in theta rhythm, we analyzed gamma amplitude and frequency distributions, using basic theta oscillation features, such as cycle amplitude, frequency and their normalized variances as explanatory variables (Fig. 4 C and D).
Strong reduction of gamma modulation in PV-Δγ2 mice was also evident after adjustment for altered theta rhythm features (at least P < 0.05, ANCOVA, see SI Results for details). Thus, PV-Δγ2 mice display two aspects of disrupted coupling between gamma and theta oscillations: first, an overall effect due to attenuated theta rhythm and, second, an effect not directly connected to alteration of theta-features, most likely because of the disruption of the coupling mechanism.

To analyze the modulation of gamma activity during theta on the level of unit activity we first established that higher amplitude gamma cycles better modulate the discharge of concurrently active pyramidal cells (r = 0.23, control, r = 0.13, PV-Δγ2, P < 0.01). The strength of this association did not differ between genotypes (z = 1.46, p = 0.15). Consistent with the LFP findings, gamma modulation of pyramidal cell (n = 41, control, and n = 39, PV-Δγ2) discharge periodically varied in theta cycles in controls but less regularly and weaker in PV-Δγ2 mice (Fig. 4E Upper, individual unit modulation coefficients, 0.24 ± 0.02 in control vs. 0.16 ± 0.02 in PV-Δγ2, P < 0.01, t test, see SI Materials and Methods for detailed description of the analysis). Therefore, the gamma modulation of spike timing of pyramidal cells as a function of the theta phase is impaired in PV-Δγ2 mice.

Removal of Synaptic Inhibition onto PV+ Cells in a Hippocampal Network Model Reproduces Experimental Results. To see whether the experimental findings could be accounted for by a theta rhythm generated within the hippocampus we have generated a biophysical CA1 network model. We adopt the same notation used by previous modeling work (21, 22, 39-41) by calling the fast spiking PV+ cells “I cells” (i.e., inhibitory cells) and the pyramidal cells “E cells” (i.e., excitatory cells). For the sake of model generality, we call the hippocampal inhibitory cells that preferentially spike at theta frequency T cells (“theta cells”), and we assume that these cells are not the PV+ interneurons. A recent computational study showed that T cells can generate a coherent population theta rhythm when they interact with the I cells, i.e., the existence of mutual T → I and I → T connections was shown to be necessary in that model for a coherent theta rhythm on the population level (40). We tested this necessity in a model containing E cells and T and I cells. Fig. 5A shows the network configuration of E, I and T cells. We represent the E-cell population as a single cell firing at the population frequency; thus this cell produces EPSPs in the I and T cells at gamma frequency, as observed experimentally (42). Fig. 5A ii–iv shows a typical result obtained (see also 22). The interplay between the subnetwork consisting of I and T cells is able to produce the theta rhythm, whereas the gamma is generated through a mechanism requiring the E and I cell subnetwork. By means of a model LFP (see Materials and Methods), we show that both gamma amplitude and frequency are modulated by the theta phase in this CA1 network model (Fig. 5C).

The PV-Δγ2 network is then simulated by breaking T → I and I → T connections (Fig. 5B). Breaking T → I attenuates the theta rhythm (Fig. 5B and C) because the T cells are no longer coherent without this connection. The T cells can occasionally generate a theta rhythm, however, this is unstable and less prominent when compared with the control network (Fig. 5B). Also, the model PV-Δγ2 network reproduces the reduction of the theta peak frequency observed experimentally (Fig. 5C). The reason for this is that the I cells are prone to spike more without the T cell inhibition and they then provide a greater level of inhibition onto the T cells, diminishing their spike frequency. Also consistent with experimental results, we observed a large reduction in gamma amplitude and frequency modulation by the theta phase in the PV-Δγ2 network (Fig. 5C). Note that the reduction of the gamma modulation by theta is not due only to the reduction of the theta power, because even for the periods when the T cells are able to create a coherent theta rhythm, the lack of T → I connections hinders the modulation of the I cells by the theta.
In summary, the present computational model is consistent with the experimental results; therefore the impairment of an intrahippocampal mechanism of theta generation could be contributing to the alterations in theta and theta-gamma coupling seen in vivo in PV−BCs. The theta rhythm becomes unstable and less prominent when only the T cells (inhibitory cells that spike preferentially at theta frequency) have been reported (21, 24). Our hypothesis is that this reduction in theta power in the hippocampus is caused by the lack of inhibition onto PV−BCs, which contributes to observed findings.

Hippocampal gamma oscillations are thought to result from interaction of pyramidal cells and interneurons but the role of mutual inhibition between PV−interneurons has been a matter of debate (2, 3, 9, 20). Our results show that mutual inhibition is dispensable for in vivo gamma oscillations and stress the importance of recurrent excitation and feed-back inhibition (9). In all mammals examined, including humans, gamma oscillations are modulated by concurrent theta rhythm (6–10, 50). This theta-gamma coupling may serve as a general coding scheme that coordinates distributed cortical areas, encodes serial information and working memory, and aids theta phase precession of place cells (5, 10–14, 21). Empirical evidence supports such proposals by showing higher levels of theta-gamma coupling during cognitive demands (10, 14). Here, we show that inhibition onto PV−interneurons is required for theta-gamma coupling, even after controlling for the level of theta power (Fig. 4). These experimental results can be reproduced by our simple computational model, in which the source of inhibition resides within the hippocampus and is constitutive by I cells (putative fast spiking, hence PV−interneurons) and T cells (inhibitory cells that spike preferentially at theta frequency), and in which the theta rhythm arises from their mutual interaction. Furthermore, by suggesting an impairment of an intrahippocampal mechanism of theta generation in PV−BC mice, this model is also consistent with the reduction of theta power observed in all CA1 layers, because interneurons targeting pyramidal cells at distinct levels (e.g., soma, apical dendrites) would present reduced theta coherence. We note, however, that the reduction of hippocampal gamma power modulation by theta phase could also be explained by the lack of theta inputs from an external pacemaker (e.g., from GABAergic septal cells) onto I cells. Also the reduction in theta oscillations could be caused by an impairment of the circuitry in the MS: GABAergic projection neurons in the MS are also PV−, and will thus have lost reciprocal GABAergic inhibition and input from hippocampo-septal projections. It is therefore likely that both extra hippocampal interneurons, including PV−BCs (18). Rhythmic inhibition of PV−hippocampal interneurons by the MS at theta frequency (19, 44) could cause rhythmic IPSPs in principal cells and the corresponding theta dipole in the pyramidal cell layer (16). GABAergic projections from the hippocampus to the MS (45) might contribute to theta rhythm generation in the hippocampus (16, 46). Our data are compatible with the prediction that interneuron-specific ablation of the inhibitory septo-hippocampal projection may severely impair theta rhythm in the hippocampus.
transgenic (PV-Cre mice; 51). All experiments were performed on mice homozygous for the conditional γ allele and hemizygous for the PVCre transgene (PV-γγ) and PVCre negative littermate controls. For details see SI Materials and Methods.

In Situ Hybridization and Immunohistochemistry. In situ hybridization experiments were carried out as described in ref. 36. See SI Materials and Methods for oligonucleotide sequences and immunohistochemical procedures.

Materials and Methods

All procedures involving experimental mice had ethical approval from the Regierungspräsidium Karlsruhe, Germany.

Generation of Mice with a Selective Loss of Synaptic Inhibition onto PV+ Neurons. Mice carrying a conditional allele of the GABA receptor γ2 gene (γ2l7flox; 35) were crossed with transgenic mice expressing Cre recombinase selectively in parvalbumin-positive cells (PVCre mice; 51). All experiments were performed on mice homozygous for the conditional γ allele and hemizygous for the PVCre transgene (PV-γγ) and PVCre negative littermate controls. For details see SI Materials and Methods.

Whole-Cell Recordings. Whole-cell voltage clamp recordings were made in acute transverse hippocampal slices (300 μm thickness) from brains of adult mice as described in ref. 20. For details see SI Materials and Methods.

In Vivo Recordings. Wire electrodes (tetrodes or arrays of single wires) were active into the hippocampus to record simultaneously the spontaneous in vivo activity of hippocampal pyramidal cells and interneurons. For details see SI Materials and Methods.

Computer Modeling. We used single compartment models for the E and T cells. All cells were modeled by using the Hodgkin-Huxley formalism. The model CA1 network consisted of 1 E, 5 I, and 15 T cells. A greater number of T cells was used to allow the analysis of their synchronization. Detailed information about the model cells and synapses, the model LFP, and numeric and random aspects of this work can be found in SI Appendix. All simulations were carried out using the NEURON simulation program (52).

ACKNOWLEDGMENTS. We thank A. Draghun for stimulating discussions and support. A. Sirvio for valuable assistance with the preparation of the manuscript; and I. Preugschauf for comments on an earlier version of the manuscript; and I. Preugschauf, U. Amtmann, and A. Sergiyenko for technical assistance. This work was supported by VolkswagenStiftung Grant I/78 554 (to W.W.) and I/78 563–564 (to M.B.); Deutsche Forschungsgemeinschaft Grant WI 1951/2 (to W.W. and P.W.); Medical Research Council Grant G0601498 (to W.W. and P.W.); a Heidelberg Young Investigator Award (to P.W.); the Royal Society (M.B.); German Science Foundation Grant FRI 650 D5; C6; the Leibniz Research Foundation (H.M.); the Burroughs Wellcome Fund (A.B.L.T. and N.K.); National Institutes of Health Grant R01 NS45658 as part of the NSF/National Institute of Mental Health Collaborative Research on Computational Neuroscience Program (N.K.), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (A.B.L.T.).
Neuronal models

**E cell:** We use the pyramidal cell model of Ermentrout and Kopell (1998), which is a reduction of a model due to Traub & Miles (1991):

\[ C \frac{dV}{dt} = g_{Na} m_\infty(V)^3 h(V_{Na} - V) + g_K n^4(V_K - V) + g_L(V_L - V) + I, \]  
\[ \frac{dn}{dt} = \frac{n_\infty(V) - n}{\tau_n(V)}, \]  
\[ h = \max(1 - 1.25n, 0), \]

with

\[ x_\infty(V) = \frac{\alpha_x(V)}{\alpha_x(V) + \beta_x(V)} \text{ for } x = m \text{ or } n, \]  
\[ \tau_x(V) = \frac{1}{\alpha_x(V) + \beta_x(V)} \text{ for } x = n, \]  
\[ \alpha_m(V) = \frac{0.32(V + 54)}{1 - \exp(-(V + 54)/4)}, \]  
\[ \beta_m(V) = \frac{0.28(V + 27)}{\exp((V + 27)/5) - 1}, \]  
\[ \alpha_n(V) = \frac{0.032(V + 52)}{1 - \exp(-(V + 52)/5)}, \]  
\[ \beta_n(V) = 0.5 \exp(-(V + 57)/40). \]

In Equations (1)–(2), the letters \( C, V, t \) and \( \tau, g, \) and \( I \) denote capacitance density, voltage, time, conductance density, and current density, respectively. The units that we use for these quantities are \( \mu F/cm^2, mV, ms, mS/cm^2, \) and \( \mu A/cm^2 \). For brevity, units will usually be omitted from here on. The parameter values of the model are \( C = 1, g_{Na} = 100, g_K = 80, g_L = 0.1, V_{Na} = 50, V_K = -100, \) and \( V_L = -67 \).

**I cells:** For fast-spiking PV-positive interneurons, we use the Wang & Buzsaki (1996) model:

\[ C \frac{dV}{dt} = g_{Na} m_\infty(V)^3 h(V_{Na} - V) + g_K n^4(V_K - V) + g_L(V_L - V) + I, \]
\[ \frac{dn}{dt} = \frac{n_\infty(V) - n}{\tau_n(V)}, \]  
\[ \frac{dh}{dt} = \frac{h_\infty(V) - h}{\tau_h(V)}, \]
with

\[ x_\infty(V) = \frac{\alpha_x(V)}{\alpha_x(V) + \beta_x(V)} \quad \text{for } x = m, h \text{ or } n, \quad (9) \]

\[ \tau_x(V) = \frac{0.2}{\alpha_x(V) + \beta_x(V)} \quad \text{for } x = h \text{ or } n, \quad (10) \]

The rate functions \( \alpha_x \) and \( \beta_x \), \( x = m, h, \) and \( n \), are defined as follows:

\[
\alpha_m(V) = \frac{0.1(V + 35)}{1 - \exp(-(V + 35)/10)} , \\
\beta_m(V) = 4 \exp(-(V + 60)/18) , \\
\alpha_h(V) = 0.07 \exp(-(V + 58)/20) , \\
\beta_h(V) = \frac{1}{\exp(-0.1(V + 28)) + 1} , \\
\alpha_n(V) = \frac{0.01(V + 34)}{1 - \exp(-0.1(V + 34))} , \\
\beta_n(V) = 0.125 \exp(-(V + 44)/80) .
\]

The parameter values, using the same units as for the E cell, are \( C = 1, g_{Na} = 35, g_K = 9, g_L = 0.1, V_{Na} = 55, V_K = -90, \) and \( V_L = -65. \)

**T cells:** For the theta interneurons, we use the model described in Tort et al. (2007), which is a reduction of the multi-compartmental O-LM cell model described in Saraga et al. (2003):

\[
C \frac{dV}{dt} = g_{Na} m^3 h (V_{Na} - V) + g_K n^4 (V_K - V) + g_{Aab} (V_A - V) \\
+ g_h r (V_h - V) + g_L (V_L - V) + I ,
\]

(11)

with

\[
\frac{dx}{dt} = \frac{x_\infty(V) - x}{\tau_x(V)} \quad \text{for } x = m, h, n, a, b, r .
\]

(12)

For \( x = m, n, h \), the functions \( x_\infty(V) \) and \( \tau_x(V) \) are the same as in (4) and (5), and the rate functions \( \alpha_x \) and \( \beta_x \), are defined as follows:
\[
\alpha_m(V) = \frac{-0.1(V + 38)}{\exp(-(V + 38)/10) - 1}, \\
\beta_m(V) = 4 \exp(-(V + 65)/18), \\
\alpha_h(V) = 0.07 \exp(-(V + 63)/20), \\
\beta_h(V) = \frac{1}{1 + \exp(-(V + 33)/10)}, \\
\alpha_n(V) = \frac{0.018(V - 25)}{1 - \exp(-(V - 25)/25)}, \\
\beta_n(V) = \frac{0.0036(V - 35)}{\exp((V - 35)/12) - 1}.
\]

For \(x = a, b, r\), we provide the functions \(x_\infty(V)\) and \(\tau_x(V)\) below:

\[
a_\infty(V) = \frac{1}{1 + \exp(-(V + 14)/16.6)}, \\
\tau_a(V) = 5, \\
b_\infty(V) = \frac{1}{1 + \exp((V + 71)/7.3)}, \\
\tau_b(V) = \frac{1}{\exp((V - 26)/18.5) + 0.0000009 + 0.04 \exp(-(V + 70)/11)}, \\
r_\infty(V) = \frac{1}{1 + \exp((V + 84)/10.2)}, \\
\tau_r(V) = \frac{1}{\exp(-14.59 - 0.086V) + \exp(-1.87 + 0.0701V)}.
\]

The parameter values are \(C = 1.3, g_L = 0.05, g_{Na} = 30, g_K = 23, g_A = 16, g_h = 8, V_{Na} = 90, V_K = -100, V_A = -90, V_h = -32, V_L = -70.\)

**Synaptic model:** Each synaptic input was modeled as a current of the form \(I_{syn,XY} = G_{XY}/N_{X}s(V - V_{syn})\), where \(X\) and \(Y\) denote the type of the pre and post-synaptic cell, respectively (ie, \(X, Y = E, I\) or \(T\)), \(G_{XY}\) is the maximal synaptic conductance, \(N_X\) is the number of \(X\) cells, \(V\) is the membrane potential of the post-synaptic cell, and \(V_{syn}\) is the reversal potential. The variable \(s\) is a normalized double-exponential function characterized by rise \((\tau_{rise})\) and decay \((\tau_{decay})\) time constants. The synapses were implemented using the \(\text{Exp2Syn()}\) built-in function of NEURON. The IPSPs originating from the T cells synapses were slower than IPSPs originating from the I cells synapses; we used \(\tau_{rise} = 2\) ms, and \(\tau_{decay} = 22\) ms for T synapses, \(\tau_{rise} = 0.07\) ms and \(\tau_{decay} = 9.1\) ms for I synapses, and \(\tau_{rise} = 0.05\) ms and \(\tau_{decay} = 5.3\) ms for E synapses. The reversal potential was set to 0 mV for E synapses and to -80 mV for T
and 1 synapses. We used \( G_{II} = 0.01, G_{TI} = 0.15, G_{IT} = 0.2, G_{IE} = 0.05, G_{TE} = 0.15, G_{EI} = 0.05, G_{ET} = 0.07, I_E = U(1.9, 2.1), I_I = 0.3, I_T = 0 \). The model KO network is obtained by setting \( G_{TI} \) and \( G_{II} \) equal to zero.

**Model Local Field Potential:** The model local field potential (LFP) consisted of the membrane potential of a “passive” E cell programmed inside the network. This cell receives the same synaptic inputs as the “active” E cell. However, this cell does not send any synaptic output onto other cells and, moreover, it also does not spike given that its external drive current is set to zero. The model LFP was analyzed in MATLAB (The MathWorks, Inc. Natwick, MA). Power spectrum density was obtained using the Welch’s averaged periodogram using a 6 s window length with 50% overlap (\textit{pwelch()} function from the Signal Processing toolbox). Gamma (40-80 Hz) and theta (5-9 Hz) filtered signals were obtained by using a linear finite impulse response (FIR) filter (\textit{eegfilt()} function from the EEGLAB toolbox; Delorme and Makeig, 2004). The amplitude and phase of the filtered signals were obtained from the the Hilbert transform. The instantaneous gamma frequency was calculated from the inter peak intervals of the gamma filtered signal. The theta-phase was binned into 18 intervals (i.e., bin size = 20°), and the mean gamma frequency and amplitude were computed for each theta-phase bin.

**Numeric and Random Aspects:** All simulations were carried out using the NEURON simulation program version 5.9 (Hines and Carnevale, 1997) with a time step of 25 \( \mu \)s. As initial conditions, the membrane potential of each cell was uniformly distributed between -85 and -60 mV and the channel-gating variables were set to their corresponding steady-state values. Each cell was further randomized using an IClamp of random uniform magnitude and random uniform duration between 0 and \( t_{\text{syn}}/2 \), where \( t_{\text{syn}} = 500 \) ms is the time when synapses were turned on. For each of the 50 trials, the total simulation time was of 12 s, and the last 10 s were used for the analysis. In each trial, the E cell drive was chosen from a uniform distribution between 1.9 and 2.1 \( \mu \)A/cm\(^2\); therefore, the exact gamma peak frequency varied from trial to trial.

**References**


Supporting Information

Wulff et al. 10.1073/pnas.0813176106

SI Results

Analysis of Theta Rhythm in Relation to the Running Speed. Reduced theta oscillations during waking can arise either from slower running (1) and/or due to a defective theta generation independently of the running speed. To establish possible contribution of the two factors, we recorded LFP and the animal’s position during exploration in an open field from additional 3 mutant and 3 control mice. The average running speed was similar in the two groups (6.8 ± 0.6, control, vs. 6.2 ± 0.1 cm/s, mutant, excluding immobility; note that theta power in the main data sample, Fig. 2B, is computed for detected theta during waking thus largely excluding immobility: 6.6 ± 0.7, control, vs. 5.8 ± 0.1 cm/s, including immobility). The regularity of running was similar between genotypes (0.10 ± 0.004, control, vs. 0.11 ± 0.021 cm/s², mutant, first derivative of the speed, including immobility; 0.11 ± 0.004, control, vs. 0.12 ± 0.022 cm/s², mutant, excluding immobility).

Theta rhythm amplitude (Hilbert transformed) and frequency (computed from interpeak intervals) were averaged within each of the 20 speed bins for each animal and pooled according to the genotype. Theta frequency was strongly correlated with the running speed in control (r = 0.7, P < 0.0001, Spearman coefficient) but not in the mutant (P ≈ 0.3). Theta amplitude was poorly correlated with the speed (P > 0.1 in either genotype) but displayed significant association in control mice when speed values < 25 cm/s were considered (r = 0.4, P < 0.01). Alterations of theta oscillations between genotypes were clearly evident when similar values of the running speed were considered (Fig. S5, P < 0.0001, for theta amplitude, F1,112 = 294, and frequency, F1,112 = 103, ANCOVA could only be performed for significantly different regression slopes).

Analysis of Coupling After Adjustment for Attenuated Theta Rhythm in PV-Δγ2 Mice. We considered awake theta rhythm features that were associated with the modulation of concurrent gamma oscillations (P < 0.1). First, theta cycle amplitude reliably predicted modulation of gamma oscillation amplitude in control mice (r = 0.6, P < 0.0001 in control and r ≈ 0.8 in PV-Δγ2 mice). Amplitude modulation of gamma oscillations was reduced in mutants after adjustment for theta amplitude (F1,59 = 65.4, P < 0.0001, for small theta cycles, F1,35 = 65.0, P < 0.0001, for large theta cycles, ANCOVA, Fig. 4D); theta cycles of small and large amplitudes - theta to delta ratio < 11 and ≥ 11 respectively - were treated separately to ensure equality of the regression slopes). Theta cycle amplitude also correlated with the modulation of gamma frequency (r ≈ 0.3, P < 0.05 in control and r = 0.4, P ≈ 0.1 for PV-Δγ2 mice) but, again, did not explain its reduction in PV-Δγ2 mice (F1,103 = 5.9, P < 0.05). Second, although theta cycles of higher frequency displayed some association with a lower index of gamma amplitude modulation in mutants (r = 0.1, p = 0.7 in control and r = −0.2, P = 0.09 in PV-Δγ2 mice), the adjusted group effect showed a significant difference between control and PV-Δγ2 mice (F1,117 = 88.3, P < 0.0001). Third, decreased variability of theta rhythm amplitude and frequency was associated with a stronger modulation of gamma amplitude (r = −0.6, P < 0.0001 and r = −0.5, P < 0.001 for theta amplitude and frequency respectively) and, to a smaller extent of gamma frequency (r = −0.2, P ≤ 0.05 and p = 0.1 for theta amplitude and frequency respectively) in control but not in PV-Δγ2 mice (p = 0.1). The reduced coupling of theta and gamma oscillations was also evident after accounting for the stability of theta rhythm (gamma amplitude modulation: F1,118 = 38.8, P < 0.0001, F1,117 = 18.4, P < 0.0001 for theta amplitude and frequency normalized variances as covariates respectively).

SI Materials and Methods

Generation of PV-Δγ2 Mice. In γ2I77lox mice exon 4 of the γ2 subunit gene is flanked by loxP sites (2). In addition a codon was changed in exon 4, resulting in a neutral amino acid substitution (F77 to I) (2). Mice homozygous for the γ2I77lox gene were crossed with mice heterozygous for γ2I77lox and hemizygous for a PVCre transgene (3). Litters of the following genotypes were used for the experiments: γ2I77lox/γ2I77lox/PVCre (PV-Δγ2) and γ2I77lox/γ2I77lox (littermate controls). Mice were genotyped by PCR analysis of genomic DNA from tail biopsies using the following primer pairs: γ2x5′-s (5′-GTCACTGCTA- AATATCTACAGTTG-3′) and γ2x5′-as (5′-GGATTG- GCA-TCAGCAACATG-3′) to test for the γ2I77lox allele (213-bp band for WT, 250-bp band for γ2I77lox), Cre1 (5′-GACGAGTTCCGCTACATGG-3′) and Cre2 (5′-AGCTAGTGCCTCTCTGAC-3′) to test for the Cre recombinase transgene (250-bp band for PVCre).

In Situ Hybridization. In situ hybridization with 35S-labeled oligonucleotide probes was as described in ref. 4. The oligonucleotide sequences were:

Parvalbumin (PV): 5′-TCTTCAAGGCCACCACTCTGGGAG-3′,
GABA_A receptor γ1 subunit: 5′-ATGCAAGGTCCGTATCCATGAGTCGT-3′,
GABA_A receptor γ2 subunit (Ex4): 5′-GTGTCGATGGATGTTTTCACCACCATATGCTATAC-3′,
GABA_A receptor γ3 subunit: 5′-AGAGGGTCTTGAAGGCTTATTCGAGG-3′

Images were generated from 4 to 12 week exposures to Biomax MR X-ray film (Eastman Kodak). To assess nonspecific labeling of the sections, each labeled oligonucleotide was hybridized to brain sections, each labeled oligonucleotide was hybridized to brain sections.

Immunohistochemistry. Adult animals were transcardially perfused with 4% paraformaldehyde in PBS, pH 7.4. Brains were removed and 60-μm-thick sagittal or coronal sections were obtained using a Leica VT1000S vibratome. Free-floating sections were washed in PBS 3 times for 10 min, endogenous peroxidase activity was reduced in PBS plus 0.5% H2O2 for 10 min. After washing twice in PBS (10 min each), sections were permeabilized and blocked in PBS plus 0.3% Triton X-100 and 2% normal goat serum for 30 min at room temperature and subsequently incubated with anti-parvalbumin (rabbit polyclonal, 1/1000 dilution, Swant) primary antibody in a solution composed of 2% NGS, 0.3% Triton X-100 in PBS for 24 h at 4°C. Incubated slices were washed 3 times in PBS plus 0.1% Triton X-100 for 10 min at room temperature, incubated for 1 h at room temperature with a 1:500 dilution of biotinylated goat anti rabbit IgG (Vector Laboratories) in PBS plus 0.1% Triton X-100, and subsequently washed twice in PBS plus 0.1% Triton X-100 and twice in PBS alone for 10 min at room temperature. DAB stainings were performed using an avidin-biotin kit ( Vectastain Elite ABC Kit, Vector Laboratories). Slices were rinsed briefly.
in Tris-HCl 10 mM, mounted on slides, dehydrated, rinsed in Xylo1 and coverslipped using Eukitt (Sigma–Aldrich).

For quantification of PV-positive hippocampal interneurons cells were counted (blind to the genotype) under a light microscope in DAB stained serial coronal sections matched for rostrocaudal level by bregma distance in PV-ΔY2 mice and littermate controls. The total number of parvalbumin positive cells per hippocampus was counted. 3 mice of each genotype (4 to 12 hippocampi per mouse) were analyzed. The numbers of parvalbumin positive cells per hippocampus were averaged for each mouse. Average cell numbers were then calculated for each genotype and compared using unpaired Student's t test.

Whole-Cell Recordings. Transverse hippocampal slices (300 μm thickness) were cut from brains of 40- to 105-day-old mice using a vibrotome (DTK-1000, Dosaka). Patch pipettes were pulled from borosilicate glass tubing (2 mm outer diameter, 1 mm wall thickness). When filled with intracellular solution, the resistance was 2.2 - 3.5 MΩ. Whole-cell recordings were obtained from basket cells and principal cells in the CA1 hippocampal subfield under visual control using infrared differential interference contrast videomicroscopy (5, 6). Basket cells had soma located in the CA1 principal cell layer or Compound IPSCs were evoked in the principle cell layer with a stimulation frequency of 0.2 Hz. Pulses were delivered by using a stimulus isolator and had an amplitude of 3 - 20 V and a duration of 200 μs. To obtain sequential recordings of compound IPSCs in basket cells and principal cells of the same slice preparation, first a basket cell recording was performed. The patch pipette was withdrawn and a subsequent principal cell recording was obtained under identical extracellular stimulation conditions. Somata of both, basket cell and principal neuron, were located in the same depth of the slice preparation. The recording temperature was 31 - 34°C.

Multic1amp 700B amplifier (Axon Instruments) was used for current clamp and voltage clamp recordings. Basket cell recordings with initial resting potentials more positive than −55 mV and principal cell recordings with initial resting potentials more positive than −65 mV were discarded. At the beginning of the recording basket cells were identified on the basis of their spiking pattern at 1 s depolarizing current injections. Basket cells generated high frequency trains of nonaccommodating action potentials (mean action potential frequency 163 ± 9 Hz at 800 pA, n = 6 basket cell recordings in CA1). Posthoc morphological analysis revealed axonal arborizations in the principal cell layer (7). Biocytin-filled neurons were visualized with avidin-biotinylated peroxidase complex and 3,3-diaminobenzidine as chromogen (8). To record compound IPSCS, cells were held in the voltage-clamp mode (holding potential −70 mV) with series resistance (Rs) compensation (65 - 90%, lag 20 μs; Rs before compensation 8 - 15 MΩ). The stationarity of the series resistance in the postsynaptic neuron was assessed from the amplitude of the capacitive current in response to a 10 mV pulse, and the compensation was readjusted during the experiment when necessary. Compound IPSCs were filtered at 6 kHz using the 4-pole low-pass Bessel filter of the amplifiers and were digitized at 20 kHz using a 1401plus laboratory interface (Cambridge Electronic Design) interfaced to a Pentium-PC. Igor-based programs (Fpulse; homemade) were used for stimulus generation and data acquisition.

Solutions. The physiological extracellular solution contained 125 mM NaCl, 25 mM NaHCO3, 25 mM glucose, 2.5 mM KCl, 1.25 mM NaH2PO4, 2 mM CaCl2, and 1 mM MgCl2 (bubbled with 95% O2/5% CO2 gas mixture). 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNOX) was added to the bath solution to block excitatory postsynaptic currents and 1 μM CGP55845A (Tocris) to block GABAA receptor-mediated currents. Pipettes used for extracellular stimulation of presynaptic axons contained Hepes-buffered Na+–rich solution (pH = 7.2) (KOH, 308 - 315 mOsm). To verify that IPSCs were mediated by GABAA receptors, we further added 20 μM bicuculline methiodide to the extracellular solution in a subset of experiments (n = 3). The intracellular solution contained 110 mM K-glucionate and 40 mM KCl, 0.1 mM EGTA, 2 mM MgCl2, 2 mM Na2ATP, and 10 mM Hepes; the pH was adjusted to 7.2 with KOH; the osmolarity was 290–310 mOsm; 0.2 biocytin was added for intracellular labeling. Bicuculline methiodide was purchased from Sigma, CNOX from Tocris. Other chemicals were from Merck, Sigma, Riedel-de Haen or Gerbu.

Data Analysis. The peak current was determined from average traces as the maximum within a window of 2 - 6 msec duration following the stimulus artifact from the preceding baseline. The decay phase of compound IPSCs was fitted with a single exponential [A exp(-t/τm)] or with the sum of 2 exponentials [A exp(-t/τ1) + B exp(-t/τ2)], using a nonlinear least-squares fit algorithm; time constants are reported as single exponential (τm) or as amplitude-weighted mean τm = (τ1 + B τ2)/(A + B). Values are given as mean ± SEM. Error bars in Figures also indicate SEMs. Significance of differences was assessed by a Mann–Whitney test at the significance level (p) indicated.

In Vivo Recordings
Implantation of Electrodes. Custom-made microd1ves were loaded with independently movable tetrodes (fabricated from 12-μm stainless steel wires, Kanthal, Mörfelden-Walldorf, Germany), linear silicon probes (100-μm intersite distance, Acro AB, Kista, Sweden) or arrays of single tungsten wires (40 μm, California Fine Wire Company, Grover Beach, CA, USA). Electrodes were implanted under isoflurane anesthesia above hippocampus (AP: 1.8, L(right):1, V: 0.7 mm) of control (n = 13) and PV-ΔY2 (n = 12) mice. Neocortical electroencephalogram (EEG) and reference electrodes were miniature stainless-steel screws in the skull. Electromyogram (EMG) electrodes were inserted in the neck muscle. Implanted electrodes and microdrive were secured on the skull with dental acrylic.

Data Acquisition. Electrodes were connected to operational amplifiers (Noted B.T., Pec5, Hungary) to eliminate cable movement artefacts. Electrophysiological signals were differentially amplified, band-pass filtered (1 Hz - 10 kHz, MCP Plus signal conditioner, Alpha Omega, Nazareth, Israel) and acquired continuously at 20 kHz (Alpha-Map, Alpha Omega). Electrodes were positioned in the CA1 str. pyramidale using depth profile of ripple oscillations and unitary activity as a reference. Recordings were performed during exploratory behavior (foraging in an open field), awake immobility and sleep. Two light-emitting diodes were attached to the headset to track animal's position (in indicated experiments).

Data Analysis. Signal processing was carried out off-line by custom-written MATLAB (Mathworks) and occasionally by C++ algorithms. EEG was obtained by low-pass filtering and down-sampling of the wide-band signal to 1250 Hz. Recordings used for further analyses were selected based on the following criteria: the location of the electrode in str. pyramidale was judged by the maximal amplitude of ripples and multiunit activity in the wide-band signal. Smaller, slower (50–100 ms) potentials often concurring with ripples had to be equal in positive and negative directions. In these recordings vigilance states (waking, slow-wave sleep, SWS, and paradoxical sleep, PS) were reconstructed with 10 s resolution similarly as reported elsewhere (9). Waking (desynchronized neocortical EEG and phasic activity in the EMG, thus largely active waking), SWS (slow-wave activity in the neocortical EEG and decreased mus-
were used in further analysis. The quality of sorted units was generally lower number of events than in LFP analysis) data: only proper modulation depth was computed (division of the difference of maximal and minimal count by their sum).

The instantaneous running speed was computed from the mouse’s position in an open field. For correlative analysis the running speed and LFP variables were first averaged for consecutive 0.5 second intervals and then for each speed bin (20 bins).

Theta oscillation epochs were manually selected during wake-ning and PS. In this signal theta oscillations were further automatically detected based on theta-delta power ratio of at least 6. Power spectral density was computed with the multitaper method (NW = 3, window length 1024). Peaks of theta and of concurrent gamma oscillations were detected in 5–10 Hz and 35–85 Hz band-pass filtered signals. Amplitudes of the peaks and interpeak intervals were computed. For the analysis of coupling between theta and gamma oscillations positive peaks and interpeak intervals in the gamma band were assigned phase of the concurrent theta epoch (obtained by Hilbert transform in 5–10 Hz band). Coefficient of modulation was estimated similarly to the LFP-gamma modulation of the discharge for each theta-phase bin for each unit. Theta modulation was estimated similarly to the LFP-gamma modulation with an adaptation for unitary (more variable along with generally lower number of events than in LFP analysis) data: only proper modulation depth was computed (division of the difference of maximal and minimal count by their sum).

Histology. After completion of the experiments, mice were deeply anesthetized, perfused intracardially with 4% PFA solution and decapitated. Brains were fixed in 30% formalin-sucrose. The brains where subsequently frozen, cut in 40-μm slices, stained with cresyl violet and recording sites were confirmed.

Statistical Analysis. Two-way ANOVA was used to estimate the significance of genotype- and vigilance state-related differences. The statistical significance of single comparisons was determined by the Wilcoxon rank sum test or t test depending on the distribution normality (determined by the Lilliefors test). The differences in the coupling of theta and gamma oscillations were further estimated with ANCOVA using theta - rhythm features as covariates. Circular statistics was performed using the Rayleigh test. P-values <0.05 were considered to indicate significance.
Fig. S1. GABA<sub>A</sub> receptor γ2 subunit gene expression in PV-Δγ2 mice. In situ hybridization (X-ray film) autoradiographs showing the expression of parvalbumin (left panel) and γ2 (middle and right panel) mRNA in brain sections from adult control (left and middle panel) and PV-Δγ2 (right panel) mice. The corresponding transgene content (genotype) of the animals is indicated above the images (arrowheads indicate loxP sites; ex 4, exon 4; PV, parvalbumin promoter). In PV-Δγ2 mice the γ2 mRNA signal is lost in areas with a high density of PV-positive cells, e.g., reticular thalamus (rt), colliculi (col), Purkinje cells and molecular layer of the cerebellum (Cb). The autoradiograph on the left was obtained with an oligonucleotide specific for parvalbumin, the autoradiographs in the middle and on the right were obtained with a γ2 (exon 4) -specific oligonucleotide. H, hippocampus. (Scale bar, 2 mm.)
Fig. S2. Adult PV-Δγ2 mice have unaltered brain morphology and show normal distribution and quantity of PV-positive interneurons. (A and B) Cresyl violet staining of sagittal brain sections from PV-Δγ2 (left) and control (right) mice reveals no difference in brain morphology between the genotypes. (B) Higher resolution images of the hippocampus. (C) PV immunoreactivity in coronal sections of PV-Δγ2 (left) and control (right) mouse hippocampi shows no differences in number and distribution of PV-positive neurons (arrows). H, hippocampus; DG, dentate gyrus. (Scale bars: B, 500 μm; C, 350 μm.)
Transcript levels and distribution of γ1 and γ3 mRNAs are unchanged in PV-Δγ2 mice. In situ hybridization (X-ray film) autoradiographs showing the expression of GABA<sub>A</sub> receptor γ1 (A) and γ3 (B) subunit mRNAs in horizontal brain sections from adult PV-Δγ2 (Left in A and B) and control (Right in A and B) mice. Autoradiographs were obtained with oligonucleotides specific for γ1 (A) and γ3 (B) mRNA. Cb, cerebellum; col, colliculi; CPu, caudate putamen; GP, globus pallidus; H, hippocampus; LS, lateral septum. (Scale bar, 2 mm.)
**Fig. S4.** Representative data examples. (A) An example of EMG, neocortical EEG and reconstructed vigilance states (W - waking, SWS - slow-wave sleep, PS - paradoxical sleep) from a PV-Δγ2 mouse. (B) Hippocampal EEG recorded during PS (in PV-Δγ2 mouse during an episode marked in (A) with an asterisk). Theta rhythmicity is indicated in 1–20 Hz bandpass filtered signals (green and blue traces) superimposed on corresponding 1–85 Hz filtered signals (black and red traces). Bottom traces show normalized 1–20 Hz filtered signals. Note reduced regularity of theta oscillations in the mutant (blue trace). (C) Interspike histograms of 2 putative pyramidal cells during theta oscillations. (D) Group average of interspike histograms for pyramidal cells during theta oscillations. Spike counts in individual unit histograms are binwise (10 ms bin) normalized by the total number of spikes in a train. (E) Average cross-correlations (CCGs) of putative pyramidal cells (157 pairs, control, 313 pairs, PV-Δγ2) obtained from complete recording sessions. Spike counts in each 5 s bin of individual unit CCGs is scaled to total spike count. Arrows indicate theta-band CCG peaks (lags of 100 to 200 ms) in control. (F) Group statistics for the theta-band firing probability (mean of the unitwise theta-band average probability ± SEM). *, *P* = 0.01. The reduction of short lags (~50 ms around 0) of firing in the mutant is not significant. Interspike histograms and cross-correlations of pyramidal cells indicate reduced theta-band rhythmicity of single cells and population discharge respectively.
Fig. S5.  (A and B) Log-power spectral density of hippocampal EEG during waking and paradoxical sleep (PS). (C and D) Association between theta rhythm features and running speed in control and PV-Δγ2 mice. A.u., arbitrary units, Hilbert transformed amplitude of theta LFP (5–10 Hz bandpass).
**Fig. S6.** Uncoupling of theta and gamma oscillations during paradoxical sleep. (Left) Altered modulation of gamma amplitude and gamma frequency (mean ± SEM) for all theta oscillation epochs detected during paradoxical sleep. (Right) Group statistics of modulation coefficients derived from histograms of part A. ***, P < 0.01.

Other Supporting Information Files

SI Appendix