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Neuregulin-1 Modulates Hippocampal Gamma Oscillations: Implications for Schizophrenia

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Alterations in gamma-frequency oscillations are implicated in psychiatric disorders, and polymorphisms in *NRG-1* and *ERBB4*, genes encoding Neuregulin-1 (NRG-1) and one of its receptors, designated ErbB4, are associated with schizophrenia. Here we show that NRG-1 selectively increases the power of kainate-induced, but not carbachol-induced, gamma oscillations in acute hippocampal slices. NRG-1β is more effective than NRG-1α, a splice variant with lower affinity for ErbB receptors, and neither isoform affects the network activity without prior induction of gamma oscillations. NRG-1β dramatically increases gamma oscillation power in hippocampal slices from both rats (2062 ± 496%) and mice (710 ± 299%). These effects of NRG-1β are blocked by PD158780, a pan-specific antagonist of ErbB receptors, and are mediated specifically via ErbB4 receptors, because mice harboring a targeted mutation of ErbB4 do not respond to NRG-1. Moreover, we demonstrate that 50% of gamma-amino butyric acidergic parvalbumin (PV)–positive interneurons, which heavily contribute to the generation of gamma oscillations, express ErbB4 receptors. Importantly, both the number of PV-immunoreactive interneurons (−31%) and the power of kainate-induced gamma oscillations (−60%) are reduced in ErbB4 knockout mice. This study provides the first plausible link between NRG-1/ErbB4 signaling and rhythmic network activity that may be altered in persons with schizophrenia.

Keywords: ErbB4, hippocampus, interneuron, mouse, parvalbumin, rat

Introduction

The synchronization of neuronal network activity in the human cortex and hippocampus at gamma frequencies (30–80 Hz) is important for cognition, learning and memory (Engel and Singer 2001). Spontaneous long-lasting gamma oscillations have been recorded in vivo in the hippocampus and their frequency is modulated by gamma-aminobutyric acidergic (GABAergic) basket cells (Bragin et al. 1995; Fisahn et al. 1998; Csicsvari et al. 2003; Mann et al. 2005). Similar gamma rhythms can be evoked in rodent acute hippocampal slices by bath application of carbachol (Fisahn et al. 1998) or kainic acid (Hajos et al. 2000; Fisahn et al. 2004) resulting in the activation of muscarinic or kainate receptors, respectively. Both carbachol-induced and kainate-induced gamma oscillations are driven by the complex network of the CA3 area and rely on interplay of excitatory and inhibitory synaptic transmission (Fisahn et al. 1998). Whereas fast inhibitory and fast excitatory neurotransmission is necessary for both types of gamma oscillations, differences are emerging. For example, carbachol-induced activity depends on the recruitment of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors whereas kainate-induced activity does not. Furthermore, it has been suggested that the dominance of inhibitory versus excitatory neurotransmission may be different in the various induction paradigms for gamma oscillation and that different paradigms recruit distinct subgroups of GABAergic interneurons (Palhalmi et al. 2004).

Recent studies indicate that the power of gamma oscillations in subjects diagnosed with schizophrenia is reduced (Kwon et al. 1999; Wilson et al. 2007), and that regional reaction time phase-lock of oscillations is correlated with either the positive or negative symptoms of the disorder (Spencer et al. 2004). In addition, the amounts of RNA and immunoreactivity for parvalbumin (PV) are reduced in post-mortem tissue from the frontal cortex and the hippocampus of schizophrenic patients (Reynolds et al. 2002; Zhang and Reynolds 2002; Hashimoto et al. 2003; Torrey et al. 2005), and a direct relation to the changes in gamma oscillations has been suggested (Lewis et al. 2005).

Neuregulin-1 (NRG-1) is a trophic and differentiation factor that signals via ErbB receptor tyrosine kinases (ErbB2–ErbB4) and that harbors an epidermal growth factor (EGF)–like domain mediating its biological effects (Buonanno and Fischbach 2001). The isoforms NRG-1α and NRG-1β are generated by alternate splicing of the EGF-like domain; NRG-1β is more highly expressed in brain and has the highest affinity for ErbB receptors. In the hippocampus pro-NRG-1β is presynaptically expressed in CA3 pyramidal neurons, and it is cleaved and released in an activity-dependent manner (Eilam et al. 1998; Loeb et al. 2002; Ozaki et al. 2004). NRG-1 binds to either ErbB3 or ErbB4, causing a conformational change that promotes receptor dimerization and autophosphorylation, and subsequent activation of downstream signaling pathways. Although ErbB2 does not bind NRG-1, it functions as coreceptor after heterodimerization with ErbB3 or ErbB4; ErbB4 can also function as a homodimer (Buonanno and Fischbach 2001). ErbB receptors are widely and differentially expressed in the adult brain. In general, ErbB2 is expressed in most cells, ErbB3 is enriched in glial populations, and ErbB4 is mainly found in neurons. ErbB4 transcripts and protein are most highly expressed in interneurons (Lai and Lemke 1991; Gerecke et al. 2001; Yau et al. 2003; Longart et al. 2007), where it localizes at glutamatergic postsynaptic densities and interacts with PSD-95 (Garcia et al. 2000; Huang et al. 2000).

NRG-1 and *ERBB4* were identified as schizophrenia susceptibility genes (Stefansson et al. 2002; Norton et al. 2006; Silberberg et al. 2006), and these finding have been supported by numerous subsequent genetic-association studies worldwide (Corfas et al. 2004; Harrison and Weinberger 2005; Li et al. 2006). Biochemical studies using post-mortem brain samples, recently revealed that NRG-1 signaling and ErbB4-PSD95 interactions are altered in dorsal prefrontal cortices of

schizophrenia patients (Hahn et al. 2006). Moreover, subjects harboring an "at-risk" NRG-1 polymorphism manifest decreased frontal and temporal lobe activation, decreased IQ and augmented psychotic symptoms (Hall et al. 2006). Animal studies have also shown that NRG-1 and ErbB4 hypomorphic mice, but not ErbB2 or ErbB3 mutant mice (Gerlai et al. 2000), exhibit deficits in sensory gating that are ameliorated by treatment with the antipsychotic clozapine (Stefansson et al. 2002).

Given the associations of gamma oscillations, basket cell function and the NRG/ErbB signaling pathway with schizophrenia, we were interested in investigating a potential role of NRG-1 in regulating gamma oscillatory activity.

Material and Methods

Animals

Electrophysiological experiments were performed with male Wistar rats (4–5 weeks), and with ErbB4$^{MHC\text{-}ErbB4}$–/– (Tidcombe et al. 2003) and C57BL/6 wild-type (WT) mice (5–8 weeks); immunohistological experiments used adult mice from both groups (9–12 weeks). ErbB4$^{MHC\text{-}ErbB4}$–/– mice were backcrossed for 15 generations into C57BL/6. Animals were raised under a 12-h light/12-h dark cycle with food and water provided ad libitum. Procedures were approved and followed the NIH guidelines for the care and use of laboratory animals.

Electrophysiology

Horizontal 300-μm-thick hippocampal slices were maintained at room temperature at the interface between humidified carbogen gas (95% O_2/5% CO_2) and artificial cerebrospinal fluid (ACSF), pH 7.3, containing (in mM): NaCl 124, KCl 3.5, NaH_2PO_4 1.25, $MgCl_2$ 1.5, $CaCl_2$ 1.5, $NaHCO_3$ 30, glucose 10, for at least 1 h prior to recording. Extracellular field recordings were made in stratum pyramidale (sp) of CA3 in an interface recording chamber (36 °C; rat slices) or a submerged recording chamber (32 °C; mouse slices) using glass microelectrodes containing ACSF (resistance 3–5 MΩ). Drugs: kainic acid (Sigma-Aldrich, St. Louis, MO), NRG-1α and NRG-1β EGF-like domain, PD158780 (R&D Systems, Minneapolis, MN). Different batches of NRG-1 peptides were tested for their capacity to elicit equal ErbB receptor phosphorylation. Data were recorded with a MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA) and stored using pClamp 9.2 software (Molecular Devices). Fast Fourier Transformations for power spectra were computed from 60-s traces using Axograph software (Molecular Devices). Power values are derived from integrating power spectra between 20 and 80 Hz.

Cell Quantification

Paraformaldehyde-fixed 50-μm horizontal sections were processed for either PV/ErbB4 double-immunofluorescence (6 sections per animal; WT: n = 2; ErbB4$^{MHC\text{-}ErbB4}$–/–: n = 2) or PV immunohistochemistry (16–20 sections per animal; WT: n = 4; ErbB4$^{MHC\text{-}ErbB4}$–/–: n = 4) using standard procedures. Primary antibodies: rabbit polyclonal anti-PV (Swant, Bellinzona, Switzerland), mouse monoclonal anti-ErbB4 (AB-1, Lab Vision, Fremont, CA). Numerical cell density, location in layers, and degree of coexpression were determined for PV and ErbB4 immunoreactive cell somata in hippocampal CA3 on either confocal or brightfield images. The specificity of the staining was tested by standard procedures, including application of the ErbB4 antibody on ErbB4$^{MHC\text{-}ErbB4}$–/– sections.

Data Analysis and Statistics

Analysis of electrophysiological data (paired and unpaired Student t-test) was carried out in KaleidaGraph software (Synergy Software, Reading, PA). Significance of the cell density of PV neurons in WT vs. ErbB4$^{MHC\text{-}ErbB4}$–/– mice (n = 4 each) was evaluated with a 2-tailed Student's t-test (Fig. 3*D*). The distribution of PV cells across layers in CA3 was analyzed with 2-way ANOVA (WT: n = 2, ErbB4$^{MHC\text{-}ErbB4}$–/–: n = 2; Fig. 3*E*). Significance level for all tests was set to P = 0.05. All data are means ± SEM.

Results

Neuregulin-1 Increases the Power of Gamma-Frequency Oscillations

Given the associations of gamma oscillations and NRG-ErbB signaling with schizophrenia, we investigated whether NRG-1 modulates hippocampal rhythmic activity. We first tested the effects of NRG-1β, the splice variant predominantly expressed in brain with highest affinity for ErbB receptors (Buonanno and Fischbach 2001), on kainate-induced (100 nM) gamma oscillations in rat hippocampal slices. Perfusion of slices with 2 nM NRG-1β dramatically increased the power of gamma oscillations (Fig. 1*A*; P = 0.015), whereas application of the less active NRG-1α isoform resulted in a smaller, but still significant increase (Fig. 1*B*; P = 0.031). NRG-1β increased oscillation power by 2062 ± 496% (n = 6), which was significantly higher than the 442 ± 220% (n = 4) increase elicited by NRG-1α (Fig. 1*D*, P = 0.027). Measurements taken at 5, 10, and 15 min after NRG-1β application indicate its effects on kainate-induced gamma oscillation power do not change significantly by prolonging perfusion beyond 10 min (5 min: 1.02 × 10^{-03} ± 0.36 × 10^{-03} V^2; 10 min: 1.30 × 10^{-03} ± 0.45 × 10^{-03} V^2; 15 min:

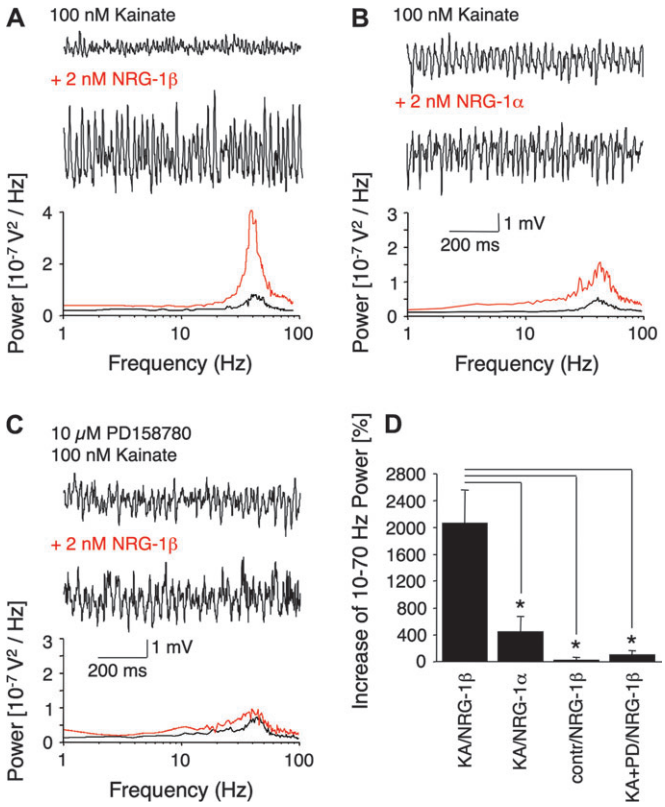

Figure 1. Modulation of kainate-induced gamma oscillations by NRG-1 in rat hippocampal slices. (*A–C*) Representative sample traces (top) and power spectra (bottom) of kainate-induced gamma oscillations in rat slices (black: control; red: NRG-1 treated); note difference in scales. (*A*) NRG-1β (n = 6; P = 0.015) and (*B*) NRG-1α (n = 4; P = 0.031) significantly increase the power of kainate-induced gamma oscillations, whereas (*C*) preincubation with the ErbB inhibitor PD158780 (PD) prior to NRG-1β treatment prevents an increase (n = 6; P = 0.13). (*D*) Quantification of relative gamma power in response to different NRG-1 and ErbB inhibitor treatments. NRG-1β increases the power of kainate-induced (KA) gamma oscillations more strongly than NRG-1α (β: n = 6, α: n = 4, P = 0.027) or NRG-1β after ErbB inhibitor treatment (KA + PD/NRG-1β, n = 6, P = 0.013). NRG-1β has no effect in control slices without prior application of kainate (contr/NRG-1β, n = 4, P = 0.73).

$1.16 \times 10^{-03} \pm 0.27 \times 10^{-03}$ V^2; $n = 8$; 5 min/10 min $P = 0.016$; 10 min/15 min $P = 0.531$). Consequently, all subsequent measurements were taken after 10-min perfusion. Interestingly, application of 2 nM NRG-1β without prior induction of gamma oscillations by kainate neither generated rhythmic network activity nor significantly increased unspecific network activity (Fig. 1*D*; 23 ± 46%, $n = 4$, $P = 0.73$). A lack of NRG-1β effect at basal activity levels is consistent with our prior work showing that basal glutamatergic synaptic transmission is not modified by NRG-1β and requires Schaffer collateral stimulation (Kwon et al. 2005).

An ErbB Receptor Antagonist Blocks the Neuregulin-1 Effect

Next, we tested for the specificity of the NRG-1β effect on gamma oscillations by treating slices with PD158780, a pan-ErbB specific antagonist that targets the intracellular tyrosine phosphorylation site of these receptors (Fig. 1*C*). When slices were pretreated with 10 μM PD158780 for 30 min, to allow penetration of the inhibitor into the cell, subsequent application of 2 nM NRG-1β did not alter significantly the power of kainate-induced gamma oscillations (Fig. 1*D*; 105 ± 58%; $n = 6$; $P = 0.13$). Taken together, our results indicate that the effects of NRG-1β are mediated specifically by ErbB receptors because the efficacy of NRG-1 isoforms to increase gamma oscillation power correlates with their binding affinities for the receptors (β isoforms being more active than α isoforms), and because NRG-1 effects are significantly blocked by PD158780. However, pharmacological approaches do not resolve which ErbB receptor subtype mediates the NRG-1 effect because, to our knowledge, selective antagonists for the distinct receptor proteins do not exist. Although the widely used antagonists AG1478 and PD158780 specifically target ErbB receptors, they are not selective for a receptor subtype. ErbB4 kinase activity, as well as those of ErbB1 (EGFR) and ErbB2, are all blocked by both antagonists (Egeblad et al. 2001; Stoll et al. 2001; Brignola et al. 2002). Because the NRG-1 ligand binds ErbB3 and ErbB4, and ErbB3 is restricted to glia in the hippocampus, we hypothesized that ErbB4 is the necessary receptor for NRG-1-mediation of hippocampal gamma oscillations. To address this issue, we had to resort to using genetically targeted mutant mice.

Neuregulin-1 Modulates Kainate-Induced, but not Carbachol-Induced, Gamma Oscillations

With the goal of investigating NRG-1 effects in genetically altered mice, we began by characterizing how NRG-1β modulates gamma oscillations in mouse hippocampal slices. As shown in Figure 2*A*,*D*, 2 nM NRG-1β also dramatically increased the power of kainate-induced gamma oscillations in WT C57BL/6 mouse hippocampal slices (710 ± 299%; $n = 12$; $P = 0.004$). The differences between rat and mouse experiments in NRG-1β-induced gamma oscillation frequency and relative power increase are due to differing recording conditions (see Methods and Supplementary Fig. 1). Consistent with previous work showing that NRG-1β action on synaptic function endures after washout (Kwon et al. 2005), we observed that its effects on gamma oscillations could not be reversed by 30-min washout (−16 ± 9%; $n = 6$, $P = 0.1$; data not shown). NRG-1β failed to generate any rhythmic network activity when applied without prior induction of gamma oscillations (−8 ± 7%; $n = 10$; $P = 0.18$; Fig. 2*D*), and its effect was blocked by 30-min preincubation of slices with 10μM PD158780 (5 ± 4%; $n = 8$; $P = 0.47$; compare NRG-1β: 710 ± 299%). However, PD158780 had no significant effect on kainate-induced gamma oscillations when it was added after NRG-1β treatment (data not shown).

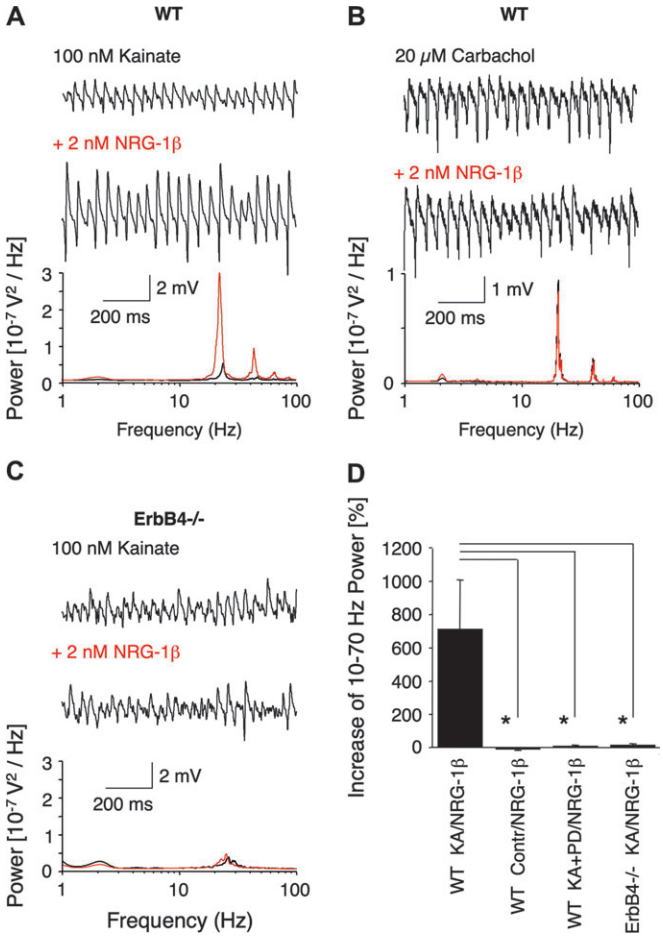

Figure 2. NRG-1β increases kainate-induced, but not carbachol-induced, gamma oscillations and its effect is absent in slices from ErbB4$^{MHC\text{-}ErbB4}$−/− mice. (*A*–*C*) Representative sample traces (top) and power spectra (bottom) of gamma oscillations; note difference in scales. (*A*) Kainate-induced ($n = 12$; $P = 0.004$) or (*B*) carbachol-induced ($n = 8$; $P = 0.2$) gamma oscillations in slices from WT mice (black: control; red: NRG-1β treated). (*C*) Kainate-induced gamma oscillations in slices from ErbB4$^{MHC\text{-}ErbB4}$−/− mice (black: control; red: NRG-1β treated). (*D*) Quantification of the effects of NRG-1β on kainate-induced gamma oscillations in hippocampal slices from WT or ErbB4$^{MHC\text{-}ErbB4}$−/− mice. NRG-1β increases significantly the power of kainate-induced oscillations ($n = 12$; $P = 0.004$), whereas NRG-1β (2 nM) has no effect in WT slices without prior application of kainate ($n = 10$; $P = 0.18$). Coapplication of 10 μM PD158780 prevents NRG-1β modulation of kainate-induced gamma oscillations ($n = 8$, $P = 0.47$), which differs from NRG-1β + kainate-induced oscillations ($P = 0.038$). The NRG-1β modulation of kainate-induced gamma oscillations in ErbB4$^{MHC\text{-}ErbB4}$−/− slices is absent ($n = 14$, $P = 0.26$) and therefore significantly different from WT littermates ($P = 0.018$), demonstrating that the effects of NRG-1β require signaling via ErbB4 receptors.

To determine whether the effects of NRG-1β on network activity are selective to kainate-induced gamma oscillations, we investigated its effects on mouse slices treated with carbachol (Fig. 2*B*), another induction paradigm for gamma oscillations (Fisahn et al. 1998). The NRG-1β-induced increase in the power of kainate-induced gamma oscillations is totally absent in carbachol-induced gamma oscillations (−10 ± 7%; $n = 8$; $P = 0.2$; Fig. 2*B*). The differential modulation by NRG-1β is most likely due to differing neuronal mechanisms involved in the kainate and carbachol induction paradigms (A. Fisahn, unpublished data), for example, recruitment of AMPA receptors in

carbachol- but not kainate-induced gamma oscillations (Bartos et al. 2007), and indicates a site-specific action of NRG-1/ErbB signaling on the kainate-related pathway.

Neuregulin-1 Modulation of Gamma Oscillations is ErbB4 Receptor Dependent

Based on the cellular and subcellular distribution of ErbB4 receptors (Gerecke et al. 2001), and the behavioral similarities between NRG-1 and ErbB4 hypomorphic mice (Stefansson et al. 2002) that is not shared by ErbB2 and ErbB3 mutant mice (Gerlai et al. 2000), we hypothesized that the ErbB4 receptor mediates the NRG-1 effects on gamma oscillations. We tested this hypothesis using adult ErbB4 knockout mice rescued from embryonic lethality by transgenic expression of ErbB4 specifically in heart (ErbB4$^{MHC\text{-}ErbB4}$–/–; Tidcombe et al. 2003). As shown in Figure 2*C,D*, the NRG-1β-induced increase in gamma oscillation power is entirely absent in hippocampal slices from ErbB4$^{MHC\text{-}ErbB4}$–/– mice (10 ± 10%; n = 14; P = 0.26; compared with WT littermate controls: 710 ± 299%; P = 0.018). This result, together with the block of NRG-1β-induced increase in gamma oscillation power in WT slices by PD158780, demonstrates that the modulatory role of NRG-1β on kainate-induced gamma oscillations specifically requires signaling via ErbB4 receptors.

We went on to investigate possible differences in kainate-induced oscillations between WT and ErbB4$^{MHC\text{-}ErbB4}$–/– mutant mice. We found that in ErbB4$^{MHC\text{-}ErbB4}$ null mice gamma oscillation power was reduced by 60% (WT: 2.78 × 10^{-04} ± 8.20 × 10^{-05} V^2, n = 20; ErbB4$^{MHC\text{-}ErbB4}$–/–: 1.09 × 10^{-04} ± 2.40 × 10^{-05} V^2, n = 14; P = 0.0386), whereas the peak frequency was not altered (WT: 28.97 ± 1.12 Hz, n = 20; ErbB4$^{MHC\text{-}ErbB4}$–/–: 27.43 ± 1.38 Hz, n = 14; P = 0.331, unpaired t-test). We also observed that hippocampal slices from ErbB4$^{MHC\text{-}ErbB4}$–/– mice were more prone to generate epileptiform activity in response to 100 nM kainate (A. Fisahn, unpublished observation). Taken together, these changes indicate a reduction or impairment of network inhibition in ErbB4$^{MHC\text{-}ErbB4}$–/– mice.

Coexpression of ErbB4 and PV in Interneurons

Because the importance of PV-immunoreactive interneurons for gamma oscillations is well established (Bartos et al. 2007), we went on to investigate the hippocampal interneuron populations that express PV and ErbB4. As shown in Figure 3*A,B*, most PV-positive cells are located close to pyramidal cell somata in strata oriens (so), sp, and lucidum (sl), whereas ErbB4 cells also reside in strata radiatum (sr) and lacunosum moleculare (slm). We found that in WT mice almost half of PV cells coexpress ErbB4 (44%, 250 of 573 cells) throughout all layers in cornu ammonis (Fig. 3*C*: slm: 50 ± 50%, sl/sr: 35.2 ± 7%, sp: 46.5 ± 5.8%, so: 41.7 ± 3.6%), whereas only 27% (250 of 938) of ErbB4 cells coexpress PV, with a strong gradient across layers (Fig. 3*D*: slm: 1.1 ± 1.1%, sl/sr: 15.8 ± 2.8%, sp: 31.3 ± 12.6%, so: 32.5 ± 15.3%). We would like to emphasize that coexpression was determined by counting only neurons that were clearly, and independently, identifiable in both the red and green color channels, rather than solely on appearance of yellow pixels in the overlay picture; therefore, it is unlikely that we overestimated the number of neurons that coexpress ErbB4 and PV.

Reduced Number of PV-Immunoreactive Neurons in Mutant Mice

Next, we compared the number of PV-positive neurons in hippocampal sections from WT and ErbB4$^{MHC\text{-}ErbB4}$–/– mice.

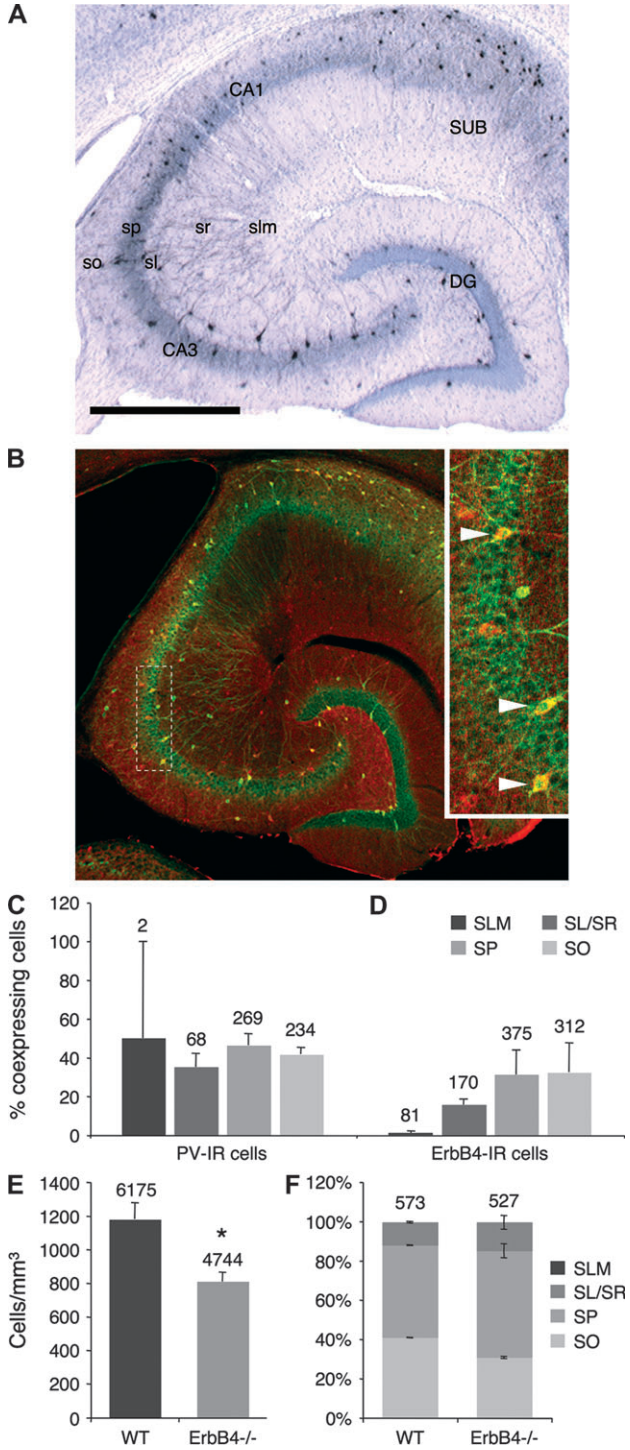

Figure 3. PV-expressing neurons coexpress ErbB4 in WT hippocampus and are reduced in ErbB4$^{MHC\text{-}ErbB4}$–/– mice. (*A*) PV immunohistochemistry, and (*B*) mounted image of ErbB4 (red) and PV (green) double-immunofluorescence on sections from WT mice. ErbB4-positive somata are distributed throughout all layers, whereas PV-expressing somata are mostly near to pyramidal cells. Scale bar = 600 μm. Insert: Pyramidal cell layer of CA3, showing coexpression of ErbB4 and PV (arrowheads). (*C*) Almost 50% of PV-immunoreactive (IR) cells coexpress ErbB4 throughout all layers; (*D*) however, less ErbB4-IR cells coexpress PV, notably in slm. (*E*) PV-expressing cells are reduced by 31% in ErbB4$^{MHC\text{-}ErbB4}$–/– mice (WT vs. ErbB4$^{MHC\text{-}ErbB4}$–/– mice, n = 4 each, 16–20 sections per animal, P = 0.017). (*F*) Quantitative analysis confirms that most PV-IR somata are near pyramidal cells, either in the so, sp, or lower stratum radiatum (sr), whereas slm is almost devoid of PV-IR cells. WT and ErbB4$^{MHC\text{-}ErbB4}$–/– mice do not differ in across-layer distribution of PV-positive cells (n = 2 each). (*C–F*) Total numbers of counted cells are presented on top of the columns.

This analysis revealed that the numerical density (cells/mm^3) of PV-positive cell bodies is reduced by 31% in cornu ammonis of ErbB4$^{MHC\text{-}ErbB4}$–/– compared with WT mice (Fig. 3*E*; WT: 1183 ± 98, n = 4; ErbB4$^{MHC\text{-}ErbB4}$–/–: 812 ± 58, n = 4; P = 0.017). Our results on the numerical density of PV cells in the hippocampus of WT animals are in line with previously published data of both mice (Jinno and Kosaka 2002) and rats (Keilhoff et al. 2004). The 31% loss in ErbB4$^{MHC\text{-}ErbB4}$–/–, however, does not change the distribution of PV cells across layers (P = 0.787; Fig. 3*F*).

Discussion

We show pharmacologically and with ErbB4$^{MHC\text{-}ErbB4}$–/– mice that NRG-1 dramatically, and in an activity-dependent fashion, increases the power of hippocampal gamma oscillations via ErbB4 receptors, which are coexpressed by half of PV-containing interneurons. As a possible mechanism, we suggest that the NRG-1–induced increase in gamma oscillation power results from an activation of ErbB4 receptors on PV-containing interneurons, because perisomatic feedback inhibition crucially contributes to extracellular gamma oscillations (Mann et al. 2005). An increase of precisely timed GABA release via ErbB4 receptor activation (Woo et al. 2007; A. Buonanno, unpublished data) from perisomatic-targeting interneurons would lead to increased synchronization of pyramidal cell activity and, via recurrent collaterals and additional mechanisms, to recruitment of more interneurons (Mann and Paulsen 2007). Consequently, inhibition of ErbB4 receptors or deleting them outright would abolish the NRG-1–induced increase of both GABA release and gamma oscillations. However, although the activation of ErbB4-expressing PV neurons provides a plausible mechanism for the NRG-1 effect, we presently cannot exclude the possibility that other interneurons also contribute to the increase in oscillation power. Given the high density of ErbB4-expressing interneurons in the hippocampus it is likely that additional populations of interneurons coexpress the receptor. Further studies are needed to identify these neurons and investigate whether they contribute to the NRG-1 effects on gamma oscillations. Our results in ErbB4$^{MHC\text{-}ErbB4}$–/– mice clearly demonstrate that ErbB4 is necessary for the NRG-1 effect; however, we currently cannot rule out if ErbB2 might contribute to the response through heterodimerization with ErbB4.

ErbB4 is crucially involved in the proliferation, differentiation and migration of interneuronal precursor cells (Yau et al. 2003; Anton et al. 2004). It was recently reported that GABA-immunoreactivity is reduced by 40% in the P20 hippocampus of ErbB4$^{MHC\text{-}ErbB4}$–/– mice (Flames et al. 2004), which is close to the 31% reduction of PV-immunoreactivity that we observe in adult mutant mice. It is therefore tempting to speculate that the interneurons that coexpress PV and ErbB4 are most susceptible to disruption of the NRG-1-ErbB4 signaling pathway. This is interesting given the selective loss of PV-positive interneurons reported in post-mortem hippocampus of schizophrenia patients (Zhang and Reynolds 2002; Lewis et al. 2005). The reduction in PV-immunoreactivity in cornu ammonis of ErbB4$^{MHC\text{-}ErbB4}$–/– mice correlates with a reduction in power of gamma oscillations by 60% in vitro, whereas the peak frequency is not significantly changed. A clear relation of a loss or impairment of PV-immunoreactivity to changes in cortical gamma oscillations has yet to be established (Vreugdenhil et al. 2003; Fuchs et al. 2007). However, ongoing experiments indicate that hippocampal slices of ErbB4$^{MHC\text{-}ErbB4}$–/– mice are also more susceptible to epileptiform activity than WT slices (A. Fisahn, unpublished observation), which supports the idea of dysfunctional changes in hippocampal circuitry of ErbB4$^{MHC\text{-}ErbB4}$–/– mice. Generally, the lack of a clear correlation between reduced PV-immunoreactivity and changes in gamma oscillation suggests that PV-expressing interneurons might be differently affected in different brain areas, different experimental paradigms, and schizophrenia.

There is increasing evidence for a role of acute NRG-1/ErbB signaling in the regulation of neural plasticity at glutamatergic synapses. At hippocampal CA3-to-CA1 synapses NRG-1 induces changes in AMPA receptor, but not NMDA receptor, surface expression and excitatory postsynaptic potentials (EPSPs; Kwon et al. 2005; Li et al. 2007). In prefrontal cortical pyramidal cells, however, NRG-1 was reported to reduce NMDA, but not AMPA, receptor currents (Gu et al. 2005). Acute reductions in neocortical glutamatergic transmission by application of NMDA antagonists can induce aberrant gamma oscillations in vivo with both simultaneously increased and decreased frequencies in different cortical areas (Pinault 2008). These regional differences suggest that NRG-1 effects on glutamatergic transmission, and on gamma oscillations, could also be region-specific, probably due to different cellular and subcellular localization of ErbB receptors in neocortical versus hippocampal areas.

Numerous studies suggest that alterations in neuronal connectivity, in particular a regional reduction of specific interneurons, may underlie the pathophysiology in schizophrenia and other psychoses (Green and Nuechterlein 1999; Ford et al. 2007) that are associated with impaired sensory information processing and reductions in the power of gamma oscillations (Kwon et al. 1999; Wynn et al. 2005; Wilson et al. 2007). This study is the first to directly link NRG-1/ErbB4 signaling and gamma band activity involved in higher brain processes. However, the single nucleotide polymorphisms (SNPs) in the *NRG-1* and *ERBB4* genes that are associated with schizophrenia will likely cause only subtle changes in the expression and the biological activity of these proteins, and it is yet unknown whether a reduction of PV neurons and gamma oscillations is a primary effect, or rather compensatory for other genetic and nongenetic risk-factors of schizophrenia.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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Notes

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