

***ErbB4* is a suppressor of long-term potentiation in the adult hippocampus**

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Abstract

ErbB4 has emerged as a leading susceptibility gene for schizophrenia but the function of the ErbB4 receptor in the adult brain is unknown. Here we show in the adult hippocampus that long-term potentiation (LTP) of transmission at Schaffer-collateral CA1 synapses was markedly enhanced in mutant mice lacking ErbB4. Concordantly, LTP was enhanced by acutely blocking ErbB4 in wild type animals, indicating that ErbB4 activity constitutively suppresses LTP. Moreover, increasing ErbB4 signaling further suppressed LTP. By contrast, altering ErbB4 activity did not affect basal synaptic transmission or short-term facilitation. Our findings suggest that cognitive deficits in schizophrenia may be a consequence of hyperfunction of ErbB4 signaling leading to suppressed glutamatergic synaptic plasticity, thus opening new approaches for treatment of this disorder.

Keywords

ErbB4; transgenic mouse; neuregulin; Schaffer collateral-CA1 synapses; long-term potentiation; theta burst stimulation; synaptic plasticity; paired-pulse facilitation

Introduction

A growing body of linkage and association evidence in humans implicates *ErbB4* as a leading susceptibility gene for schizophrenia [10,21], but a critical unresolved issue is defining the essential functions of ErbB4 in the brain and the link to schizophrenia [22]. Because deficits in cognition are a core feature of schizophrenia [12] and because a critical cellular substrate for normal cognitive functioning is synaptic plasticity at glutamatergic synapses [13], we used a loss-of-function approach to investigate the hypothesis that ErbB4 may function to regulate glutamatergic synaptic transmission or plasticity.

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ErbB4 is expressed in adult CNS [5], and in the adult hippocampus, a brain region increasingly implicated in schizophrenia [3,10,20], ErbB4 is abundantly expressed by pyramidal cells (eg. [4,7,14] and Fig. 1a, b, c) and is enriched at glutamatergic synapses in the post synaptic density [7]. ErbB4 associates with the scaffolding protein PSD-95 [4,6,7] and through this with the NMDA receptor [4,6,7], a subtype of glutamate receptor having a key role in synaptic plasticity. Our evidence indicates that tonic activity of ErbB4 constitutively suppresses long-term potentiation (LTP) of glutamatergic synaptic transmission in the adult hippocampus. Our findings reveal a previously unknown essential function of ErbB4 in the brain, suggesting that cognitive deficits in schizophrenia may be a consequence of hyperfunction of ErbB4 signaling thereby suppressing plasticity at glutamatergic synapses.

Methods

ErbB4 null mutant mice

ErbB4 null mice were kindly provided by Martin Gassmann [23]. Embryonic lethality of *ErbB4*^{-/-} mice was genetically rescued by expressing ErbB4 under a cardiac-specific myosin promoter (*ErbB4*^{-/-}HER4^{heart}). These mice do not express ErbB4 in the brain or non-cardiac tissues.

Immunocytochemistry

Anesthetized adult male *ErbB4*^{+/+}HER4^{heart} and *ErbB4*^{-/-}HER4^{heart} mice were perfused transcardially with 4% paraformaldehyde. Brains were removed, post-fixed, and placed in 30% sucrose. Para-sagittal 50 µm frozen sections were collected in cold 0.1 M phosphate-buffered saline and stained with Nissl or processed for immunohistochemistry using anti-NeuN antibody (1:2000, Chemicon) or anti-ErbB4 antibody (SC-283; 1:500, Santa Cruz) with or without its immunogenic peptide. Immunostaining was revealed using fluorescently-conjugated secondary antibodies.

Immunoblotting

Hippocampal or heart tissue from *ErbB4*^{+/+}HER4^{heart} and *ErbB4*^{-/-}HER4^{heart} mice was homogenized in buffer (in mM): 20 Tris-HCl, pH 8, 137 NaCl, 1% NP-40, 10% glycerol, and the protease inhibitor cocktail (Sigma). Proteins were resolved in SDS-PAGE, transferred onto a nitrocellulose membrane, and visualized using enhanced chemiluminescence. Antibodies used were ErbB4 (Cell Signaling Technology) and β-actin (Sigma).

Electrophysiology

Hippocampal slices (300 µm) prepared from anesthetized (20% urethane, i.p.) ~ 20 week-old *ErbB4*^{+/+}HER4^{heart} (n = 4 mice) or *ErbB4*^{-/-}HER4^{heart} (n = 4 mice) mice or 21 day old male Sprague Dawley rats (Charles River) were placed in a holding chamber for ≥ 1 hr prior to recording. A single slice was then transferred to a recording chamber and superfused with artificial cerebral spinal fluid (ACSF; at 2 ml/min) composed of (in mM): 132 NaCl, 3 KCl, 1.25 NaH₂PO₄, 2 MgCl, 11 D-glucose, 20 NaH₂CO₃ and 2 CaCl₂ saturated with 95% O₂ (balance 5% CO₂) at 28 ± 2 °C (pH 7.40; 315–325 mOsm). ACSF was supplemented with

bicuculline methiodide (5 μ M). Field excitatory postsynaptic potentials (fEPSPs) were evoked using bipolar tungsten electrodes located \sim 50 μ m from the CA1 cell-body layer and were recorded using ACSF-filled glass micropipettes placed in the stratum radiatum 60–80 μ m from the cell body layer. Stimulation of Schaffer collateral afferents consisted of single pulses (0.08 ms duration) at 0.1 Hz; intensity was set to 30–35% of that which produced maximum synaptic responses. Theta burst stimulation consisted of 15 bursts of 4 pulses at 100 Hz, delivered at an interstimulus interval of 200 ms. ACSF was supplemented as indicated with neuregulin-1 β , NRG-1 β , (2 nM), which was stored as single-use aliquots in aqueous solution at -80 $^{\circ}$ C. ACSF was also supplemented as indicated with PD158780 (10 μ M; dissolved in DMSO), which was made fresh immediately before the experiment. fEPSP slope was calculated as the slope of the 10–60% rising phase. Raw data were amplified using a MultiClamp 700A amplifier and a Digidata 1322A acquisition system sampled at 10 KHz, and analyzed with Clampfit 9.0 and Sigmaplot 7 software. Recordings were done with the experimenter blind to mouse genotype. Statistical comparison of data, presented as mean (\pm SEM), was done using two-way analysis of variance (ANOVA) with the Tukey Test. Experiments were in accordance with policies of The Hospital for Sick Children Animal Care Committee and the Canadian Council on Animal Care.

Results

In our experiments we used mutant mice [23] in which *ErbB4* had been deleted but in which expression of the human ErbB4 was driven in the heart by the α -myosin heavy chain promoter (*ErbB4*^{-/-}HER4^{heart}) which rescued an otherwise lethal cardiac defect (Fig. 1d). In adult *ErbB4*^{-/-}HER4^{heart} mice, we found that the size, anatomy and gross morphology of the hippocampus were not different from wild-type littermate controls (*ErbB4*^{+/+}HER4^{heart}; Fig. 1e). Moreover, in electrophysiological recordings using adult *ErbB4*^{-/-}HER4^{heart} mice, we found that at Schaffer collateral-CA1 synapses in acute hippocampal slices, the basal stimulus-response relationships for afferent fiber volleys and fEPSPs (Fig. 2a,b) and for paired-pulse facilitation, a measure of presynaptic function (Fig. 2c), were not different from wild-type littermates. We thus next investigated synaptic plasticity at these synapses by studying LTP induced by theta-burst stimulation (TBS), a stimulation paradigm mimicking the endogenous theta rhythm that is critical for normal cognitive processing [8]. We found that theta-burst-induced LTP (tbLTP) was dramatically increased in the *ErbB4*^{-/-}HER4^{heart} mice (Fig. 2d): in ErbB4 mutant animals fEPSP slope was 210 ± 20 % ($n = 14$) 60 min after TBS compared with 162 ± 6 % ($n = 14$) in wild-type mice ($p < 0.01$). Thus, while *ErbB4*^{-/-}HER4^{heart} mice had no abnormality of basal synaptic transmission or pre-synaptic function, they showed greatly increased tbLTP. Therefore, these findings indicate that *ErbB4* genetically suppresses a prominent form of synaptic plasticity at Schaffer collateral-CA1 synapses.

To determine whether the enhancement of tbLTP seen in the *ErbB4*^{-/-}HER4^{heart} mice was due to loss of ErbB4 signaling in the adult or to an abnormality due to lack of ErbB4 during development, we examined the effect of acutely inhibiting ErbB4 receptors on tbLTP in normal adult animals. For this purpose we used the potent and membrane-permeable ErbB kinase inhibitor, PD158780, which is known to prevent receptor autophosphorylation and signaling [19]. We found that bath-applied PD158780 (10 μ M) had no effect on volley

amplitude, basal fEPSPs or on paired-pulse facilitation (Fig. 3a,b,c). However, tbLTP was greatly enhanced in slices treated with PD158780 (Fig. 3d): with PD158780 application fEPSP slope was $189 \pm 11\%$ ($n = 21$) 60 min after TBS compared with $149 \pm 4\%$ ($n = 13$) in control, untreated slices ($p < 0.001$). As PD158780 may act on ErbB receptors in addition to ErbB4, we tested it in slices from *ErbB4*^{-/-}HER4^{heart} mice and found that it had no effect on tbLTP (data not illustrated), indicating that the enhancement of tbLTP by PD158780 is dependent upon ErbB4. Thus, in the adult hippocampus ErbB4 signaling constitutively suppresses synaptic plasticity.

To determine whether increasing ErbB4 signaling beyond the constitutive level may further suppress tbLTP we compared the effect of neuregulin, NRG-1 β , an agonist for receptors of the ErbB family, in *ErbB4*^{-/-}HER4^{heart} versus wild-type mice. We and others have shown that NRG-1 β impairs synaptic plasticity [1,7,9]. We found here that bath-applying NRG-1 β (2 nM) nearly abolished tbLTP in slices from wild-type mice but had no effect on tbLTP in those from *ErbB4*^{-/-}HER4^{heart} mice (Fig. 3e), indicating that NRG-1 β inhibits tbLTP through activating ErbB4. Importantly, NRG-1 β had no effect on basal synaptic transmission or paired-pulse facilitation (Fig. 3a,b,c) and PD158780 prevented the suppression of tbLTP by applying NRG-1 β in slices from normal animals (Fig. 3f). Thus, under basal conditions the effect of ErbB4 signaling on tbLTP is not maximal in normal animals, and causing hyperfunction in this signaling pathway further suppresses synaptic plasticity.

Discussion

To study the non-redundant functions of ErbB4 in the hippocampus we used *ErbB4*^{-/-}HER4^{heart} mice. We found that basal synaptic transmission and paired-pulse facilitation were not different from wild type whereas LTP was increased in the mice lacking ErbB4. There were no detectable developmental abnormalities in the hippocampus of the ErbB4 mutant mice that might have accounted for this enhanced LTP, and moreover, acutely administering PD158780 to adult hippocampal slices from wild type animals was without effect on basal synaptic transmission but caused an increase in LTP. We note that the increase in LTP by PD158780 appears to be mediated by blockade of ErbB4 as this compound had no effect on LTP in the ErbB4 mutant mice. The cognate ligand for ErbB4 receptors is NRG1 [18] and we found that the suppression of LTP by administering NRG-1 β [1,7,9] was prevented in the ErbB4 mutant mice and also by PD158780. The most parsimonious explanation for these findings taken together is that a non-redundant function of NRG1-ErbB4 signaling in the hippocampus is to constitutively suppress LTP at Schaffer collateral-CA1 synapses.

The enhancement of LTP in the ErbB4 null mutant mice, and the enhancement by acutely administering PD158780, is consistent with predictions from the suppression of LTP produced by administering exogenous NRG-1 β [1,7,9]. Recently, it has been reported in hippocampal slice cultures that synaptic spine density and pairing-induced LTP are reduced by decreasing the expression of ErbB4 or by overexpressing a kinase dead ErbB4 mutant [11]. While it is conceivable that the difference between this study and those in which NRG-1 β was administered is possibly due to exogenous NRG-1 β not mimicking the endogenous ligand [11], this explanation cannot account for the enhancement of LTP seen

presently in the mice lacking ErbB4 nor the enhancement by applying PD158780. Rather, the apparently divergent results may reflect differences between acute slices and cultures, or between NRG1-ErbB4 signaling during development as opposed to in the adult.

ErbB4 receptors are known to be expressed by GABAergic neurons [25] and activation of ErbB4 enhances presynaptic release of GABA in prefrontal cortex [24]. In present study, recordings were carried out in the presence of the GABA_A receptor antagonist, bicuculline. Thus, our findings are independent of GABA_A-mediated inhibitory mechanisms.

Both *ErbB4* and *NRG1* have emerged as leading susceptibility genes for schizophrenia [15,16,21]. One prominent hypothesis regarding the pathogenesis of schizophrenia is NMDA receptor hypofunction [2,17]. Recently, it has been found in post-mortem prefrontal cortex in schizophrenia that NRG1-ErbB4 signaling is enhanced and that there is increased NRG1-induced attenuation of NMDA receptor signaling [6]. Here we find in mice and rats that a constitutive function of ErbB4 is to suppress activity-dependent glutamatergic synaptic plasticity without altering synaptic transmission *per se* and that this plasticity, which is NMDA receptor-dependent, can be further suppressed by enhancing NRG1-ErbB4 signaling. Taken together, we propose that a cellular substrate for the cognitive dysfunction in schizophrenia is suppression of the plasticity of glutamatergic synaptic transmission by a gain-of-function of NRG1-ErbB4 signaling.

Conclusion

We find that NRG1-ErbB4 signaling constitutively suppresses LTP at Schaffer collateral synapses in CA1 hippocampus without affecting basal synaptic transmission. This provides a unifying concept for understanding the mechanistic basis of schizophrenia risk pathways in that cognitive deficits may result from excessive function of NRG1-ErbB4 signaling further suppressing synaptic plasticity. Interfering with the suppression of synaptic plasticity caused by NRG1-ErbB4 signaling thus represents a novel strategy for treatment of this prevalent and disabling disorder.

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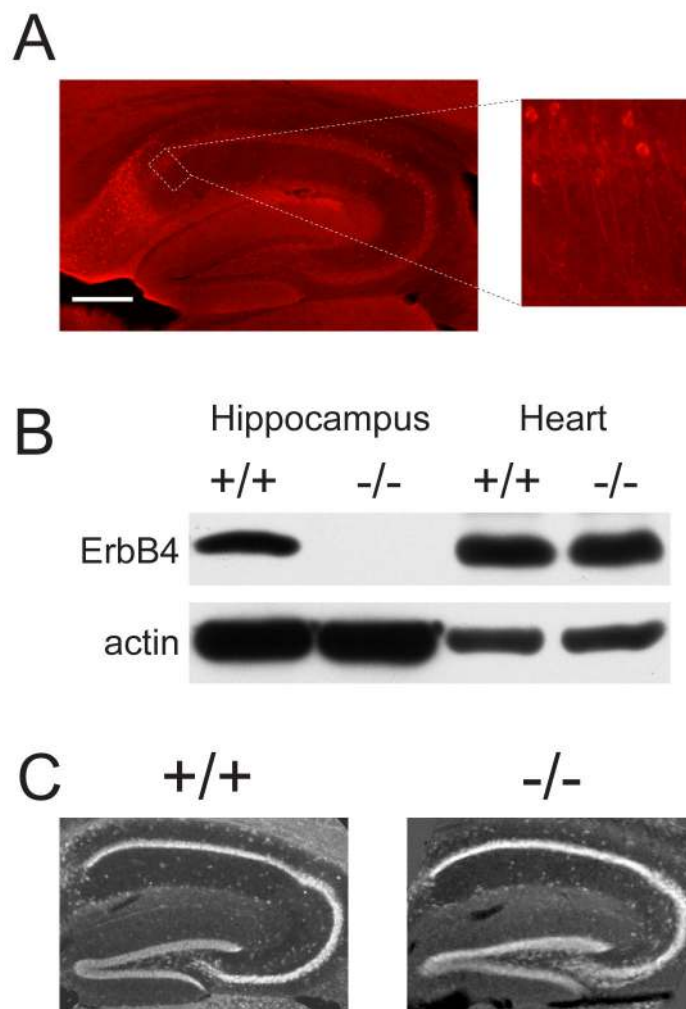


Fig. 1. Hippocampal ErbB4 expression and morphology in *ErbB4*^{+/+}HER4^{heart} and *ErbB4*^{-/-}HER4^{heart} mice. (a) ErbB4 immunoreactivity (shown in red) in a parasagittal hippocampal section from an adult *ErbB4*^{+/+}HER4^{heart} mouse. Scale bar, 200 μ m. Right: Higher magnification fluorescent micrograph representing CA1 region indicated by the dotted line. Scale bar, 150 μ m. (b) Adjacent *ErbB4*^{+/+}HER4^{heart} hippocampal coronal sections incubated with anti-ErbB4 antibody (left) or with anti-ErbB4 antibody pre-incubated with its antigenic peptide (right). ErbB4 immunoreactivity in red and NeuN immunoreactivity is shown in green. Scale bar, 100 μ m. (c) Photomicrograph of hippocampal CA1 from an adult *ErbB4*^{+/+}HER4^{heart} mouse showing distribution of immunoreactivity for ErbB4 (red), MAP2 (blue) and NeuN (green). Scale bar, 25 μ m (d) Western blot analysis of ErbB4 protein expression in *ErbB4*^{+/+}HER4^{heart} (+/+) and *ErbB4*^{-/-}HER4^{heart} (-/-) mice. (e) Nissl staining in parasagittal hippocampal sections from *ErbB4*^{+/+}HER4^{heart} (+/+) and *ErbB4*^{-/-}HER4^{heart} (-/-) mice. Scale bar, 200 μ m.

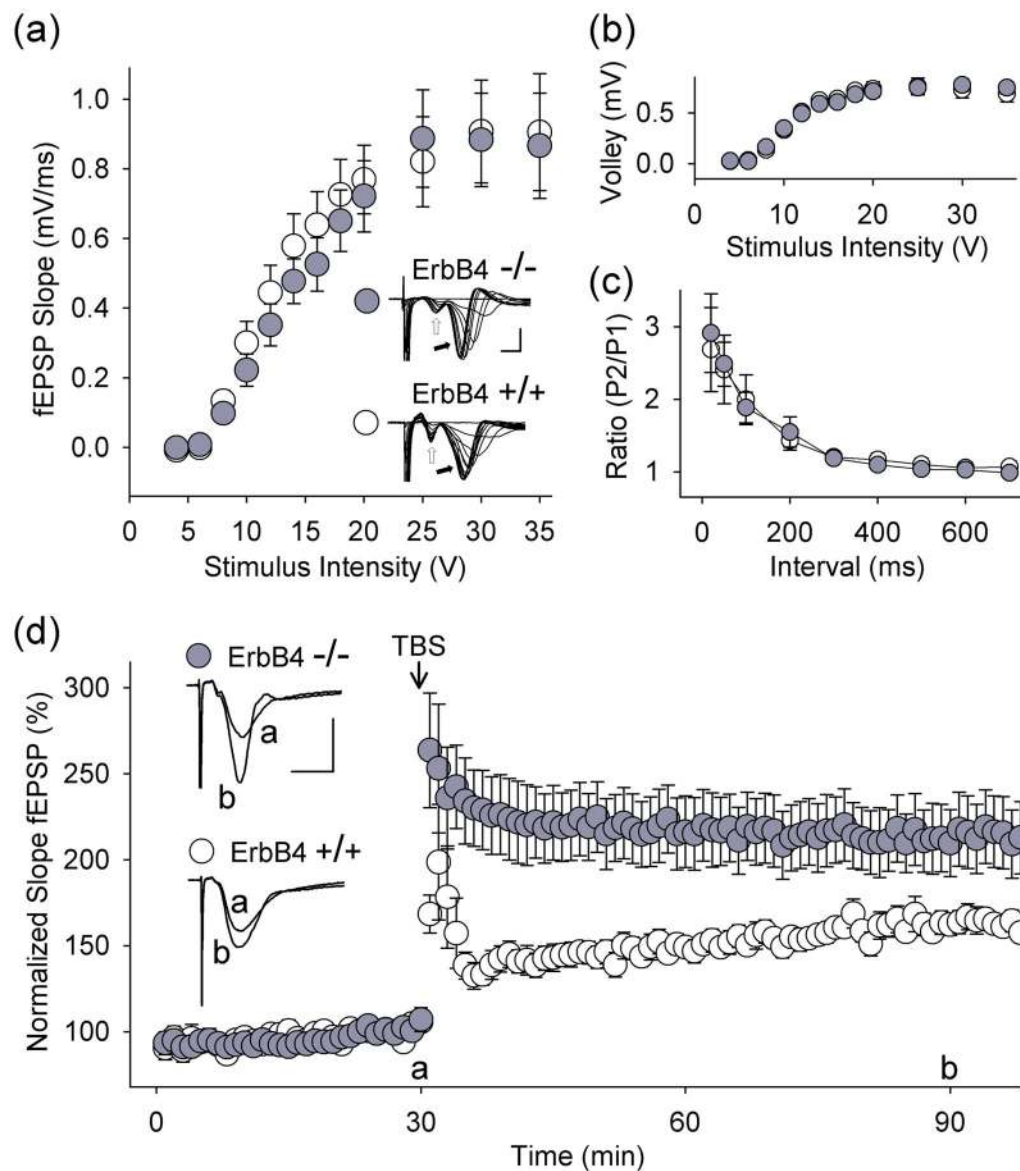


Fig. 2. Loss of function of ErbB4 enhances tLTP in CA1 hippocampus. (a) fEPSP slope and (b) fiber volley amplitude plotted as a function of stimulus intensity (*ErbB4*^{+/+}HER4^{heart} (*ErbB4*^{+/+}), open circles; *ErbB4*^{-/-}HER4^{heart} (*ErbB4*^{-/-}), filled circles). Strength of Schaffer collateral stimulation is indicated on the horizontal axis. Representative traces show fiber volley (open arrow) and fEPSPs (filled arrow; scale bars: 2 ms, 1 mV). In all panels, data are shown as mean \pm SEM. (c) Paired-pulse facilitation of fEPSPs in slices from *ErbB4*^{+/+}HER4^{heart} (open circles) and *ErbB4*^{-/-}HER4^{heart} (filled circles) mice. Interstimulus interval is indicated on the horizontal axis. P1, first response; P2, second response. (d) Summary scatter plot shows grouped normalized fEPSP slope every 1 min in slices from *ErbB4*^{+/+}HER4^{heart} (open circles, $n = 14$ slices) and from *ErbB4*^{-/-}HER4^{heart} (filled circles, $n = 14$ slices) mice. Theta-burst stimulation was delivered to Schaffer collateral-CA1 synapses at the 30 min time point. fEPSP slope was normalized with respect to the mean

slope of fEPSPs recorded during the 10 min period immediately before TBS. Inset: average of six consecutive fEPSPs recorded before or after TBS ('a' or 'b', respectively; scale bars: 10 ms, 0.5 mV). $p < 0.01$, *ErbB4*^{+/+}HER4^{heart} vs. *ErbB4*^{-/-}HER4^{heart}, 60 min after TBS.

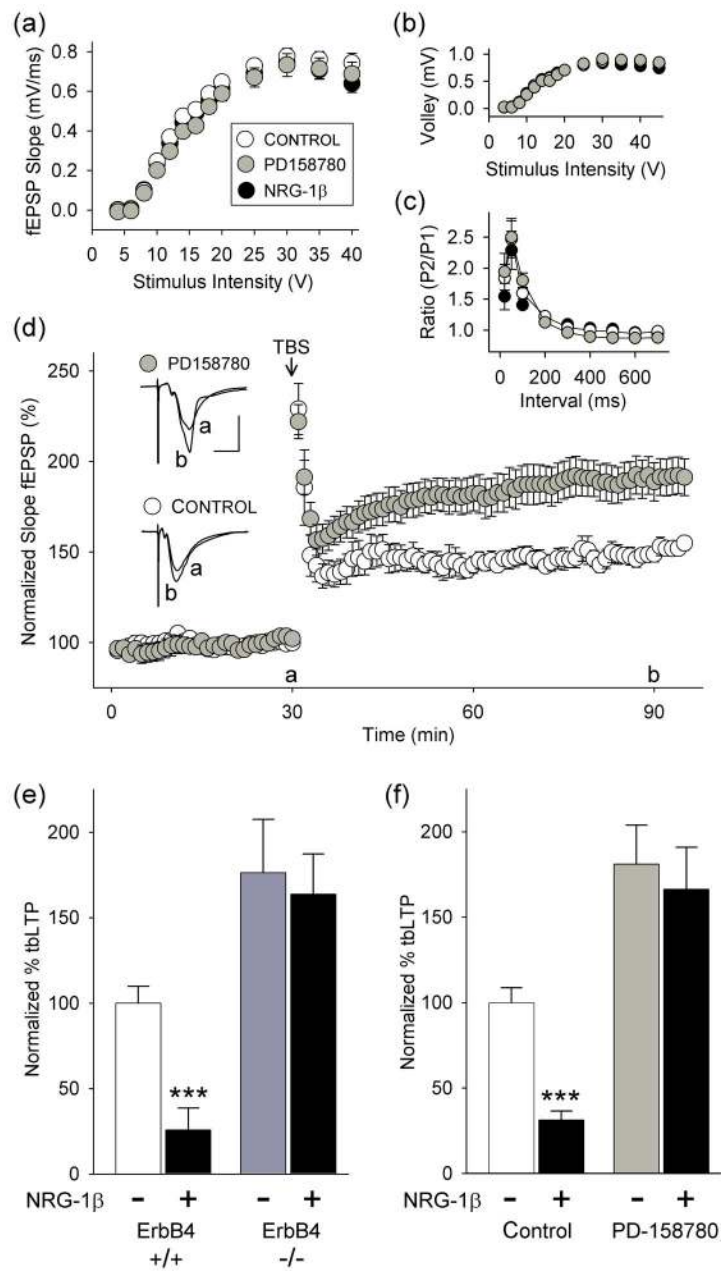


Fig 3. Pharmacological inhibition of ErbB4 increases tbLTP in CA1 hippocampus and prevents the suppression of tbLTP by NRG-1 β . (a) fEPSP slope and (b) fiber volley amplitude plotted as a function of stimulus intensity at rat Schaffer collateral-CA1 synapses in control slices (open circles) and in slices treated with NRG-1 β (2 nM; black circles) or PD158780 (10 μ M; gray circles). Strength of Schaffer collateral stimulation is indicated on the horizontal axis. In this and the subsequent panel, data are shown as mean \pm SEM. (c) Paired-pulse facilitation of fEPSPs in control slices and in slices treated with NRG-1 β (black circles) or PD158780 (gray circles). Interstimulus interval is indicated on the horizontal axis. P1, first response; P2, second response. (d) Summary scatter plot shows grouped normalized fEPSP

slope plotted every 1 min in control (open circles, $n = 13$) and PD158780-treated (filled circles, $n = 21$); in ACSF beginning 25 min before TBS with final concentration of 10 μM) slices from rats. Inset: average of six consecutive fEPSPs recorded before or after TBS ('a' or 'b', respectively; scale bars: 10 ms, 0.6 mV). $p < 0.001$, control vs. PD158780, 60 min after TBS. (e) Histogram shows TBS-induced increase in fEPSP slope 60 min after TBS in slices from *ErbB4^{+/+}HER4^{heart}* (white bar) and *ErbB4^{-/-}HER4^{heart}* (dark gray bar) mice without (-) and with (+; black bars) NRG-1 β treatment (in ACSF beginning 20 min before TBS with final concentration of 2 nM). Results are expressed as a percentage of TBS-induced increase in fEPSP slope (% tbLTP) with tbLTP in *ErbB4^{+/+}HER4^{heart}* slices (white bar) normalized to 100 %. *** $p < 0.001$ vs. *ErbB4^{+/+}HER4^{heart}* (white bar); $p < 0.05$, *ErbB4^{-/-}HER4^{heart}*/NRG-1 β (black bar) vs. *ErbB4^{+/+}HER4^{heart}* (white bar). (f) Histogram shows normalized TBS-induced mean increase in fEPSP slope in control (white bar) and PD158780-treated slices (light gray bar) without (-) and with (+; black bars) NRG-1 β (2 nM administered as above). *** $p < 0.001$ vs. control (white bar); $p < 0.01$, PD158780/NRG-1 β (black bar) vs. control (white bar). Data are taken 60 min after TBS.