# Impaired maturation of dendritic spines without disorganization of cortical cell layers in mice lacking NRG1/ErbB signaling in the central nervous system

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**Neuregulin-1 (NRG1) and its ErbB2/B4 receptors are encoded by candidate susceptibility genes for schizophrenia, yet the essential functions of NRG1 signaling in the CNS are still unclear. Using CRE/LOX technology, we have inactivated ErbB2/B4-mediated NRG1 signaling specifically in the CNS. In contrast to expectations, cell layers in the cerebral cortex, hippocampus, and cerebellum develop normally in the mutant mice. Instead, loss of ErbB2/B4 impairs dendritic spine maturation and perturbs interactions of postsynaptic scaffold proteins with glutamate receptors. Conversely, increased NRG1 levels promote spine maturation. *ErbB2/B4*-deficient mice show increased aggression and reduced prepulse inhibition. Treatment with the antipsychotic drug clozapine reverses the behavioral and spine defects. We conclude that ErbB2/B4-mediated NRG1 signaling modulates dendritic spine maturation, and that defects at glutamatergic synapses likely contribute to the behavioral abnormalities in *ErbB2/B4*-deficient mice.**

cerebral cortex | dendritic spines | migration | neuregulin | schizophrenia

NRG1 activates receptor tyrosine kinases consisting of dimers formed by ErbB2, ErbB3, and ErbB4. Both ErbB3 and ErbB4 bind NRG1, whereas ErbB2 and ErbB4 have intrinsic tyrosine kinase activity. Functional NRG1 receptors therefore consist of ErbB4 homodimers, or of heterodimers between ErbB2, ErbB3 and ErbB4 (1). NRG1 and its receptors are expressed in the CNS and the *NRG1*, *ErbB2*, and *ErbB4* genes are candidate susceptibility genes for schizophrenia (1, 2). In the central nervous system (CNS), NRG1 is thought to regulate the differentiation of radial glia and neurons, myelination, neuronal migration and synaptic function (1). However, mice with targeted deletions of *NRG1*, *ErbB2* and *ErbB4* die during embryogenesis, whereas mice lacking *ErbB3* die perinatally (3–6). Thus, our knowledge of NRG1-ErbB functions in the CNS has been derived from studies using cultured cells, dominant-negative ErbB receptors, and mice partially defective in NRG1 signaling (1). No animal model lacking all NRG1 signaling specifically in the CNS has been described. We have therefore engineered *ErbB2/B4-CNSko* mice that lack both ErbB2 and ErbB4 (the only ErbBs with intrinsic tyrosine kinase activity) in the CNS. Surprisingly, the mutant mice show no defects in brain morphology and in the layered structure of the cerebral cortex, hippocampus, and cerebellum. Instead, the maturation of dendritic spines is affected. *ErbB2/B4*-deficient mice also display behavioral abnormalities that have been associated with schizophrenia-like symptoms. Clozapine treatment reverses behavioral and spine defects, indicating that perturbation of glutamatergic synapses might contribute to the behavioral abnormalities. Interestingly, reduced spine density has been observed in the brain of schizophrenia patients (7), suggesting that defects in spine maturation may constitute a risk factor for the development of schizophrenia.

## Results

**Normal Cortical Development and Glial-Guided Migration.** To inactivate NRG1/ErbB signaling in the CNS, we crossed a mouse line homozygous for loxP-flanked (flox) *ErbB2* and *ErbB4* alleles with *hGFAP-CRE* mice that expresses CRE in neural precursors starting at embryonic day (E) 13.5 (8). Mutant offspring will be referred to as *ErbB2/B4-CNSko* mice. Littermates lacking CRE or floxed alleles were indistinguishable from wild-type (WT) mice, served as controls, and will be referred to as WT. We confirmed recombination of the flox alleles and absence of ErbB2/ErbB4 proteins in the brains of mutant mice (Fig. 1 *A* and *B*). *ErbB2/B4-CNSko* mice were viable, had brains of normal size, and showed no defects in the layered structure of the cerebral cortex, hippocampus, and cerebellum (Fig. 1 *C* and *D*; [supporting information (SI) Fig. S1*A*]). Analysis with the pan-neuronal marker NeuN and the layer specific markers Cux1 (layers II–IV) and Tbr1 (layer VI) revealed no differences in neuronal density across all and within specific cortical layers (Fig. 1 *E* and *F*). These findings were unexpected, as NRG1/ErbB signaling was thought to be essential for the formation of cortical cell layers (9, 10).

Thus, to confirm our observations, we generated *ErbB2/B4* double mutants using additional mice expressing CRE in neural precursors from E8.5 (Nestin8-CRE) and E10.5 (Nestin-CRE, EMX1-CRE) onward (11–13). ErbB2/B4 proteins were absent in the developing CNS of the mutants (data not shown), but brain morphology and neuronal cell layers were unaffected (Fig. S1 *B–D* and Fig. S2*A*). 5′-Bromo-2′-deoxyuridine (BrdU) pulse-labeling experiments confirmed that cell migration also progressed normally (Fig. S2*B*). It has been suggested that radial glial development depends on NRG1/ErbB (10, 14, 15), yet stainings with the radial glial marker RC2 revealed no obvious defects in radial glia morphology (Fig. 1*G* and Fig. S3*A*). We also observed no changes in cell proliferation (Fig. S2*C*) and in the timing and levels of GFAP expression (Fig. 1*H* and Fig. S3 *B–E*), indicating that the generation of neurons and astrocytes was not affected.

**Defects in Dendritic Spine Maturation.** Golgi staining revealed no obvious defects in dendritic morphology in neurons of the cortex and hippocampus of *ErbB2/B4-CNSko* mice (Fig. 2 *A* and *D*), but dendritic spine density in pyramidal neurons in the hippocampus and cortex was reduced by 28.1% ± 3.9% and 15.8% ± 4.8%, respectively (Fig. 2 *B* and *C*; Fig. 2 *E* and *F*). To determine the mechanism causing this defect, we analyzed spine development in hippocampal cultures. After 11 days in culture, neurons from

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The authors declare no conflict of interest.

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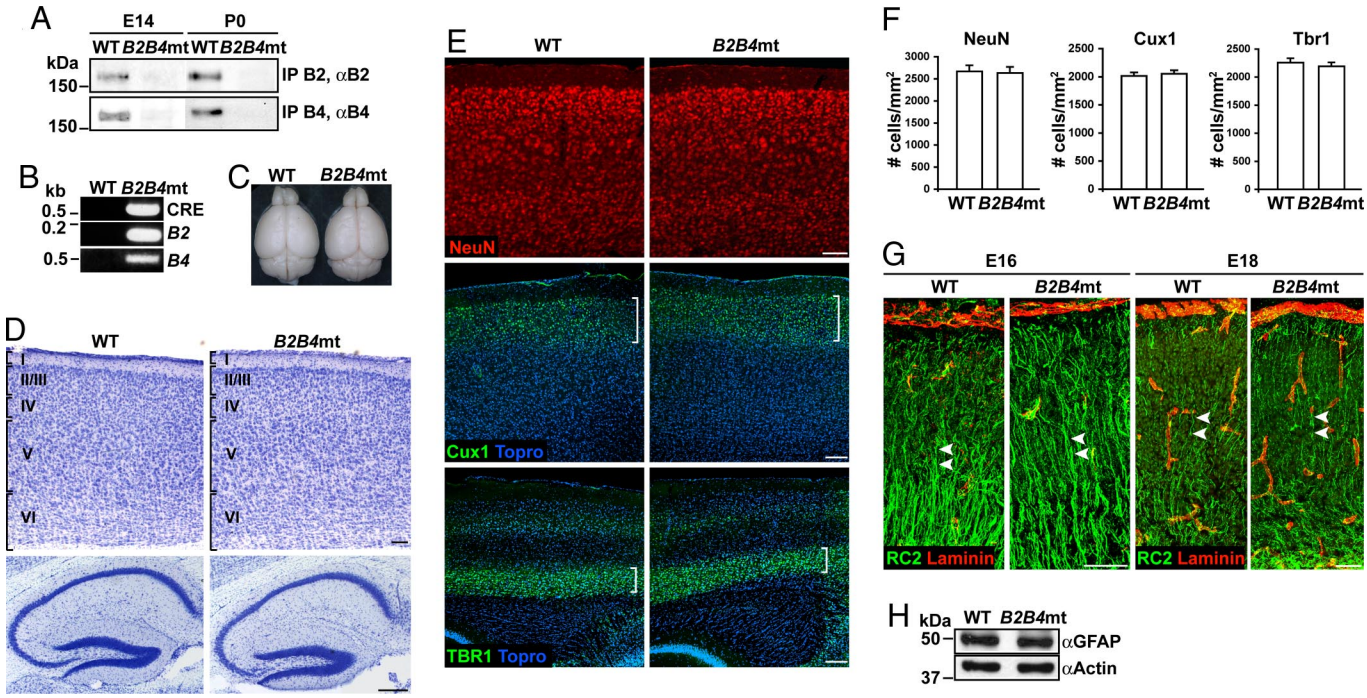


**Fig. 1.** Cortical development in *ErbB2/B4-CNSko* mice. (*A*) Immunoprecipitation of ErbB2 and ErbB4 from E14 and P0 brain extracts followed by Western blotting demonstrated an absence of ErbB2 and ErbB4 proteins; IP, immunoprecipitation. (*B*) Polymerase chain reaction (PCR) analysis of CRE-mediated recombination with DNA from P0 brains. In *ErbB2/B4-CNSko* mutants, the hGFAP-CRE transgene was detected, as well as a 170-kb (ErbB2) and a 507-kb (ErbB4) band indicating recombination of floxed alleles. (*C*) Brain size and morphology were unchanged in *ErbB2/B4-CNSko* mice at P249. (*D*) Sagittal brain sections at P38 stained with Nissl revealed no defects in cortical layers (roman numerals) and hippocampal structure. (*E*) Sagittal sections stained with NeuN (red) at P38, Cux1 at P256 (green), and Tbr1 at P249 (green). Nuclei were stained with Topro-3 (blue). (*F*) No differences were seen in NeuN, Cux1 or Tbr1-positive neurons in *ErbB2/B4-CNSko* mice (NeuN [cells/mm$^2$]: WT 2665 ± 139, *ErbB2/B4-CNSko* 2630 ± 140, $P > 0.05$; Cux-1: WT 2015 ± 62, *ErbB2/B4-CNSko* 2052 ± 62, $P > 0.05$; Tbr1: WT 2257 ± 80, *ErbB2/B4-CNSko* 2192 ± 71, $P > 0.05$). (*G*) Coronal sections stained with the radial glia marker RC2 (green) and with antibodies to laminin-α2 (red) at embryonic ages E16 and E18. No difference in the gross morphology of radial glia was detected. (*H*) Immunoblotting for GFAP using brain extracts from P0 mice. Actin served as loading control. Scale bars, 50 μm

mutant mice had normal density of filopodia (Fig. 3*A*), which are believed to be spine precursors (16). Filopodial width was also unaffected, whereas their length was decreased (Fig. 3*A*). However, after 21 days, mature neurons from mutant mice had 54.2% ± 4.2% less spines, which were also thinner than controls but of normal length (Fig. 3*B*). The density of excitatory presynaptic nerve terminals, as analyzed by staining for the vesicular glutamate transporter (VGLUT) was also decreased, but VGLUT cluster size was unchanged (Fig. 3*C*). TUNEL staining confirmed that cell death was not affected in neurons from *ErbB2/B4-CNSko* mice (Fig. S4), indicating that the defects in spines were not a secondary consequence of cell death. Interestingly, spine density in ErbB2 and *ErbB4* single mutants was less severely reduced (Fig. S5).

To test whether NRG1 might be limiting for spine development, we next added recombinant NRG1 (rNRG1) to WT hippocampal neurons starting on the first day of culture. After 11 days in culture, the density of filopodia-like protrusions was increased by 16.6% ± 5.34% (Fig. 4*A*). The protrusions had a mushroom-like appearance indicative of accelerated maturation toward spines (Fig. 4*A*). After 21 days, spine density was increase by 20.6% ± 4.5% (Fig. 4*B*) and paralleled by an increase in VGLUT cluster staining (Fig. 4*C*). When rNRG1 was added only at days 18 and 20, after dendritic development, spine density was similarly increased (Fig. S6). Our results show that ErbB2/B4-mediated Nrg1 signaling facilitates the maturation of dendritic spines and excitatory presynaptic nerve terminals in hippocampal neurons.

**Loss of NRG1/ErbB Signaling Disrupts Association of NMDA Receptors with PSD-95.** ErbB2 and ErbB4 associate with PSD-95, which also binds and clusters NMDA receptors (NMDAr) to promote spine maturation (17–20). Thus, we hypothesized that NRG1/ErbB signaling may modulate PSD-95/NMDAr interactions to promote the formation of mature dendritic spines. Consistent with this model, the number of PSD-95 and NMDAr NR1 clusters was reduced in mutant hippocampal neurons (Fig. 5 *A* and *B*). Cluster size was unchanged (Fig. S7*A*), but co-localization between NR1 and PSD-95 clusters was affected (Fig. 5 *A* and *C*). Co-immunoprecipitation experiments confirmed that complex formation between NMDAr and PSD-95 was decreased (Fig. 5*D*), even though NMDAr and PSD-95 levels were unchanged in the mutants (Fig. S7 *B* and *C*). We conclude that interactions of NMDAr with PSD-95 are perturbed in the absence of NRG1/ErbB signaling, which is predicted to cause spine loss.

**Clozapine Reverses Behavioral and Spine Defects.** Defects in synaptic density are expected to affect the function of neuronal circuits. We therefore analyzed the performance of *ErbB2/B4-CNSko* mice in several behavioral paradigms, including those that have been reported to function as read-outs for schizophrenia-like symptoms. In the open-field task, *ErbB2/B4-CNSko* mice showed normal locomotor behavior (Fig. 6*A*) but stayed longer in the central zone of the open field (Fig. 6*B*), indicative of decreased anxiety. Next, we tested the mice in the resident-intruder assay. Mutants engaged for longer times in aggressive activities (Fig. 6*C*). Finally, the prepulse inhibition (PPI) test, a psychometric measure for sensory gating, showed that mutant males had lower PPI levels than WT males (Fig. 6*D*). No difference was observed among females (data not shown). Interestingly, increased aggression and lower PPI levels are abnormalities observed in some schizophrenia patients (21–23) and gender differences in PPI have been reported in both healthy and

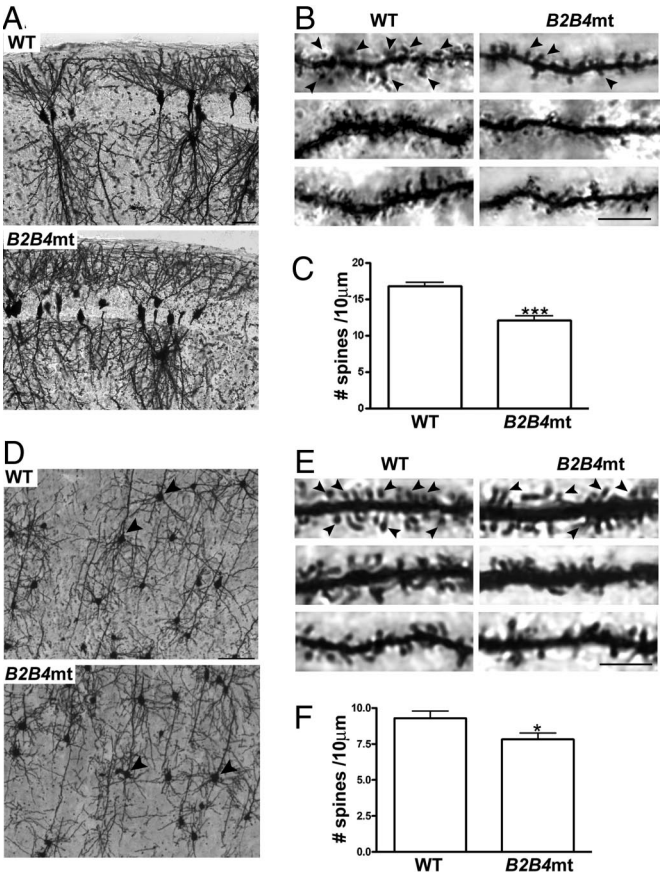


**Fig. 2.** Dendritic spine loss. (*A*, *B*) CA1 pyramidal neurons at P26 stained with Golgi. (*A*) Dendrites of CA1 neurons. (*B*) Spines (arrowheads) along apical dendrites of CA1 neurons. (*C*) Spine density along apical dendrites was reduced in *ErbB2/B4-CNSko* mice (WT: 16.82 ± 0.49 spines/10 μm, $n = 1367$ spines from 26 neurons; *ErbB2/B4-CNSko*: 12.09 ± 0.66 spines/10 μm, $n = 983$ spines from 24 neurons; $^{***}P < 0.001$). (*D*, *E*) Pyramidal neurons in the prefrontal cortex stained by Golgi. (*D*) Cell bodies (arrowheads) and dendrites of pyramidal neurons. (*E*) Spine density along dendrites was reduced in *ErbB2/B4-CNSko* mice (WT: 9.29 ± 0.5 spines/10 μm, $n = 483$ spines from 20 neurons; *ErbB2/B4-CNSko*: 7.82 ± 0.44 spines/10 μm, $n = 427$ spines from 20 neurons; $^{*}P < 0.05$). Scale bars, (*A*), 50 μm; (*D*), 100 μm; (*B* and *E*), 10 μm.

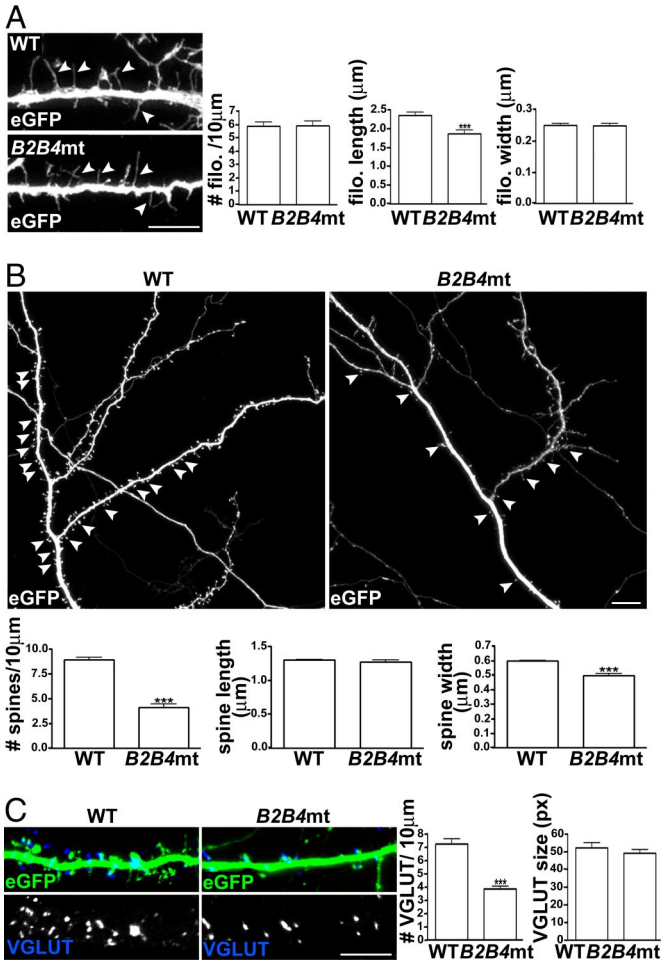


**Fig. 3.** Spine development in cultured neurons. (*A*) Hippocampal neurons transfected with eGFP, cultured for 11 days (DIV11) and imaged to reveal filopodia (arrows). Filopodial density and width were unaltered in mutants, but length was reduced (density [filopodia/10 μm]: WT 5.85 ± 0.31, *ErbB2/B4-CNSko* 5.88 ± 0.37, $P > 0.05$; width [μm]: WT 0.25 ± 0.01, *ErbB2/B4-CNSko* 0.25 ± 0.01, $P > 0.05$; length [μm]: WT 2.35 ± 0.08, *ErbB2/B4-CNSko* 1.86 ± 0.1, $^{***}P < 0.001$; $n = 650$ filopodia from 14 WT neurons, $n = 530$ filopodia from 12 *ErbB2/B4-CNSko* neurons). (*B*) Hippocampal neurons were imaged at DIV21 to reveal spines (arrowheads). Spine density and width were reduced in neurons from mutants. No difference was detected in spine length: (density [spines/10 μm]: WT 8.92 ± 0.25, *ErbB2/B4-CNSko* 4.09 ± 0.37, $^{***}P < 0.001$; width [μm]: WT 0.6 ± 0.01, *ErbB2/B4-CNSko* 0.5 ± 0.01, $^{***}P < 0.001$; length [μm]: WT 1.3 ± 0.01, *ErbB2/B4-CNSko* 1.27 ± 0.04, $P > 0.05$; $n = 442$ spines from eight *ErbB2/B4-CNSko* neurons, $n = 2852$ spines from 34 WT neurons). (*C*) Immunostaining with VGLUT (blue) in hippocampal neurons transfected with eGFP (green). Blue channel is shown separately in gray. The density of VGLUT clusters was reduced in *ErbB2/B4-CNSko* neurons. No difference was observed in cluster size: (density [clusters/10 μm]: WT 7.23 ± 0.4, $n = 509$ clusters from 10 neurons; *ErbB2/B4-CNSko* 3.84 ± 0.22, $n = 295$ clusters from 10 neurons, $^{***}P < 0.001$; (size [pixels]: WT 52.13 ± 3.06 pixels, *ErbB2/B4-CNSko* 49.12 ± 2.23 pixels, $P > 0.05$). Scale bars, 10 μm.

schizophrenic humans (24). Moreover, clozapine administration, which is used to treat schizophrenia in humans (23), alleviated behavioral responses in *ErbB2/B4-CNSko* mice (Fig. 6 *C* and *D*), and restored dendritic spine numbers and width in mutant neurons (Fig. 6*E*).

## Discussion

Our findings challenge the current view of the role of NRG1/ErbB signaling in the CNS. Studies with cells in culture, organ explants, and mice expressing dominant negative ErbB receptors suggest that NRG1 might promote the generation of radial glia and the migration of neurons along glial fibers (9, 10, 14, 15). However, these processes were unaffected in *ErbB2/B4-CNSko* mice, where all endogenous ErbB-mediated NRG1 signaling was abolished specifically within the CNS. ErbB2/B4 were effectively inactivated from early stages of cortical development, and similar results were obtained with four CRE lines. One of the lines, Nestin8-CRE, expresses CRE already at E8.5 in the neuroepithelium, before formation of radial glial cells (11–13). One explanation for the difference from earlier findings is that NRG1 may have redundant functions with other signaling pathways. Overexpression of dominant negative ErbB receptors that were used in previous studies may have affected these pathways as well.

Instead, we demonstrate that NRG1/ErbB signaling regulates dendritic spine maturation. ErbB4 binds to PSD-95 and ErbB2 associates with PSD-95 via Erbin (19, 20). PSD-95 in turn binds to NMDAr and controls the incorporation of glutamate receptors into spines, promoting spine formation (17, 18). In cultured hippocampal neurons, NRG1/ErbB4 can facilitate activity-dependent plasticity of glutamatergic synapses (25). We now show that NRG1/ErbB signaling is critical for the consolidation of dendritic filopodia

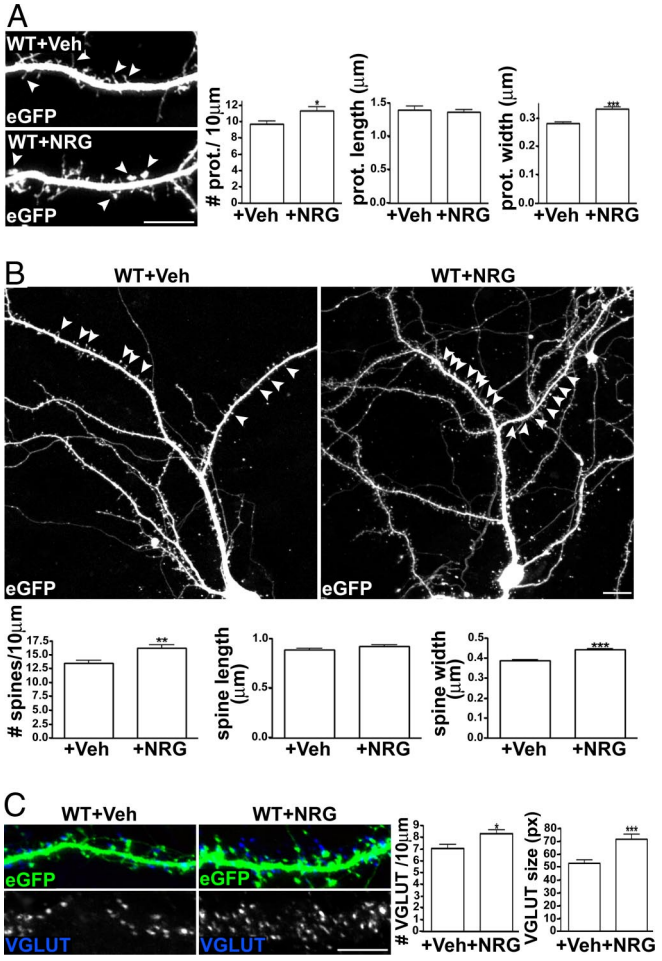


**Fig. 4.** Exogenous rNRG1 accelerates dendritic spine maturation. (*A*) WT hippocampal neurons at DIV11 transfected with eGFP (green) were treated with rNRG1 or vehicle (veh). In the presence of rNRG1, the density and width of filopodia-like protrusions (arrows) increased, but not their length: (density [protusions/10 $\mu$m]: WT+rNRG1 11.3 ± 0.53, WT+veh 9.71 ± 0.37, $^{*}P < 0.05$; width $\mu$m]: WT+rNRG1 0.33 ± 0.01, WT+veh 0.28 ± 0.01, $^{***}P < 0.001$; length $\mu$m]: WT+rNRG1 1.36 ± 0.04, WT+veh 1.39 ± 0.06, $P > 0.05$; $n = 768$ protrusion from eight WT+rNRG1 neurons, $n = 536$ protrusions from 8 WT+veh neurons). (*B*) WT hippocampal neurons at DIV21 treated with rNRG1 or vehicle. The density and width of spines (arrowheads) was increased by rNRG1. Spine length was unaffected (density [spines/10 $\mu$m]: WT+rNRG1 16.2 ± 0.63, WT+veh 13.4 ± 0.57, $^{**}P < 0.01$; width, $\mu$m]: WT+rNRG1 0.44 ± 0.01, WT+veh 0.39 ± 0.01, $^{***}P < 0.001$; length, $\mu$m]: WT+rNRG1 0.92 ± 0.01, WT+veh 0.88 ± 0.01, $P > 0.05$; $n = 1142$ spines from 9 WT+rNRG1 neurons, $n = 861$ spines from nine WT+veh neurons). (*C*) Immunostaining for VGLUT (blue) in DIV21 WT hippocampal neurons treated with rNRG1 or vehicle. Blue channel is shown separately in gray. In the presence of rNRG, VGLUT cluster density and size was increased (density [clusters/10 $\mu$m]: WT+rNRG1 8.31 ± 0.34, $n = 851$ clusters from 18 neurons; WT+veh 7.05 ± 0.35, $n = 587$ clusters from 13 neurons, $^{*}P < 0.05$; size, pixels: WT+rNRG1 71.46 ± 3.98, WT+veh 52.68 ± 2.76, $^{***}P < 0.001$). Scale bars, 10 $\mu$m.

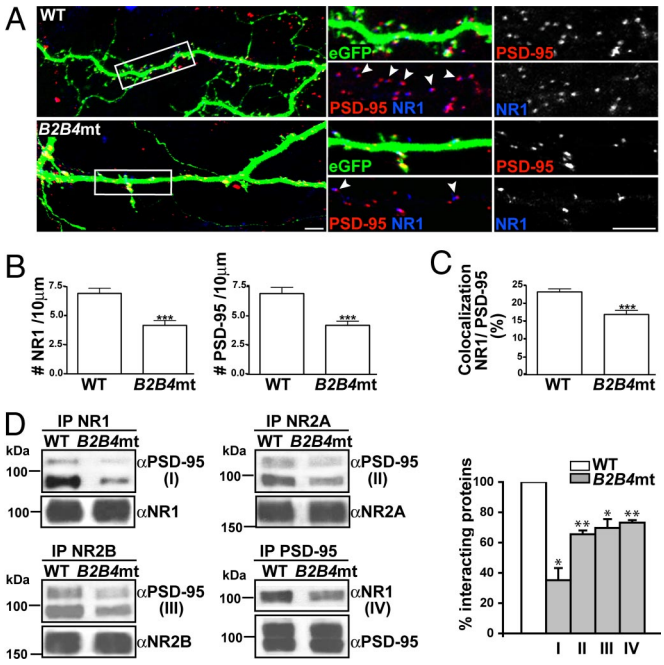


**Fig. 5.** Loss of ErbB2/B4 affects interactions between NMDAr and PSD-95. (*A*) Hippocampal neurons from WT and *ErbB2/B4-CNSko* mice transfected with eGFP (green) were stained at DIV21 with antibodies to PSD-95 (red) and NMDAr subunit NR1 (blue). Higher magnifications with red and blue channels are also shown separately in gray. (*B*) The density of PSD-95 and NR1 clusters was reduced in mutants. NR1 density (clusters/10 $\mu$m]: WT 6.92 ± 0.45, $n = 465$ clusters from 10 neurons; *ErbB2/B4-CNSko* 4.17 ± 0.43, $n = 353$ clusters from 11 neurons, $^{***}P < 0.001$; PSD-95 density (clusters/10 $\mu$m): WT 6.9 ± 0.52, $n = 464$ clusters from 10 neurons; *ErbB2/B4-CNSko* 4.19 ± 0.36, $n = 347$ clusters from 11 neurons, $^{***}P < 0.001$. (*C*) In *ErbB2/B4-CNSko* neurons the percentage of co-localization of NR1 and PSD-95 clusters was reduced (%): WT 23.25 ± 0.85, *ErbB2/B4-CNSko* 16.88 ± 1.17, $^{***}P < 0.001$). (*D*) Co-immunoprecipitation of NR1, NR2A or NR2B with PSD-95 revealed a 64.8 ± 8.1% ($^{*}P < 0.05$), 34.5 ± 2.5% ($^{**}P < 0.01$), and 30.2 ± 5.7% ($^{**}P < 0.01$) decrease of protein interactions in mutants. Reverse co-immunoprecipitation of PSD-95 with NR1 also showed a reduction of 18.2 ± 1.7% ($^{**}P < 0.01$). For each co-immunoprecipitation assay, the amount of immunoprecipitated protein revealed by immunoblotting is also presented. Negative controls for co-immunoprecipitation assays are shown in Fig. S7*C*. Scale bars, 10 $\mu$m.

into mature spines and the association of NMDAr with PSD-95. These findings suggest that NRG1/ErbB signaling promotes spine maturation by regulating NMDAr/PSD-95 interactions. Although ErbB4 has been implicated in mediating NRG1 actions at glutamatergic synapses (25), we show that ErbB2 and ErbB3 are important as well. Accordingly, spines were affected in *ErbB2* and *ErbB4* single mutants, albeit less than in double mutants. Significantly, ErbB3 has no intrinsic catalytic activity. Therefore, in *ErbB2-CNSko* mice, only ErbB4/B4 and ErbB4/B3 form functional NRG1 receptors, both of which could participate in spine maturation. In *ErbB4-CNSko* mice, ErbB2/B3 heterodimers are the only functional NRG1 receptors, suggesting that ErbB2 and ErbB3 are also required for spine development.

*ErbB2/B4-CNSko* mice showed enhanced aggression and decreased PPI levels. These behavioral defects were reversed by clozapine, which alleviates schizophrenia symptoms in humans (23). Clozapine also increased spine density in *ErbB2/B4*-deficient neurons, suggesting that defects at glutamatergic synapses contribute to the behavioral defects. Interestingly, mouse lines hypomorphic for NRG1 signaling or lacking specific NRG1 isoforms show variable defects in PPI, social interaction, or even motor function (1, 26), indicating that different perturbations in the NRG1/ErbB pathway cause distinct behavioral outcomes. A decrease in the expression of some NRG1/ErbB pathway components has been observed in the brains of some schizophrenia patients, but an increase in NRG1/ErbB4 activity has also been reported (1). Our findings suggest that different changes in NRG1/ErbB signaling are likely translated into distinct alterations at excitatory synapses that may contribute to the pathophysiology of schizophrenia.

## Materials and Methods

An extended section is provided as *SI Materials and Methods*.

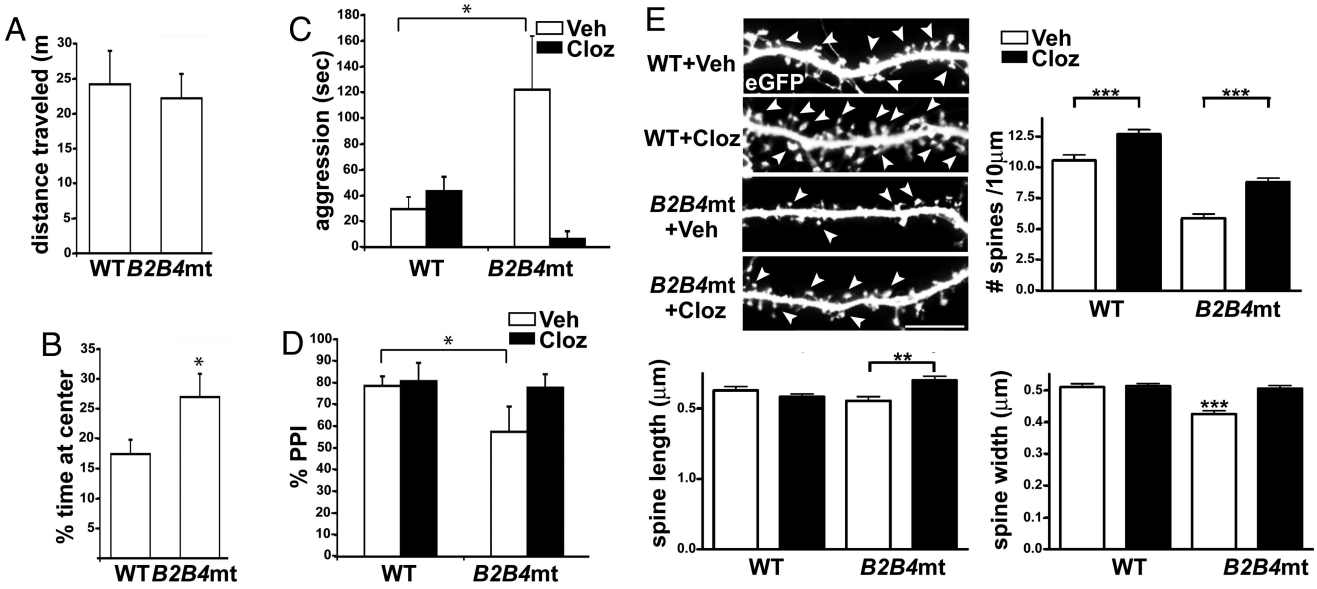


**Fig. 6.** Behavioral abnormalities in *ErbB2/B4-CNSko* mice and spine loss are attenuated by clozapine treatment. (*A*, *B*) In open-field test, *ErbB2/B4-CNSko* mice showed no difference in distance traveled compared with WT (*A*; WT 2418 ± 500 cm, $n = 17$ animals; *ErbB2/B4-CNSko* 2218 ± 374 cm, $n = 15$ animals; $P > 0.05$), but remained longer in the central zone of the open field (*B*; WT 17 ± 2% of time, *ErbB2/B4-CNSko* 27% ± 4% of time, $*P < 0.05$). (*C*) Resident intruder assay showed that *ErbB2/B4-CNSko* mice engaged for longer time in aggressive behavior (biting, kicking, wrestling) compared with WT (WT 29.5 ± 9.3 seconds, $n = 7$ animals; *ErbB2/B4-CNSko* 122.8 ± 41.6 seconds, $n = 5$ animals; $*P < 0.05$). Clozapine (cloz) treatment decreased aggression in *ErbB2/B4-CNSko* mice to WT levels (*ErbB2/B4-CNSko*+cloz: 6.37 ± 5.99 seconds; WT without clozapine: 29.5 ± 9.3 seconds, $P > 0.05$), without affecting basal aggression in WT (WT+clozapine: 43.3 ± 11.2 seconds; WT without clozapine: 29.5 ± 9.3 seconds, $P > 0.05$). (*D*) In a PPI test, *ErbB2/B4-CNSko* mice showed reduced PPI compared with WT (WT 78.6% ± 4.3% PPI, $n = 7$ animals; *ErbB2/B4-CNSko* 57.4% ± 8.4% PPI, $n = 5$ animals; $*P < 0.05$). This difference was abolished after clozapine treatment (WT 80.7% ± 11.6% PPI, *ErbB2/B4-CNSko* 77.7% ± 6.2% PPI; $P > 0.05$). (*E*) Hippocampal neurons from WT and *ErbB2/B4-CNSko* mice at DIV21 treated with clozapine or vehicle (veh). In neurons from WT mice, clozapine treatment resulted in an increase in spine density, but spine width and length were unaffected: (density, spines/10 μm: WT+veh 10.56 ± 0.46, WT+cloz 12.7 ± 0.37, $***P < 0.001$; (width, μm): WT+veh 0.51 ± 0.01, WT+cloz 0.51 ± 0.01, $P > 0.05$; (length, μm): WT + veh 1.13 ± 0.03, WT + cloz 1.08 ± 0.02, $P > 0.05$; $n = 641$ spines from 11 WT+veh neurons, $n = 1058$ spines from 13 WT+cloz neurons). In neurons from *ErbB2/B4-CNSko* mice, clozapine increased spine density, width, and length: density, spines/10 μm): *ErbB2/B4-CNSko*+veh 5.88 ± 0.35, *ErbB2/B4-CNSko*+cloz 8.83 ± 0.29, $***P < 0.001$ (width, μm): *ErbB2/B4-CNSko*+veh 0.42 ± 0.01, *ErbB2/B4-CNSko*+cloz 0.5 ± 0.01, $***P < 0.001$ (length, μm): *ErbB2/B4-CNSko*+veh 1.06 ± 0.03, *ErbB2/B4-CNSko*+cloz 1.2 ± 0.03, $**P < 0.01$; $n = 518$ spines from 13 *ErbB2/B4-CNSko*+veh neurons, $n = 678$ spines from 11 *ErbB2/B4-CNSko*+cloz neurons. Scale bars, 10 μm

**Generation and Analysis of Mice.** *ErbB2/B4-CNSko* mice were generated by crossing mice homozygous for floxed (fl) *ErbB2* and *ErbB4* alleles (27, 28) with different CRE lines as indicated. Histology and biochemistry were carried out as described (12, 29). The source of all antibodies used is listed in the supplement. Behavioral analysis was carried out with 1.5–7-month-old mice following published procedures (30, 31). All values are shown as mean ± SEM. After initial behavioral analysis, mice rested for 48 hours before being injected with clozapine (0.3 mg/kg body weight) 30 minutes before retesting.

**Hippocampal Cultures and Imaging.** Hippocampal neurons from P1 mice were cultured, transfected, and stained as described (32). Ten-μM human NRG1-β1 EGF domain (R&D Systems) or one μM clozapine (Sigma) was added when indicated. Treatments started at DIV1 and were repeated every 4 days or, when indicated, were administered only at DIV18 and 20. MetaMorph (Universal Imaging Corporation) and ImageJ (National Institutes of Health) were used for morphometry. Student's *t* test and one-way analysis of variance (ANOVA), followed by the Dunnett *post hoc* test, were performed with GraphPad Prism. All values are shown as mean ± SEM. $*P < 0.05$ (significant), $**P < 0.01$ (very significant), and $***P < 0.001$ (highly significant).

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