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Neuregulin signaling, cortical circuitry development and schizophrenia

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Neuregulin-1 (Nrg1) and its receptor ErbB4 are encoded by genes that have been repeatedly linked to schizophrenia. Both genes are thought to play important roles in the development of brain circuitry, but their precise contribution to the disease process remains unknown. In this review, we summarize novel findings on the biological function of Nrg1 and ErbB4 in mice, with a focus on the development of inhibitory circuits in the cerebral cortex. We will also discuss how this basic knowledge may help us to understand the etiology of schizophrenia, and eventually lead to the development of novel therapies for treating the disorder.

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Introduction

Schizophrenia is a severe mental illness that affects approximately 1% of the population worldwide. Genetic factors play a major role in the development of schizophrenia, with an estimated heritability of around 80% [1]. In addition, environmental stressors seem to be key determinants in the initiation of the disorder. Schizophrenia is formally diagnosed by the presence of three distinct symptom clusters: positive (e.g. delusions, hallucinations), negative (e.g. apathy, social withdrawal) and cognitive (e.g. attention, working memory). Positive symptoms are the most dramatic manifestations of the disease, but cognitive deficits are perhaps the most distinctive features. Individuals that suffer from schizophrenia have important deficits in memory, attention and executive function, and some of these disturbances appear before the onset of psychosis [2]. Milder cognitive deficits are often present in unaffected relatives [3], which reinforce the view that cognitive impairment lies at the core of the disorder. Although the pathophysiological mechanism underlying these cognitive disturbances has yet to be determined, recent work indicates that abnormal inhibitory function and a concomitant disruption of synchronized oscillatory activity in the cerebral cortex may play an important role in this process [4–6,7•].

Several lines of evidence suggest that schizophrenia arises as a consequence of abnormal brain development [8,9]. According to this view, genes linked to the disease encode for proteins that play a major role in the development of specific brain circuitries, most notably in the cerebral cortex. This would lead to a suboptimal conformation of cortical circuitries that is susceptible to deteriorate beyond normal performance levels in the presence of additional environmental factors, such as drug abuse or stress. Consequently, unraveling the mechanisms underlying schizophrenia depends, at least in part, on characterizing the function that risk genes may play in the development of the cerebral cortex.

Genetic studies over the past few years have found a strong association between the gene encoding for *Neuregulin-1* (*NRG1*) and schizophrenia. Since Stefansson *et al.* [10] identified *NRG1* as a candidate gene for the disorder, numerous studies have obtained supporting evidence for this hypothesis [11]. The recent identification of the NRG1 receptor ErbB4 as an additional candidate risk gene for schizophrenia has added support to the hypothesis that this signaling pathway plays an important role in the pathophysiology of the disorder [12••,13–15]. These findings have fueled research in this area, most notably in the search for a plausible biological mechanism that could explain their involvement in the disease process. Although NRG1 and ErbB4 have been implicated in multiple developmental events, recent work has focused on the function of these genes in the wiring of the cerebral cortex. Here, we summarize recent findings illustrating how abnormal Nrg1/ErbB4 signaling disrupts GABAergic function in the cerebral cortex, the main topic of the present review. These results are also analyzed in the context of recent studies that highlight the relevance of cortical interneurons in cognitive function, and their possible implication in the pathophysiology of schizophrenia.

Neuregulin-1 and its receptors

NRG1 is a member of a family of four genes encoding for structurally related proteins characterized by an EGF-like domain that mediates their interaction with ErbB receptor tyrosine kinases [16]. Alternative splicing of

NRG1 generates over 30 isoforms, which can be grouped into six types of proteins with different structural and functional personalities [17•]. Most NRG1 isoforms contain an immunoglobulin (Ig)-like domain and are synthesized as single-pass transmembrane molecules with the EGF-like domain facing the extracellular side (types I, II, IV and V). Subsequently, these pro-NRG1 isoforms are processed releasing diffusible NRG1 proteins. By contrast, type III NRG1 isoforms transverse twice the plasma membrane due to the presence of a characteristic hydrophobic cysteine-rich domain (CRD). Consequently, type III Nrg1 (also known as CRD-Nrg1) remains attached to the membrane after being proteolytically processed.

NRG1 function is thought to be largely mediated by a family of tyrosine kinase receptors related to the epidermal growth factor receptor (EGFR or ErbB1). NRG1 binding to its receptors leads to dimerization and kinase activation, which initiates several downstream signaling events [18]. Interestingly, ErbB4 is the only receptor that binds neuregulins and is catalytically active, and so ErbB4 homodimers may function as signaling units in the brain. In contrast, ErbB3 can bind NRG1, but its kinase domain is inactive, while ErbB2 and EGFR are catalytically active but do not seem to bind NRG1. Consequently, these receptors can only function as heterodimers with a binding partner (ErbB3 or ErbB4). Multiple studies indicate that ErbB2/B3 heterodimers mediate the function of NRG1 primarily in the peripheral nervous system, whereas ErbB4 seems to be the main signaling partner of NRG1 in the brain [19]. In light of the implication of both genes in schizophrenia, unraveling the precise sites of action for NRG1 in the developing cortex — that is, the location of ErbB4 receptors — seemed decisive to clarify the involvement of this signaling system in the pathophysiology of the disorder.

ErbB4 expression in the developing cerebral cortex

ErbB4 is probably the most abundant neuregulin receptor in the mouse forebrain [20,21], but its cellular distribution has been a matter of debate until very recently. Thus, while ErbB4 expression has been largely attributed to GABAergic neurons in the embryonic telencephalon [22,23], ErbB4 receptors have been associated with both glutamatergic pyramidal cells and GABAergic interneurons in the postnatal cortex [20,22,24–29]. This apparent discrepancy has led to contradictory views on the possible role of ErbB4 in the cerebral cortex, adding confusion to an already complex matter. Recent studies have solved this uncertainty by unambiguously demonstrating that ErbB4 expression in the postnatal mouse neocortex and hippocampus is largely — if not completely — restricted to GABAergic interneurons [30,31•,32••]. ErbB4 receptors are primarily confined to interneurons that also contain parvalbumin (PV+) [23,30,32••], a calcium binding protein that identifies the two major classes of fast-spiking interneurons, basket and chandelier cells. ErbB4 also appears to be present in a relatively small number of other classes of interneurons [23,30,32••].

Electron microscopy analyses have also shed light on the subcellular location of ErbB4 receptors, which appear to be distributed in two distinct compartments within cortical interneurons: somatodendritic and axonal domains. ErbB4 receptors are highly abundant in the dendrites of cortical interneurons [31•], where they integrate into postsynaptic densities receiving glutamatergic synapses [32••]. This finding comes to resolve an apparent contradiction with previous results in the field, which supported the idea that ErbB4 was also present in pyramidal cells based on the biochemical observation that this receptor interacts with PDS-95, a postsynaptic marker of glutamatergic synapses [24,25]. Thus, ErbB4 is indeed present at postsynaptic sites of excitatory synapses, but only in those targeting the dendrites of GABAergic interneurons [31•,32••]. The population of interneurons with dendritic ErbB4 expression has not been fully characterized, but it likely includes a large number of basket cells, the most common type of PV+ fast spiking interneurons (Figure 1).

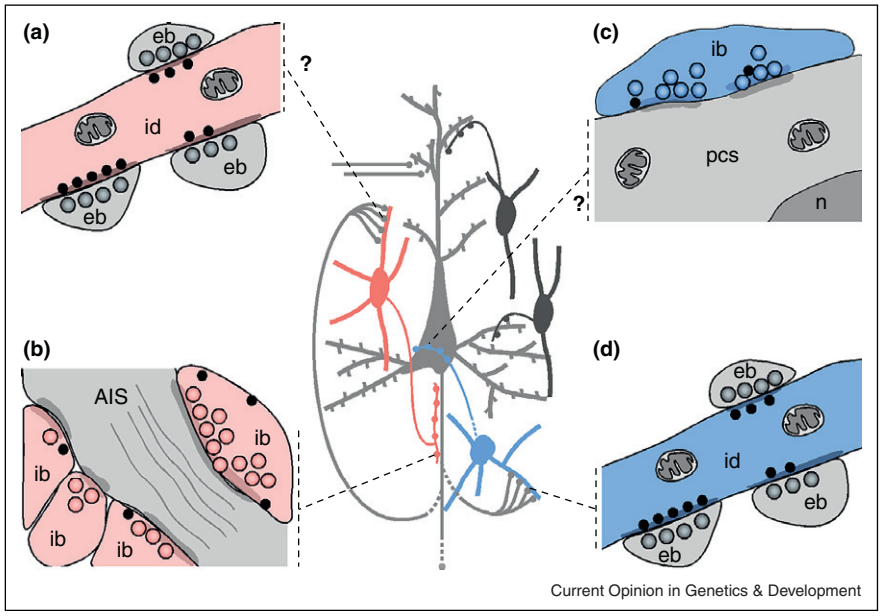
ErbB4 receptors also localize to axon terminals in at least some classes of interneurons [28,32••] (Figure 1). Electron microscopy analyses found ErbB4 receptors in axon terminals contacting the axon initial segment (AIS) of pyramidal cells and, less frequently, their soma [32••]. This suggests that ErbB4 may also play a presynaptic role in chandelier cells, a class of interneurons that specifically targets the AIS of pyramidal cells, and perhaps also in subsets of basket cells, which target the soma. Interestingly, the combination of pre-embedding and post-embedding electron microscopy techniques suggests that ErbB4 receptors have an extra-synaptic location in axon terminals, which is in contrast with their precise synaptic arrangement in dendrites [32••].

What is the precise location of ERBB4 receptors in the developing human brain? Although multiple splice isoforms of *ERBB4* have been identified in human fetal and adult brains [33], their cellular distribution has not yet been explored in detail. However, the distribution of *ERBB4* mRNA in the cortex of juvenile and adult non-human primates follows a scattered pattern [34], a finding that is consistent with the location of this receptor in GABAergic interneurons.

Wiring of inhibitory circuitries by Nrg1 and ErbB4

As summarized above, ErbB4 seems to be expressed by the same population of interneurons at both embryonic and postnatal stages, which suggests that neuregulin

Figure 1



Cellular and subcellular distribution of ErbB4 in cortical neurons. Schematic drawing of a cortical microcircuitry containing a pyramidal cell (light gray) and interneurons (red, blue and dark gray). ErbB4 is largely confined to PV-expressing interneurons, which includes basket (red) and chandelier (blue) cells. ErbB4 is also expressed, to a minor extent, in other classes of interneurons (not depicted). At the subcellular level, ErbB4 is expressed at the postsynaptic density of basket cell inhibitory dendrites receiving excitatory input **(d)**. ErbB4 may have a similar distribution in the dendrites of chandelier cells **(a)**, although this remains to be experimentally tested (question mark). In addition, ErbB4 is present in inhibitory boutons contacting the axon initial segment **(b)** and the cell soma of pyramidal cells **(c)**. It remains to be elucidated whether the somatic synapses containing ErbB4 originate from basket cells expressing PV (as depicted) or the neuropeptide cholecystokinin (question mark). Note that the expression of ErbB4 in inhibitory boutons seems to be primarily extra-synaptic, whereas ErbB4 expression in the dendrites of interneurons is largely restricted to the postsynaptic density. AIS, axon initial segment; eb, excitatory bouton; ib, inhibitory bouton; id, interneuron dendrite, n, nucleus; pcs, pyramidal cell soma.

signaling may regulate different aspects of their development. Consistent with this view, Nrg1 and ErbB4 have been shown to regulate the migration of some interneurons derived from the medial ganglionic eminence (MGE), a transitory proliferative region in the subpallium that is the main source of PV+ interneurons [23]. Immature interneurons fail to reach the cortex in normal numbers in the absence of ErbB4, and consequently the postnatal cortex of *ErbB4* null mutant mice contains reduced numbers of GABAergic interneurons that express PV [23,30]. Thus, Nrg1/ErbB4 signaling at embryonic stages controls the allocation of normal numbers of PV+ interneurons in the cerebral cortex.

Does Nrg1/ErbB4 signaling also play a role in the postnatal development of cortical interneurons? Recent work suggests that this is the case (Figure 2). Indeed, Nrg1/ErbB4 signaling seems to regulate two different aspects of the wiring of PV+ interneurons, in line with the subcellular distribution of ErbB4 within these cells. First, ErbB4 seems to modulate synapse formation in inhibitory neurons, as shown both *in vitro* and *in vivo* [32••,35]. In particular, recent *in vivo* experiments have shown that ErbB4 is cell-autonomously required for chandelier cell axons to establish or maintain a normal complement of inhibitory synapses. In the absence of ErbB4, chandelier cells make fewer synapses than normal, leading to inefficient synaptic transmission [32••]. Of note, this finding is highly reminiscent of the reported loss of axon candlesticks — the distinctive arrays of synaptic boutons made by chandelier cells — in the prefrontal cortex of schizophrenia subjects [36,37]. The role of ErbB4 in this process seems to depend, at least in part, on Nrg1, because overexpression of this gene in pyramidal cells increases the number of inhibitory synapses these neurons receive [32••].

The second role of ErbB4 receptors in the wiring of cortical inhibitory circuitries seems related to their location in the dendrites of PV+ interneurons. Morphological analyses revealed that loss of ErbB4 function in these cells causes a reduction in the number of glutamatergic synapses they receive *in vivo*. Furthermore, electrophysiological experiments confirmed that the excitatory drive to interneurons is reduced in interneuron-specific *ErbB4* mutants [32••]. Since activation of PV+ interneurons has been shown to be essential for their recruitment into specific cortical assemblies and the generation of gamma rhythm in the cortex [38••,39], disruption of Nrg1/ErbB4 signaling in this location is likely to have major functional consequences.

Figure 2

Summary of genetic studies on the function of Neuregulin-1 and ErbB4 in the cerebral cortex

Control	*Nrg1*$^{+/-}$	• Decreased number of functional NMDA receptors [10] • Hypophosphorylated NMDA receptors [59] • Defective short-term synaptic plasticity and long-term potentiation [59] • Locomotion- and exploration-related hyperactivity [10, 46] • Impaired prepulse inhibition [10]
Control	*Type III Nrg1*$^{+/-}$ *Type III Nrg1*$^{-/-}$	• Decreased dendritic spine density in pyramidal cells [45] • Disrupted dendritic morphology [41•] • Impaired prepulse inhibition [45] • Impaired working memory [45]
Control	*ErbB4*$^{+/-}$	• Hypophosphorylated NMDA receptors [59] • Locomotion-related hyperactivity [10] • Impaired prepulse inhibition [10]
Control	*ErbB4*$^{-/-}$;*HER4*^{heart}	• Reduced number of GABAergic interneurons [23] • Reduced number of PV+ interneurons [43•] • Neuregulin-1 modulates gamma oscillations via ErbB4 [43•]
Control	*Nestin-Cre;ErbB4*$^{flox/flox}$	• Impaired working memory [44]
Control	*Nestin-Cre;ErbB2*$^{flox/flox}$*;ErbB4*$^{flox/flox}$	• Decreased dendritic spine density in pyramidal cells [40•] • Reduced number of excitatory synapses in vitro [40•] • Disrupted scaffolding of postsynaptic proteins [40•] • Impaired prepulse inhibition [40•]
Control	*PV-Cre;ErbB4*$^{flox/flox}$	• Increased firing rates in pyramidal cells [42••] • Impaired GABAergic neurotransmission [42••] • Locomotion-related hyperactivity [42••] • Deficit in prepulse inhibition [42••] • Impaired working memory [42••]
Control	*rv::Cre;ErbB4*$^{flox/flox}$ *Dlx5/6-Cre;ErbB4*$^{flox/flox}$	• Decreased number of chandelier synapses [32••] • Decreased number of excitatory synapses to PV+ interneurons [32••] • Decreased mIPSCs frequency in pyramidal cells [32••] • Decreased mEPSCs frequency in interneurons [32••]

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Summary of genetic studies on the function of *Neuregulin-1* and *Erbb4* in the cerebral cortex. In this table, morphological and electrophysiological phenotypes are depicted on the left for each type of genetic analysis. A summary of main findings is provided on the right. *Nrg1*$^{+/-}$ and *ErbB4*$^{+/-}$ are heterozygous mice for a null mutation in the Nrg1 and ErbB4 genes, respectively. *Type III Nrg1*$^{-/-}$ and *Type III Nrg1*$^{+/-}$ are homozygous and heterozygous mice for a null mutation that only disrupts type III isoforms of the *Nrg1* gene. *ErbB4*$^{-/-}$;*HER4*^{heart} are homozygous mice for a null mutation on the ErbB4 gene that also express the human ERBB4 gene in the heart. *Nestin-Cre;ErbB4*$^{flox/flox}$ and *Nestin-Cre;ErbB2*$^{flox/flox}$*;ErbB4*$^{flox/flox}$ are CNS-specific conditional mutant mice for *ErbB4* and both *ErbB2* and *ErbB4*, respectively. *PV-Cre;ErbB4*$^{flox/flox}$ are PV-specific conditional mutant mice for *ErbB4*. *rv::Cre;ErbB4*$^{flox/flox}$ are mice in which retrovirus expressing Cre has been injected in the origin of PV+ interneurons, the MGE. *Dlx5/6-Cre;ErbB4*$^{flox/flox}$ are forebrain GABAergic-specific conditional mutant mice for *ErbB4*.

Previous studies have suggested that Nrg1 signaling may also control the development of pyramidal cells and their connections. In particular, conditional inactivation of both ErbB2 and ErbB4 in the CNS has been shown to disrupt the maturation of dendritic spines in pyramidal cells [40•] (Figure 2). Since loss of ErbB4 in pyramidal cells alone does not seem to affect the morphology or number of dendritic spines [32••], the function of Nrg1 in this process might be mediated by ErbB2 receptors. Alternatively, the defects reported in pyramidal cells of *ErbB2;ErbB4* double mutants might be secondary to the disruption in inhibitory synapses, a hypothesis that remains to be experimentally tested. In addition, a recent study has shown that reverse signaling mediated by type III Nrg1 (i.e. via the intracellular domain of Nrg1 and independent of ErbB kinase activity) is required for the dendritic development of pyramidal cells [41•].

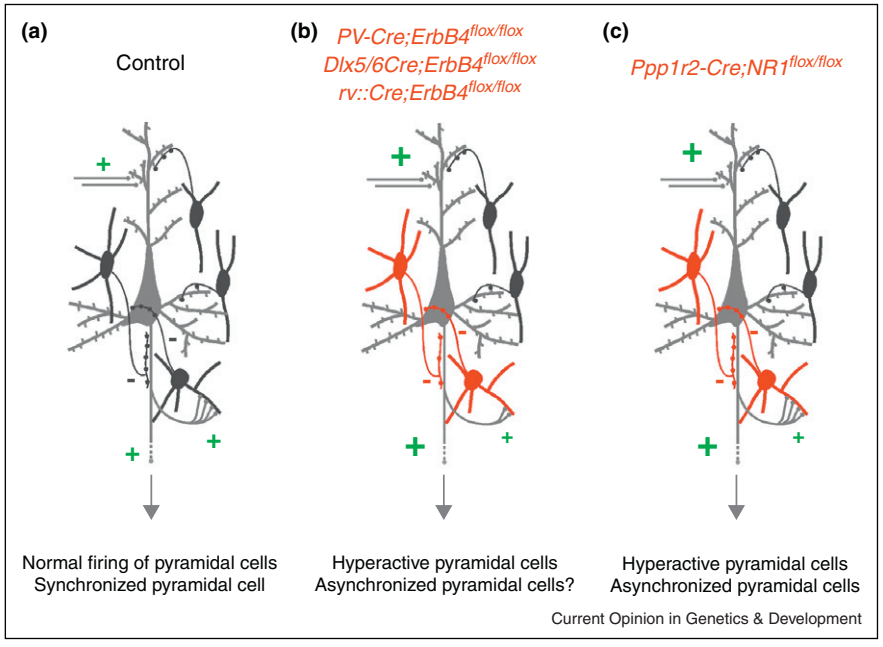
Neural circuitry disruption in schizophrenia

The experiments summarized above demonstrate that ErbB4 mediates consecutive functions of Nrg1 in the establishment of specific inhibitory circuitries in the neocortex and hippocampus of mice. Depending on the timing of the perturbation, loss of ErbB4 signaling may disrupt inhibitory circuitries in different ways. Thus, early loss of ErbB4 reduces the number of interneurons that reach the cortex, while late disruption of Nrg1/ErbB4 signaling impairs synapse formation. In addition, Nrg1 regulates GABAergic transmission in the adult cortex [28,42••], which represents an additional mechanism through which this signaling pathway influences inhibitory cortical function (Figure 2).

What are the functional consequences of perturbing Nrg1/ErbB4 signaling in mice? Analysis of the different mutant strains indicates that disruption of Nrg1/ErbB4 consistently leads to disinhibition of cortical excitatory neurons and reduced neuronal synchrony. For example, embryonic deletion of ErbB4 reduces the power of kainate-induced gamma oscillations [43•], while conditional deletion of ErbB4 during the second and third postnatal weeks (using a PV-Cre mouse strain) leads to an abnormal increase in the activity of pyramidal cells [42••]. Thus, independently of the severity of the perturbation, disruption of Nrg1/ErbB4 signaling leads to hyperactive pyramidal cells and compromised network activity (Figure 3).

Behavioral analyses have consistently found important defects in *ErbB4* mutants [10,44]. Most notably, PV-specific *ErbB4* mutant mice exhibited schizophrenia-relevant phenotypes similar to those observed in *Nrg1* or *ErbB4* null mutant mice, including hyperactivity, impaired working memory, and deficient prepulse

Figure 3



ErbB4 and NMDAR in inhibitory circuitry formation and function. **(a)** Basket and chandelier cells control pyramidal cell output by forming inhibitory synapses onto the soma and AIS, respectively, of pyramidal cells. In normal cortical circuitries, PV+ interneurons regulate the excitability of pyramidal cells and contribute to their synchronization. **(b)** Loss of ErbB4 causes a decrease in inhibitory inputs to the AIS of the pyramidal cells [32••], a decrease in excitatory inputs received by PV+ interneurons [32••], and an increase in pyramidal cell firing [42••]. Although not experimentally tested yet, this is also likely to lead to reduce the synchronization of pyramidal cells. **(c)** Loss of a subunit of the NR1 subunit of the NMDA receptor in PV+ interneurons during early postnatal life (chandelier and basket cells) also causes hyperactive but asynchronic pyramidal cells [48••].

inhibition (PPI) [42••,45,46] (Figure 2). These results position the defects in inhibitory transmission mediated by PV+ interneurons at the center of the physiopathology of schizophrenia, and suggest that disruption of the Nrg1/ErbB4 signaling pathway is a plausible pathophysiological mechanism that may contribute to the disorder.

The involvement of glutamatergic transmission in the pathophysiology of schizophrenia is a long-standing hypothesis that seems to dispute the central role of inhibitory circuits in the disorder, as outlined above. This hypothesis is largely based on the observation that NMDA receptor (NMDAR) antagonists induces schizophrenia-like phenotypes in mice and enhances the symptoms in schizophrenia subjects [47]. Recent works on the mechanisms regulating the activation of cortical PV+ interneurons through NMDARs have come to shed light into this apparent discrepancy. Thus, conditional deletion of the NR1 subunit of the NMDAR specifically from cortical interneurons (mostly PV+) causes disinhibition of cortical excitatory neurons and reduced neuronal synchrony, which leads to the emergence of schizophrenia-related symptoms similar to those described after systemic treatment with NMDAR antagonists [48••] (Figure 3). These results indicate that deficient activation of interneurons alone is sufficient to induce behavioral defects in mice that are reminiscent of schizophrenia, and reinforce the view that disruption of PV+ interneuron function is central to the disorder. These findings are also consistent with the observation that GABAergic interneurons are disproportionally more sensitive to NMDAR antagonists than pyramidal neurons [49,50]. In sum, considering that ErbB4 associates with PSD-95 [24,25], and that this protein interacts with the NMDAR complex primarily at the synapses that mediate the activation of PV+ interneurons [31•,32••], these findings nicely reconcile both hypotheses (glutamatergic and GABAergic) on the pathophysiology of schizophrenia (Figure 3).

Timing is perhaps the most important predictor of the behavioral outcome resulting from a disruption in the excitatory drive of cortical interneurons. Thus, while deletion of NR1 from PV+ interneurons early in life causes social behavioral deficits [48••], as outlined above, removal of the same gene after the second postnatal week [51•] or in adult mice [48••] does not cause the same impairment. This strongly suggests that disruption of inhibitory circuits during embryonic or early postnatal development is required to elicit schizophrenia-related symptoms. This observation might be explained by the dynamic expression of NMDAR in cortical PV+ interneurons, which decreases prominently after adolescence [52•]. These results further suggest that disruption of Nrg1/ErbB4 signaling in adult mice may not produce behavioral deficits, a hypothesis that remains to be elucidated.

New perspectives for treatment?

It is presently unclear how genetic variation in NRG1 and ERBB4 may predispose to schizophrenia. Several studies have suggested that risk haplotypes may increase NRG1/ERBB4 signaling in adult humans [53,54]. At this point, however, interpretation of these studies is problematic, because we do not know whether the reported defects are directly caused by the risk haplotypes, or a consequence of compensatory mechanisms which are ongoing in the patient brain. In addition, recent studies in animal models indicate that antipsychotic treatment may also influence Nrg1 and ErbB4 levels [55], which therefore represents another possible confounding factor. In any case, if we assume that disruption of NRG1/ERBB4 signaling in humans leads to similar defects to those described in mice, enhancing the function of PV+ interneurons may have some therapeutic value. This could be achieved, for example, by using NMDAR agonists that would selectively activate glutamatergic receptors present in PV+ interneurons. Of note, if the lessons we have learned from the work of Belforte *et al.* [48••] hold true in humans, then palliative treatments should start early in life, way before the onset of psychosis.

A look ahead

There are many important questions that remain to be addressed on the precise role of neuregulin signaling in the organization of inhibitory circuitries and its involvement in schizophrenia. For example, it is unclear whether ErbB4 is present in the dendrites and axons of precisely the same population of interneurons. It is possible, for example, that postsynaptic ErbB4 expression is restricted to basket cells, while presynaptic ErbB4 receptors concentrate primarily on chandelier cells. Solving this question should help to distinguish between possible presynaptic and postsynaptic functions of ErbB4. In any case, the mechanisms that underlie the function of Nrg1/ErbB4 signaling in the presynaptic and postsynaptic wiring of cortical interneurons remain largely unknown. ErbB4 signaling has been suggested to control the expression of NMDAR subunits in the cerebellum [56,57], but recent results indicate that this might not be the main function of this receptor *in vivo* [58•]. Alternatively, ErbB4 may modulate the channel properties of NMDARs by regulating their phosphorylation status [59]. Another open question is the possible role of Nrg1/ErbB4 in other neural circuitries that are thought to be relevant for schizophrenia, such as the amygdala and basal ganglia. Finally, recent genetic studies have also linked NRG3 to schizophrenia [60•,61•,62•,63•], but the role of this gene in the development of the cerebral cortex is completely unknown. Inroads into these topics should help us to clarify the function of neuregulin signaling, both in health and in disease.

Note added in proof

While this review was in press a manuscript by Ting and colleagues [64] was published. In this study, the authors

found that *in vivo* deletion of ErbB4 in parvalbumin-positive interneurons led to deficits in the excitatory inputs receive these cells, and that Nrg1 increases the number, size, frequency and amplitude of mEPSCs *in vitro*. Together, these results add support to the idea that Nrg1/ErbB4 signalling is important for excitatory synaptogenesis in interneurons.

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