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The Neuregulin Signaling Pathway and Schizophrenia: From Genes to Synapses and Neural Circuits

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Abstract

Numerous genetic linkage and association studies implicate members of the Neuregulin-ErbB receptor (NRG-ErbB) signaling pathway as schizophrenia “at risk” genes. An emphasis of this review is to propose plausible neurobiological mechanisms, regulated by the Neuregulin-ErbB signaling network, that may be altered in schizophrenia and contribute to its etiology. To this end, the distinct neurotransmitter pathways, neuronal subtypes and neural network systems altered in schizophrenia are initially discussed. Next, the review focuses on the possible significance of genetic studies associating NRG1 and ErbB4 with schizophrenia, in light of the functional role of this signaling pathway in regulating glutamatergic, GABAergic and dopaminergic neurotransmission, as well as modulating synaptic plasticity and gamma oscillations. The importance of restricted ErbB4 receptor expression in GABAergic interneurons is emphasized, particularly their expression at glutamatergic synapses of parvalbumin-positive fast spiking interneurons where modulation of inhibitory drive could account for the dramatic effects of NRG-ErbB signaling on gamma oscillations and pyramidal neuron output. A case is made for reasons that the NRG-ErbB signaling pathway constitutes a “biologically plausible” system for understanding the pathogenic mechanisms that may underlie the complex array of positive, negative and cognitive deficits associated with schizophrenia during development.

Keywords

ErbB4; neuregulin; GABAergic interneuron; schizophrenia; bipolar depression; hypoglutamatergic; gamma oscillation; LTP; depotentiation

INTRODUCTION

Schizophrenia is a complex psychiatric disorder generally characterized by positive symptoms that include auditory hallucinations, running thoughts and delusions, as well as negative symptoms such as social withdrawal, anhedonia and blunted affect. Neurocognitive deficits in working memory, attention and executive function are additional parameters associated with schizophrenia. The combined effects of multiple susceptibility genes, with small effects from individual genes, are believed to contribute to the risk of developing schizophrenia (rev. (1)). The degree of concordance for schizophrenia in monozygotic twins has been reported to be between 50 – 80%, indicating that, while genetic background is

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important, genetic liability on its own is not sufficient to cause schizophrenia. It is generally accepted that genetic liabilities in combination with environmental contributions during development, such as frequency of cannabis use, infection or stress during gestation, stress during parturition and paternal age at conception, increase the likelihood of developing schizophrenia later in life (rev. (2,3)). While the positive symptoms of the illness (i.e. psychosis) frequently become apparent in the second decade of life, schizophrenia is generally considered a neurodevelopmental disorder (rev. (2,4,5)). The late onset of symptoms are thought to arise from earlier developmental alterations, such as deficits in neuronal migration, maturation of neuronal processes, deficits in synaptic pruning and myelination of selective axonal tracts projecting to frontal cortical areas (rev. (6)), which in turn can affect synaptic connectivity. Therefore, a future challenge in understanding the neurobiological deficits in schizophrenia is to functionally dissect the molecular, cellular, synaptic and neural circuits that contribute to the complex array of positive, negative and cognitive deficits, in particular the cognitive deficits that ultimately have the highest impact in the lives of persons with schizophrenia.

Neurotransmitter Systems and Genes Implicated in Schizophrenia

Multiple neurotransmitter systems are implicated in schizophrenia—Several lines of evidence on the neurochemical changes that may underlie the deficits observed in schizophrenia strongly implicate disturbances in glutamatergic and dopaminergic neurotransmission (7–12), as well as GABAergic interneurons (rev. (13–15)). Human and animal studies using *N*-methyl-D-aspartate (NMDA) receptor competitive antagonists, such as ketamine and PCP, suggest that hypofunction of NMDA receptors may underlie the functional deficits observed in schizophrenia (rev. (12,16–18); *see article by D. Javitt in this issue*). The glutamate hypofunction hypothesis of schizophrenia originated from the observation that NMDA receptor antagonists elicit cognitive deficits in healthy individuals that are similar to the positive (i.e. hallucinations) and negative (i.e. social withdrawal) symptoms observed in persons diagnosed with schizophrenia (rev. (19)); however, it has been suggested that the effects observed with PCP could result from its partial agonism on D2-type dopamine receptors and interference with dopaminergic transmission (20). An extension of the glutamate hypofunction hypothesis emphasizes the selective reduction of NMDA receptor activity in GABAergic interneurons (rev. (13,14,17,21)). Although these pharmacological and genetic findings primarily implicate NMDA receptors, it is important to note that, because of the intimate functional interactions between NMDA and AMPA receptors, in particular the requirement for membrane depolarization by AMPA receptors to relieve the voltage-sensitive magnesium block of NMDA receptors, perturbations in either receptor type might be expected to contribute to glutamate hypofunction.

A role for altered dopaminergic transmission in schizophrenia, predominantly the positive symptoms of the disorder, was supported initially by the observation that most antipsychotics are dopamine D2-type receptor antagonists or partial agonists (see (10,23)). This hypothesis has been revised recently to account for the positive and negative symptoms resulting from dopamine imbalances and for the cortical hypofrontality observed in schizophrenia, and proposes that cortical hypoglutamatergia enhances subcortical hyperdopaminergia and cortical hypodopaminergia (see (8,24)). Importantly, it is difficult to assign the systemic effects of drugs targeting dopamine receptors specifically to this neurotransmitter system, because there is extensive cross-talk between dopamine and glutamatergic or GABAergic neurotransmission both at a neural systems level (24), as well as by indirect and direct physical interactions between distinct types of receptors (rev. in (25)). Dopamine receptor activation regulates glutamate and GABA neurotransmission at synapses by regulating ion channel properties and subcellular targeting of receptors (26–32). Direct physical interactions have been reported, in particular between dopamine D1 and

NMDA receptors (33–35). The reciprocal interactions between glutamate, GABA and dopamine neurotransmission at the system, cellular and molecular level make it difficult to directly assign imbalances in any one transmitter system to deficits observed in schizophrenia and stress the importance of genetics for identifying the heritable underlying neurobiological liabilities that contribute to the risk of developing the disorder. While there have been numerous studies reporting association of genes involved in the synthesis, degradation and binding of different neurotransmitters with schizophrenia (see below), replication of these findings have been few and genome wide association studies have not borne out a clear link between neurotransmitter-associated genes and the etiology of schizophrenia. It is thus conceivable that genes that subserve a modulatory rather than an effector role in the dopamine, glutamate and GABA neurotransmission pathways significantly contribute to increased risk for schizophrenia (see below).

Neuronal network activity is altered in schizophrenia—Numerous studies suggest that neuronal circuitry and network activity, in particular gamma oscillations, are altered in schizophrenia (see (21,36–39). 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 (40). Gamma oscillations in rodents have been recorded *in vivo* (41,42) and their frequency is modulated by GABAergic basket cells, as suggested by studies performed in acute slices where gamma oscillations are induced by either carbachol or kainate (43,44). Studies performed in hippocampal slices showed that the power (amplitude) and frequency of carbachol- and kainate-induced gamma oscillations are driven by the complex recurrent network of the CA3 area and rely on the interplay of fast inhibitory and fast excitatory synaptic neurotransmission (43). The power of gamma oscillations in subjects diagnosed with schizophrenia is reduced (45,46), and the regional reaction time phase-lock of oscillations is correlated with either positive or negative symptoms (38). Also of importance is the repeated observation that GABAergic fast-spiking interneurons and expression of GAD67, the rate limiting enzyme in the GABA synthesis pathway, are reduced in parvalbumin-expressing interneurons in postmortem brains from affected individuals, suggesting that specific neural circuits may be associated with schizophrenia (47–49); see (21)). Altered functionality of parvalbumin expressing interneurons, which provide perisomatic innervation to pyramidal neurons, may account for the observed reduction in neural network oscillations that are important for working memory (14,36). Of significance, we recently reported that the Neuregulin-ErbB signaling pathway has a strong modulatory effect on the power of kainate-induced gamma oscillations (50); see below).

Genes encoding proteins regulating synaptic transmission and plasticity associated with schizophrenia—There is general agreement that schizophrenia is a polygenic disorder, with each “at risk” locus having low penetrance and/or contributing small effects. At this time it is not known if the effects of the “at risk” genes converge to affect common neural processes, such as neuronal migration, differentiation, synapse formation or maturation, or if they encode proteins in a common signaling pathway. The identification of a handful of susceptibility genes by genetic linkage and association studies, and whose products are plausible elements of the neurobiological processes altered in schizophrenia, represents a major advance in studying the etiology of this disorder (rev. (1,51,52)). Many of these genes encode proteins involved in synaptic structure and plasticity, or regulate neurodevelopment. Among them are the neurotrophic and differentiation factor Neuregulin-1 (NRG1), and to a lesser extent one of its receptors, ErbB4. They have reproducibly emerged as important candidate schizophrenia susceptibility genes in numerous case-control association studies across different ethnic groups, as corroborated in meta-analyses of up to 24 independent studies ((53–55); *also see Hahn et al. in this issue*) but for which conclusions must be tempered by considerations of population

stratification (see (56)). Analysis of *de novo* rare structural chromosomal variants found that microdeletions and microduplications are relatively more common in cases of schizophrenia and childhood onset schizophrenia (57), and that genes encoding signaling networks proteins controlling neurodevelopment, including the NRG1-ErbB4 pathway, are disrupted disproportionately (discussed below). In addition to NRG1, *disrupted in schizophrenia-1* (*DISC1*) was identified as a risk gene based on a family study in which a translocation was shown to disrupt the locus (58,59). Interestingly, contrary to what may have been expected from pharmacological and neuroimaging data, case-control studies associating schizophrenia with genes for dopamine, glutamate or GABA neurotransmission have generally not been successfully replicated. However, family-based studies have implicated metabolic and receptor genes for dopamine (*COMT*, *DRD2*, *DRD4*), glutamate (*GRM7*, *SLC1A3*) and GABA neurotransmission (60–62). While it has been proposed that schizophrenia-associated genes can generally be separated into two groups that affect glutamate-dependent plasticity (for example, NRG1) or dopamine metabolism (see (63)), there may in fact be a more direct relationship. As discussed below, we recently have shown a direct functional link between NRG1, glutamatergic transmission and dopamine signaling, and suggested that alterations in the NRG1-ErbB signaling pathway could account for dysregulation of the glutamatergic and dopaminergic system in schizophrenia (64).

Implications of the NRG/ErbB signaling pathway in schizophrenia—Stefansson and colleagues originally identified the NRG-1 HAP_{ice} haplotype as an “at risk for schizophrenia” locus (65). Interestingly, the single nucleotide polymorphism (SNP) SNP8NRG243177, which maps within the HAP_{ice} haplotype (see Fig. 1A), is in disequilibrium with other SNPs within the haplotype and has characteristics of a functional polymorphism. SNP8NRG243177 [T/T] has been associated with impairment of frontal and temporal lobe activation, deficits in cognitive function and predisposition to the development of psychotic symptoms in individuals at high risk for developing schizophrenia (66–68); however, some of these findings were not replicated by another group (see (69)). In addition, in normal individuals the [T/T] allele was associated with reduced spatial working memory (70) and white matter in the anterior limb of the internal capsule (71). These studies suggest that SNP8NRG243177 is a functional polymorphism, since no other SNPs or microsatellites from this haplotype were associated with these behavioral or functional deficits. Intriguingly, SNP8NRG243177 maps upstream of the NRG1 type IV 5'-exon (72), one of the NRG1 isoforms generated by differential promoter usage (see below), and our recent analysis failed to identify transcripts that harbor the SNP8NRG243177 polymorphic site therefore arguing against a NRG1 protein variant (Shamir and Buonanno, J. Neurochem., *in press*). Weinberger and colleagues reported elevated mRNA levels of NRG1 type IV in schizophrenia patients and in healthy controls homozygous for SNP8NRG243177 [T/T] (73), and suggested that this polymorphism may affect transcription rates.

While the HAP_{ice} haplotype is the most studied polymorphism in *NRG1*, haplotypes encompassing sequences downstream of the NRG1 types I and IV promoters (Fig. 1A) have been associated with schizophrenia in populations of Han Chinese (74) and Portuguese descent (75). Moreover, an approximately 4-fold increase of NRG1 SMDF transcripts, as well as elevation of other NRG1 splice variants, were found in peripheral leukocytes from schizophrenia patients of Portuguese descent (75). All NRG1 SNPs associated with schizophrenia map to non-coding DNA sequences, with exception of a polymorphic site in an exon that encodes the NRG1 transmembrane domain (Val→Leu) which was identified in families from the Central Valley of Costa Rica (76); however, at this time the possible functional significance of this conserved amino acid change is unknown. Therefore, as is the case for other susceptibility genes (i.e., dysbindin, *DISC1*), no known schizophrenia-associated NRG1 polymorphisms have been shown to modify protein function, but could affect transcript levels based on analyses of postmortem tissue and peripheral leukocytes.

Besides NRG1, no other gene in the NRG family has been reported to be associated with schizophrenia. Recent analysis of the NRG3 gene in a family-based study of Ashkenazi Jews identified 3 SNPs mapping to the first intron that, while not associated with schizophrenia, had positive association with “delusion”, one of the quantitative trait factors for schizophrenia (77).

Genetic linkage and meta-analyses, as well as analyses of rare *de novo* structural chromosomal variants also implicate ErbB4 in schizophrenia (57,78–80), but few studies replicated the association of ErbB4 with schizophrenia compared with NRG1. Navon and colleagues performed an in-depth analysis of ErbB4 because it encodes the major receptor for NRG1 in CNS neurons and numerous studies reported linkage of chromosome 2q with schizophrenia across multiple populations. In a case-control study of Ashkenazi Jews they identified a highly significant association of a linkage disequilibrium block, defined by three SNPs, with schizophrenia (78). The association of this 3-SNP ErbB4 haplotype with schizophrenia was replicated for two other Caucasian and African American family-based groups (79). Interestingly, this haplotype, which maps close to exon 3, is associated with increased cortical and hippocampal expression of ErbB4 transcripts containing the CYT-1 exon (exon 26) that encodes a phosphatidylinositol-3 kinase (PI3K) interacting domain (78,81). In addition, the same haplotype and genotypes are associated with impairment of certain cognitive functions (79) and left frontal-temporal structural connectivity (82) in healthy individuals. In addition to the genetic association studies, ErbB4 was identified in a study of *de novo* rare chromosomal structural variants in schizophrenia (57). The chromosomal deletion identified at the *ERBB4* locus had the potential to encode a truncated form of the receptor that, if expressed, could have similar dominant-negative properties described for a truncated receptor generated by molecular manipulation (83). Biochemical functional evidence for an involvement of NRG-ErbB4 signaling in schizophrenia comes from a recent study in which a decrease in the association of ErbB4 and PSD-95 proteins was measured in prefrontal cortex lysates from postmortem brains of persons with schizophrenia as compared to controls (84). Interestingly, this change was accompanied by a decrease in the tyrosine phosphorylation of the NR2A subunit of the NMDA receptor in response to NRG-1 stimulation, suggesting that this NMDA receptor subunit may be a downstream target of ErbB4.

On the other hand there are suggestions from animal studies using NRG1 and ErbB4 hypomorphic mice, that the NRG-ErbB signaling pathway affects behaviors that have been associated with schizophrenia. Discussion of the large number of animal studies performed with different types of NRG1 and ErbB4 mutant and transgenic (over-expressing) mice is beyond the scope of this review, but they are discussed by *Waddington and colleagues in this issue*. In the context of this review, however, it is important to mention that heterozygous NRG-1 and ErbB4 mutant mice exhibit behavioral abnormalities in numerous tasks that may result from hyperactivity and abnormalities in sensory gating, behavioral phenotypes shared with NMDA receptor NR1 hypomorphic mice. Treatment of NMDA receptor NR1 hypomorphs, as well as NRG-1 and ErbB4 heterozygotes, with the antipsychotic drug clozapine used to treat schizophrenia, partially restores their normal behavior (65).

Temporal, Regional and Subcellular Expression of Neuregulin and ErbB4 Receptor

An in-depth discussion of the family of NRG ligands and ErbB receptors, and their functions in the developing brain, are beyond the scope of this article. Readers are referred to prior reviews that have covered these areas (refer to (85–88)).

Neuregulin ligands and their receptors—The Neuregulin family, comprised of NRG 1–4, are growth and differentiation factors characterized by a conserved EGF-like domain of ~55 amino acids. The EGF-like motif is necessary and sufficient to bind and elicit autophosphorylation of their cognate transmembrane receptor tyrosine kinases, known as ErbB2, ErbB3 and ErbB4 that are evolutionarily related to the EGF receptor, ErbB1 (89). While NRGs bind directly only to ErbB3 or ErbB4, once activated, these receptors heterodimerize with ErbB1 or ErbB2, or with each other, to activate the downstream signaling pathways that mediate their biological effects (i.e., MAPK and PI3K). The differential expression, processing and expression of NRGs and their receptors greatly contributes to the daunting complexity and multitude of neural functions regulated by the NRG-ErbB signaling pathway.

NRG1 is by far the most extensively studied family member. Alternative promoter usage of the NRG1 gene gives rise the different NRG1 “types” and among these NRG1 types I through IV have been the most widely studied (see (72,85,88)). Three promoters were originally identified in the NRG1 gene that generate type I (ARIA, NDF, heregulin), type II (GGF) and type III (SMDF, CRD); refer to Fig. 1B. A partial cDNA denoted type IV, obtained by RT-PCR and primer extension (72), was later shown to encode a full-length NRG1 transcript selectively expressed in the human brain ((90,91). The full-length transcript encodes pro-NRG1 type IV β 1, and like the type I isoform, is cleaved in a PKC-dependent fashion to release an active extracellular domain (Shamir and Buonanno, J. Neurochem., *in press*). NRG1 type IV has recently been the center of much attention because of its proximity to polymorphism SNP8NRG243177 in the HAP_{ICE} haplotype (Fig. 1A), its possible specificity to primates, and the association of SNP8NRG243177 with several endophenotypes common to schizophrenia (see above). The major functional consequence of alternative usage of NRG1 promoters is differences in trans-membrane topology and types of extracellular domains. Whereas type I, II and IV NRG1 isoforms are single-pass transmembrane proteins, type III NRG1 harbors an additional transmembrane domain (TM) that keeps it attached to the membrane following cleavage and thereby enabling it to act as a juxtacrine ligand. Moreover, the N-terminal cytoplasmic domain of NRG1 type III (CRD) also affects its targeting to axons (Shamir and Buonanno, J. Neurochem., *in press*). The different patterns of NRG1 isoform expression and processing led to the idea that they could serve distinct functions (92,93), a view that is consistent with the recent demonstration that local cues by NRG1 type III regulate GABAergic interneuron development in the medial ganglionic eminence, whereas type I/II isoforms signal from a distance to promote their migration into the neocortex (94). While the extracellular domain of NRG1 proteins is critical for forward signaling, its intracellular domain has been implicated in proper trafficking of pro-NRG1 to the cell surface (see (86)), and, upon shedding of the extracellular domain, in retrograde signaling to inhibit neural apoptosis (95) and to regulate cell surface expression of the α -7 nicotinic acetylcholine receptors (96,97).

ErbB receptors (ErbB1 to ErbB4) comprise a family of receptor tyrosine kinases long been recognized as critical mediators of cell fate, proliferation, migration and differentiation processes in the developing peripheral and central nervous system (rev. (85,86,98–100). ErbB4 is expressed in the developing and adult cerebral cortex, and represents the major NRG receptor in central neurons (101–105), and therefore is the focus of this review. ErbB4 transcripts are differentially spliced at sequences encoding the juxtamembrane extracellular domain (alternative exons JM-a or JM-b) and can exclude (CYT-2) or include an exon encoding a short 16 amino acid cassette (CYT-1) in the intracellular domain. JM-a but not JM-b containing receptors, are substrates for TACE- or BACE-mediated proteolytic cleavage, which can function as alternative mechanism to internalization to attenuate ErbB4 signaling in response to NRG binding. CYT-1 isoforms contain a binding site for phosphoinositide 3-kinase and their degradation is regulated by mono-ubiquitination (106).

As discussed above, their levels are different between postmortem brain of normal individuals and schizophrenics (78,81).

Developmental Expression of NRG1 and ErbB4—In general, expression of NRG-1 transcripts in the brain is highest during embryonic, fetal and early postnatal development, and decreases with age. Postnatal expression of NRG1 mRNAs is largely confined to motor nuclei in the brainstem, septal-hippocampal projection neurons (107) and hippocampal pyramidal cells (108–110), and NRG1 type III is the predominant isoform expressed in the adult brain. Because of extremely low levels of NRG1 expression in the adult brain, and the absence or poor quality of antibodies that distinguish between specific NRG1 isotypes, at the present time the distribution of distinct NRG1 proteins in the brain is not known. Based on in vitro studies and analysis of BACE knockout mice (111), there is evidence for extracellular processing of pro-NRG-1 by matrix metalloproteinases to generate soluble (type I and II) or membrane-bound (type III) forms. Work in cerebellar granule cell cultures and on the neuromuscular junction indicate that soluble NRG1 can be released from presynaptic nerve terminals in response to neuronal activity (112–115), suggesting that the NRG-ErbB pathway functions in the brain in an activity-dependent fashion.

ErbB4 is a postsynaptic density protein and is a selective marker for glutamatergic synapses onto GABAergic neurons—In situ hybridization studies in the adult rodent and monkey cortex have consistently demonstrated a pattern of ErbB4 mRNA consistent with expression in interneurons (101,102,104,105,116,117). In agreement with these findings, most immunofluorescence analyses identified strong ErbB4-immunoreactivity in GABAergic interneurons in dissociated hippocampal neuron cultures and in the cerebral cortex (102,103,117–119). By contrast, the presence of ErbB4 in pyramidal neurons has been a matter of debate. Several histological studies reported ErbB4 immunoreactivity in pyramidal neurons in the rodent (102,120,121), monkey (116) and human cortex (122), but these findings are at variance with the distribution of ErbB4 mRNA, as noted by some authors (102,116). Because of the rapidly growing number of studies linking NRG-1/ErbB signaling to the modulation of pyramidal neuron properties, such as dendritic spine morphology (123–125), glutamate receptor trafficking (126) and synaptic plasticity (64,117,127–129), it was imperative to investigate this apparent inconsistency. Using novel monoclonal rabbit antibodies that were extensively characterized by Western blotting and immunohistochemistry with tissues obtained from wild-type and ErbB4 null mice, as well as single-cell PCR from electrophysiologically identified neurons, we recently demonstrated that in the hippocampal CA1 region ErbB4 expression is confined to GABAergic interneurons and is not detected in glutamatergic principal cells (130), an observation that was recently replicated (131). These results suggest that the reported effects of NRG1 and ErbB4 activation on dendritic spine morphology (123,124) and synaptic plasticity at principal cells in the hippocampus (64,117,127–129), and possibly other brain areas (125), result from indirect effects of NRG/ErbB signaling in local interneurons and are not intrinsic to the glutamatergic neurons.

The postsynaptic density (PSD) is an important structural element of glutamatergic synapses and serves as a scaffold to organize the intricate meshwork of receptors, ion channels and signaling proteins that help to effectively juxtapose presynaptic release sites and postsynaptic glutamate receptors and to anchor signaling proteins that bidirectionally regulate the strength of the postsynaptic response. The C-terminal tail of ErbB4 interacts directly with the MAGUK family of postsynaptic proteins including PSD-95 (117,118) that also interact with the cytoplasmic tails of the NMDA receptor subunits NR2A and NR2B. Initial studies in cultured cells demonstrated that, consistent with these interactions, ErbB4 colocalized with NMDA receptors and PSD95 at synaptic sites (117,118). Later studies using double-immunofluorescence with markers for GABAergic neurons showed that in

cultured neurons and in vivo, ErbB4 accumulates at synaptic puncta on inhibitory neurons (119,130,132). Ultrastructural analysis in CA1 interneurons using immunoelectron microscopy revealed abundant ErbB4 expression in the somatodendritic compartment where it accumulates at, and adjacent to, glutamatergic postsynaptic sites (130). By contrast, we found no evidence for presynaptic expression in cultured GAD67-positive hippocampal interneurons and in CA1 basket cell terminals (119,130,132). The localization of ErbB4 at excitatory synapses on GABAergic neurons, but not excitatory neurons, identifies these synapses as a primary target of NRG signaling in the hippocampus and indicates that ErbB4 serves as a selective marker for PSDs on GABAergic neurons (130). The cellular and subcellular distribution of ErbB4 receptors also is restricted to GABAergic interneurons in the mouse cortex (131), and the monkey and human prefrontal cortex (Neddens, Vullhorst, Tricoire, Shamir, McBain and Buonanno, in preparation).

NRG-ErbB Signaling Regulates Development, Synaptic Function and Neural Network Activity: A “Biologically Plausible” Pathway That May Underlie Neuropathology

Processes in Schizophrenia: Numerous lines of evidence indicate that schizophrenia is an early neurodevelopmental disorder, with the emergence of positive and negative symptoms frequently presenting during the second decade of life (recent rev. (3–5);133–135)). NRG1 regulates distinct aspects of neural determination and differentiation in the peripheral and central nervous system, affecting neuronal and glial fate, migration, differentiation and maturation; this has been the subject of numerous reviews (85–88,136,137). Because the multitude of glial and neuronal functions regulated by the NRG-ErbB signaling are beyond the focus of this review, I will focus on neuronal migration, synaptic transmission and plasticity, and neural network activity because these are the processes most relevant to schizophrenia.

NRG-ErbB signaling regulates cortical, olfactory and tangential interneuron migration—The NRG-ErbB4 signaling pathway is important for cell migration, regulating neuronal radial glial migration in the developing cerebral cortex (138) and cerebellum (139) (but see (123)). Both NRG1 and NRG2, signaling through ErbB4 receptors, are necessary for the formation of the rostral migratory stream and differentiation of GABAergic interneuron precursors in the adult mouse brain (140). Deficits in the migration and differentiation of interneurons in the olfactory system could be expected to influence olfactory perception. Interestingly, alterations in smell discrimination have been reported in patients with schizophrenia, depression and bipolar depression (rev. (141)). If schizophrenic patients indeed have a common deficit in odor identification, recognition and discrimination, these deficits could serve as an endophenotypic marker for the disorder (rev. (142)).

Another important role of NRG-ErbB signaling that could have a major impact on deficits associated with schizophrenia is in tangential migration of cortical GABAergic interneurons, and their possible differentiation and/or survival. Distinct populations of cortical GABAergic interneurons are generated in the medial, lateral and caudal eminences of the subpallidum during development (rev. (143)). Interestingly, the interneuron precursors from the medial and lateral ganglionic eminences express high levels of ErbB4 (103,132). The soluble NRG1 (type I) was proposed to function as a chemoattractant and the juxtracrine NRG1 (type III) to provide guidance cues for GABAergic interneurons as they tangentially migrate from the subpallidum to the developing cortex (94). Analysis of GAD-67-expressing neurons in adult ErbB4 knockout mice in the cortex (94) and in the hippocampus (50), showed a reduction of 50% and 30%, respectively. Interestingly, our double-immunolabeling experiments with markers for distinct GABAergic interneuron classes in the adult hippocampus showed that absence of ErbB4 results in the selective loss of parvalbumin- and nNOS-positive, but not CCK-positive interneurons. Because parvalbumin and nNOS interneurons are generated in the medial ganglionic eminence (MGE), in contrast

to CCK interneurons that arise from the lateral ganglionic eminence (LGE), our results suggest that NRG1-ErbB4 signaling is critical for GABAergic interneuron migration, differentiation and/or survival for neurons originating from the MGE, but not the LGE (132). As discussed below, reductions in the number or functionality of parvalbumin interneurons, that perisomatically innervate glutamatergic principal cells, have major effects on the power of gamma oscillatory network activity in the hippocampus of ErbB4 mutant mice in ways that are reminiscent of those reported for the loss of cortical gamma power in schizophrenia patients (see above). The effects of NRG-ErbB signaling on olfactory and cortical interneuron migration, differentiation and/or survival, and later neural network functions, represent an excellent example of how pathogenic processes during early development can underlie functional network deficits in the mature brain.

ErbB4 receptor is expressed in a subset of GABAergic interneurons—

Immunohistochemical analysis in the hippocampus of ErbB4 and markers that differentiate distinct GABAergic interneuron subtypes, indicate that ErbB4 is expressed in a substantial fraction of CCK-, PV-, and nNOS-immunoreactive interneurons in an area- and layer-specific manner, whereas ErbB4 expression in SOM-immunoreactive cells is negligible. Moreover, a selective reduction of nNOS- and PV-positive cells was observed in ErbB4 null mice, whereas the numerical densities of CCK- and SOM-positive cells showed no major changes (132). As discussed below, the expression of ErbB4 in distinct interneuron subtypes is important for understanding the direct regulatory effects of NRG1 on gamma oscillatory network activity and its implications for schizophrenia. Moreover, the area- and layer-specific loss of certain interneuron subtypes has been reported in the hippocampus of persons with schizophrenia. (13,144–146).

The NRG-ErbB signaling pathway regulates synaptic transmission and plasticity—

While the NRG-ErbB signaling pathway is known for its role in early developmental processes, some NRG ligands and its receptors continue to be expressed at high levels in the adult brain, suggesting that they have additional functions in the mature nervous system. One of these functions is synaptic plasticity, a cellular process believed to represent a substrate for cognitive processes such as learning and memory. Proteins associated with synaptic plasticity have been identified in numerous genetic screens for schizophrenia (see (1,63)) and hypotheses of circuit “dysconnectivity” in schizophrenia are based largely on the premise that imbalances in glutamate receptor-dependent plasticity result in associative learning failures that account for cognitive function impairments in schizophrenia (147). Long-term potentiation (LTP) vs. long-term depression (LTD) and depotentiation (i.e. LTP reversal) are opposing activity-dependent mechanisms that bidirectionally maintain glutamatergic synapses within a dynamic range. Depotentiation regulates the extent of bidirectional plasticity at glutamatergic synapses in adult freely moving rats and hippocampal slices (148–150).

NRG1 was recently shown to block the induction (127) and expression (117,128) of LTP at Schaeffer collateral to CA1 hippocampal synapses. Interestingly, NRG1 does not affect basal glutamatergic transmission but effectively reverses (depotentiates) LTP expression (Fig. 2); its effects are time- and activity-dependent manner. In addition, the pan ErbB-specific inhibitor PD158780 blocks LTP depotentiation by brief electrical stimuli that effectively reverse LTP in slices and in vivo, consistent with a role of endogenous NRG1 in this process (128). NRG1 depotentiates LTP in the hippocampus by promoting the internalization of AMPA type glutamate receptors (128). Interestingly, in medial prefrontal pyramidal neurons NRG1 was reported to reduce NMDA, and not AMPA, receptor currents (126). Given the dependence of NMDA receptor opening on AMPA receptor currents to overcome the voltage-dependent block by magnesium at neuronal resting potentials, these results support a major role of the NRG-ErbB signaling pathway in regulating glutamatergic

transmission at hippocampal and cortical synapses. These studies were the first to propose a plausible link of NRG1 signaling in glutamate hypofunction and its relevance to schizophrenia. Only one study, performed in cultured hippocampal organotypic slices, reported that NRG1 increases LTP and synaptic maturation at CA1 synapses on glutamatergic neurons (124). These results are difficult to reconcile with the lack of ErbB4 receptors in these neurons (130) (see above) and with the observation that LTP is enhanced in ErbB4 mutant mice (129). The weight of evidence instead favors a model in which NRG signaling via ErbB4 receptors suppresses induction and expression of LTP in the hippocampus (64,117,127–129,151) and diminishes glutamatergic transmission in cortical neurons (126).

NRG Effects on LTP and Glutamatergic Function Mediated Indirectly via Dopamine:

Dopamine is an important modulator of LTP and LTD at glutamatergic synapses throughout the brain (see (152)). Its function has been investigated mostly in the striatum and PFC, areas heavily innervated by dopaminergic fibers. Most studies in the hippocampus (see (152,153)), which receives diffuse inputs from the ventral tegmental area, have focused on the role of D1-type dopamine receptors (D1R and D5R). These receptors are positively coupled to adenylate cyclase and are required for the stabilization of late-phase LTP *in vitro* (154,155) and *in vivo* (156). However, the role of D2-type receptors (D2R–D4R), the major pharmacological targets of antipsychotics, in hippocampal synaptic plasticity remains largely unknown (see (152)). Interestingly, mice with reduced levels of NRG1, ErbB4 and NMDA receptor subunits share certain behavioral abnormalities, such as hyperactivity and impaired sensory inhibition (65,157). The antipsychotic clozapine reverses or ameliorates these behaviors, which suggested an involvement of dopamine signaling in mediating the responses of NRG1. These observations, combined with our work on the effects of NRG1 on plasticity at CA1 glutamatergic synapses, prompted us to investigate a possible functional link between NRG1/ErbB signaling, dopamine transmission and the regulation of early-phase LTP.

Consistent with a cross-talk between the NRG-ErbB and dopamine signaling systems, delivery of NRG1 (1 nM) using reverse microdialysis in freely moving rats causes a dramatic and rapid accumulation of dopamine and its metabolites in the dorsal hippocampus, and this increase is blocked by PD158780 (64); refer to Fig. 3. These results are consistent with an earlier report showing that supranigral injection of NRG1 (10 µg) induced striatal dopamine overflow (158), but that effect was not shown to be blocked by ErbB inhibition. The effects of NRG1 on dopamine release could be direct because, as we (102) and others (104) have shown, ErbB4 mRNA hybridization in rodents is high in the ventral tegmental area and substantia nigra; a similar pattern has been observed in primates (159).

What is the functional significance of cross-talk between NRG-ErbB and dopamine signaling? NRG1-induced increases in dopamine modify LTP at glutamatergic synapses by activating D4 receptors (64), a D2-type dopamine receptor that is negatively coupled to adenylate cyclase and a target for numerous antipsychotics (Fig. 4A). The effects of NRG1 on LTP depotentiation are blocked by clozapine (Fig. 4B), an antipsychotic that inhibits D4Rs, as well as other more selective D4R antagonists. Moreover, the effects of NRG1 on LTP are absent in hippocampal slices from D4R null mice. Conversely, direct D4R activation by selective agonists mimics the effects of NRG1 by reducing AMPA receptor currents and surface expression. Using double-immunofluorescence histochemistry, we found that while ErbB4 receptor protein was expressed on GABAergic interneurons, it was not detectable on dopaminergic processes in the hippocampus (64). This was an unexpected result because in earlier studies we detected strong ErbB4 mRNA expression in the ventral tegmental area (102), which sends afferent dopaminergic projections to the hippocampus (160,161). While we presently cannot exclude the possibility that ErbB4 is present on

dopaminergic axons but too low to detect, another possibility is that NRG1 promotes dopamine release by acting indirectly via GABAergic neurons.

The novel functional link between NRG1, dopaminergic and glutamatergic function is important, given the genetic association of *NRG-1* (65) and its receptor *ERBB4* (78) with schizophrenia, and the clinical pharmacological studies showing imbalances in glutamatergic and dopaminergic neurotransmission in this psychiatric disorder. These results also suggest that dopamine could modify neural network oscillatory activity, which is altered in schizophrenia and regulated by NRG/ErbB4 signaling (see below).

Regulation of neural network activity by the NRG-ErbB signaling pathway—

Alterations in network oscillatory activity, especially in the gamma frequency range, are implicated in psychiatric disorders but to date no schizophrenia susceptibility genes have been identified that modulate this network activity. Because polymorphisms in *NRG-1* and *ERBB4* are associated with schizophrenia, we investigated a possible role of this pathway in gamma oscillations using rat and mouse hippocampal slices (50). NRG1 dramatically increases kainate-induced gamma oscillation power in hippocampal slices. This effect is blocked by the ErbB receptor blocker PD158780 and entirely absent from ErbB4 null mice. Moreover, we demonstrated that 50% of GABAergic parvalbumin interneurons, which heavily contribute to the generation of gamma oscillations, express ErbB4. Importantly, both the number of parvalbumin interneurons and the power of kainate-induced gamma oscillations are reduced in ErbB4 null mice (132). These studies provided the first plausible link between NRG/ErbB4 signaling and rhythmic network activity that may be altered in persons with schizophrenia (50).

Consistent with the expression of ErbB4 receptor protein in interneurons and their functional relevance for network activity and importance for behavior, NRG1 promotes the depolarization-dependent release of GABA purportedly by activation of ErbB4 receptors located at presynaptic terminals of basket cells innervating prefrontal cortical pyramidal neurons (162). However, as discussed earlier, immunohistological evidence for presynaptic ErbB4 receptors at these synapses may need to be interpreted carefully due to concerns regarding antibody specificity (see (130)). While others have focused on the role of *presynaptic* ErbB4 receptors on parvalbumin-positive GABAergic interneurons for regulation of network activity and behavior (131,162), we favor an alternate hypothesis. We have proposed that *postsynaptic* ErbB4 receptors expressed on dendrites of parvalbumin-positive GABAergic interneurons, which receive glutamatergic inputs and exhibit the highest levels of ErbB4 immunoreactivity, are a major site for modulation neuronal network activity and excitatory/inhibitory balance by the NRG-ErbB4 signaling pathway (50,130,132). Consistent with this hypothesis, prior studies have shown that the selective ablation of the AMPA receptor GluR1 subunit at glutamatergic postsynaptic sites of parvalbumin-positive interneurons, as well as mutation of GluR4 which is selectively expressed by GABAergic neurons in the hippocampus, results in the reduction of kainate-induced gamma oscillation power (163); similar reductions in gamma power were observed in ErbB4 null mice (see above; (50)). In addition, GluR1 and GluR4 mutant mice, the former restricted to parvalbumin-positive GABAergic interneurons, exhibited behavioral impairments that suggested deficits in working and episodic memory (163). In another study that focused on the role of NMDA receptor transmission at glutamatergic synapses onto GABAergic neurons, Belforte et al. (22) found that elimination of the obligatory NR1 subunit in approximately 50% of cortical and hippocampal GABAergic interneurons resulted in reduced neuronal synchrony and disinhibition of cortical excitatory output. In these studies perinatal, but not adult, ablation of NR1 resulted in mice exhibiting “schizophrenia-like” behaviors and early postnatal stress worsened these behaviors (22). Studies of selective AMPA and NMDA receptor mutations in GABAergic neurons are consistent with the

hypoglutamatergic model of schizophrenia and bring together two neurotransmitter pathways associated with the disorder. Interestingly, selective elimination of ErbB4 in cortical parvalbumin interneurons, using floxed *ErbB4* mice expressing cre-recombinase under control of the parvalbumin promoter, exhibit behavioral deficits that are similar to those reported in NRG1 and ErbB4 heterozygous mice and that are associated with rodent behavioral models for schizophrenia ((164); Shamir and Buonanno, unpublished). While Wen et al. (164) have focused on the role of presynaptic ErbB4 receptors to account for these findings, we have proposed a role for *postsynaptic* dendritic ErbB4 receptors on parvalbumin-positive GABAergic interneurons in modulating glutamatergic drive onto these cells which consequently could account for the reduction in gamma oscillation power and “schizophrenia-like” behaviors observed in ErbB4 mutant mice (50,130,132). In this context it is interesting to note that changes in the activity of either AMPA, NMDA or ErbB4 receptors, which converge on this single type of synapse, could account for many of the neuronal network and cognitive behavioral deficits associated with schizophrenia.

SUMMARY

In this review I briefly introduce the ligands and receptors that comprise the Neuregulin/ErbB receptor (NRG-ErbB) signaling network and their temporal-regional expression in the maturing brain, but its main purpose is to outline why the NRG-ErbB signaling pathway represents an excellent model to study the possible pathogenic mechanisms that may underlie a complex psychiatric disorder like schizophrenia. The recent demonstration that the ErbB4 receptor is highly expressed in GABAergic interneurons, including parvalbumin- and CCK-expressing basket cells that tightly control firing of hippocampal and cortical pyramidal neurons, and that NRG signaling regulates dopaminergic and GABAergic transmission, glutamatergic synaptic plasticity and gamma frequency neural network oscillatory activity, makes this signaling pathway (as well as proteins that associate with it) an important plausible biological system that conceivably contributes to cognitive deficits and other symptoms associated with schizophrenia. While these studies begin to elucidate the complexities underlying the downstream effects of NRG action, further progress will require the convergence of basic neuroscience and molecular psychiatry to properly evaluate the proposed roles of this signaling pathway in the etiology of, and/or involvement in, schizophrenia and other psychiatric disorders.

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Figure 1. *NRG1* gene structure, splice variants and haplotypes associated with risk for schizophrenia

(A) The exon-intron structure of the human *NRG-1* gene locus. The location of the core at-risk NRG-1 HAP_{ice}, HAP_{Chinese} and HAP_{Portuguese} haplotypes, as well as the single nucleotide polymorphism SNP8NRG243177 and a missense mutation in the exon encoding the transmembrane domain (TM) identified in Costa Rica, are indicated. (B) Schematic view of differential promoters and splicing events underlying NRG1 isoform diversity (adapted with permission from (88)).

Figure 2. NRG-1 β elicits a rapid and pronounced increase of extracellular DOPAMINE
 Levels of dopamine were measured every 2 min before and after drug infusion using reverse microdialysis in the dorsal hippocampus. Basal levels represent the mean of 5 points preceding treatment (set as 100%), and time=0 indicates the onset of NRG-1 β infusion (*black bar*). Dopamine levels were measured before, during and after a 5-min infusion of 1 nM NRG-1 β (■). In a separate cohort, PD158780 (10 μ M) was continuously applied starting 10 min prior to NRG-1 β infusion (□). The control group (▲) received vehicle (ACSF). Adapted with permission from (64).

Figure 3. NRG-1 depotentiates LTP at SC-CA1 hippocampal synapses. A

) LTP was depotentiated rapidly by a 10min perfusion with 1nM NRG-1 β ($\circ$), while it was stable for the 1 hr recording period in vehicle-treated slices ($\bullet$). **B**) The antipsychotic clozapine (*black bar*), which preferentially targets D4R receptors, did not affect LTP on its own ($\bullet$) but completely blocked NRG-1 β (*blue bar*) - mediated depotentiation ($\circ$). Adapted with permission from (64).

Figure 4. Pharmacological activation of D4Rs depotentiates LTP at SC-CA1 hippocampal synapses

The selective D4R agonist PD168077 (0.2 μ M) rapidly depotentiates LTP (*gray bar*), in a manner that is indistinguishable from NRG-1 β ; depotentiation by PD168077 is blocked by specific D4R antagonists (not shown). Adapted with permission from (64).