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# A New Beginning for a Broken Mind: Balancing Neuregulin 1 Reverses Synaptic Dysfunction

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**In this issue of *Neuron*, Yin et al. (2013) demonstrate that overexpression of Neuregulin 1 causes synaptic dysfunction and schizophrenia-like behavioral deficits. These abnormalities can be reverted by restoring normal levels of Neuregulin 1, opening possibilities for the treatment of mental disease.**

Schizophrenia is a severe mental illness that affects approximately 1% of the population worldwide. Genetic factors play a major role in the etiology of schizophrenia, with an estimated heritability of around 80%. Genetic variation in *Neuregulin 1* (*NRG1*) has been repeatedly linked to the disorder in multiple human populations. In particular, more than 80 single nucleotide polymorphisms localized in noncoding regions of this gene have been identified (Mei and Xiong, 2008). These observations led to the hypothesis that expression of *NRG1* might be altered in schizophrenia.

Alternative splicing of *NRG1* generates more than 30 isoforms classified in six different types (I to VI) depending on their structure (Mei and Xiong, 2008). They all share an epidermal growth factor (EGF)-like domain, which is required for the activation of several members of the ErbB family of receptor tyrosine kinases. In schizophrenia, the expression of particular isoforms of NRG1 seems altered, although reports are contradictory. For example, several studies reported a reduction in the levels of the isoform 1 alpha of NRG1 in the brain of schizophrenia patients (e.g., Bertram et al., 2007), while others showed elevated levels of NRG1 in a particular risk haplotype (e.g., Weickert et al., 2012). Consistent with this later view, several other studies have shown increased NRG1 mRNA and protein levels in the hippocampus and prefrontal cortex of schizophrenia patients (Hashimoto et al., 2004; Petryshen et al., 2005). These contradictory findings have been surprisingly replicated in mice: both loss and gain of NRG1

function cause similar behavioral phenotypes, including impaired memory and hyperactivity (Deakin et al., 2012; Stefansson et al., 2002). Altogether, these observations suggest that unbalanced expression of specific *NRG1* isoforms might cause some of the synaptic deficits that are commonly associated with schizophrenia.

In this issue of *Neuron*, Yin et al. (2013) tested this hypothesis through a very elegant strategy based on the generation of mice in which NRG1 is selectively overexpressed in the brain. Using the tetracycline-off system (i.e., gene expression in the absence of doxycycline), Yin et al. (2013) generated mice in which overexpression of the type I NRG1 isoform (a cleavable, diffusible protein) can be specifically induced in pyramidal cells of the cerebral cortex and other neuronal populations of the forebrain, thereby mimicking the elevated levels found in schizophrenia patients. Consistent with previous reports based on a broad NRG1 overexpression model (Deakin et al., 2012), Yin et al. (2013) found that exacerbated NRG1 levels in the forebrain from early postnatal stages cause some key schizophrenia-related behavioral deficits, such as hyperactivity and impaired sensorimotor gating, social behavior, and cognitive function. Yin et al. (2013) found that these alterations are primarily due to glutamatergic hypofunction, as revealed by a pronounced reduction in field excitatory postsynaptic potentials in the Shaffer collateral pathway of the hippocampus.

Since type I NRG1 is secreted, overexpression of this protein could lead to glutamatergic hypofunction through both cell and non-cell-autonomous mechanisms. Using a comprehensive array of electrophysiological measurements, Yin et al. (2013) found that the defects in glutamatergic neurotransmission are probably due to impaired glutamate release rather than to postsynaptic defects. For example, no defects were found in the amplitude of miniature excitatory postsynaptic currents (mEPSCs), but both mEPSC frequencies and paired-pulse ratios were altered in mice with type I NRG1 overexpression. The effect of excessive NRG1 signaling on excitatory vesicle release seems to be mediated by changes in the cytoskeleton (Figure 1). They found that NRG1 overexpression increases the levels of synaptic LIM domain kinase 1 (LIMK1), which in turn phosphorylates and, as a result, inactivates cofilin, a protein that modulates vesicle fusion at the active zone through the regulation of the actin depolymerization (Arber et al., 1998; Morales et al., 2000). This is an important step forward in our understanding of the molecular mechanisms through which neuregulin signaling regulates glutamatergic neurotransmission in pyramidal cells.

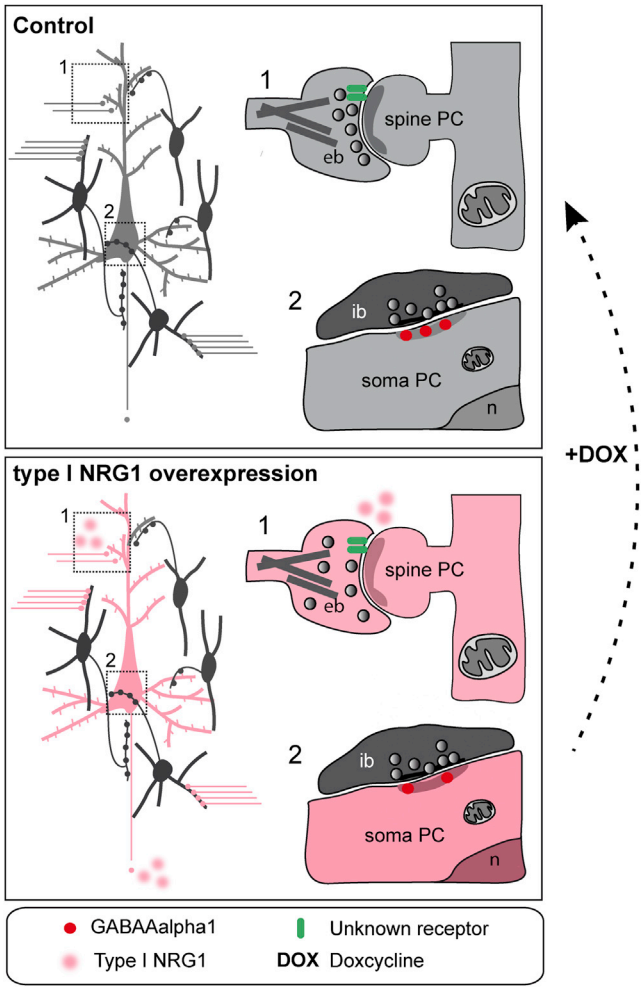


**Figure 1. Synaptic Dysfunction by Overexpression of Type I NRG1**
Overexpression of type I NRG1 in pyramidal cells (PCs) reduces mEPSC frequencies and prepulse pair ratios, which leads to decreased vesicle release in glutamatergic terminals (1). Overexpression of type I NRG1 also decreases the amplitude of mIPSC due to a reduction in the number of GABAAalpha1 receptors (2). DOX treatment (+DOX) in the adult reverts NRG1 to basal levels and restore synaptic function. eb, excitatory bouton; ib, inhibitory bouton; n, nucleus; PC, pyramidal cell.

Overexpression of NRG1 also alters GABAergic neurotransmission in the cortex but in a different manner. Yin et al. (2013) found no presynaptic abnormalities in GABAergic connections. However, they observed a decrease in the amplitude of miniature inhibitory postsynaptic currents (mIPSCs) and a reduction in expression of $GABA_A\alpha1$ receptors in pyramidal cells, both consistent with a postsynaptic defect in inhibitory neurotransmission in mice with type I NRG1 overexpression (Figure 1). These results are intriguing, because overexpression of type III NRG1 in pyramidal cells enhances the number of GABAergic synapses contacting these neurons (Fazzari et al., 2010). Thus, it seems that membrane-bound (type III) and diffusible (type I) isoforms of NRG1 differentially influence GABAergic circuits through the modulation of independent presynaptic and postsynaptic mechanisms, respectively.

The most plausible explanation for the differential effects of the various NRG1 isoforms in overexpression experiments is that they are mediated by different receptors. Consistent with this idea, Yin et al. (2013) found that the ErbB4 receptor does not mediate the defects found in pyramidal cells after overexpression of type I NRG1. Indeed, ErbB4 seems to be exclusively expressed by interneurons in rodents (Fazzari et al., 2010; Vullhorst

et al., 2009), and so the function of type I NRG1 must necessarily be mediated by another receptor in pyramidal cells. However, how is it possible that overexpression of type I NRG1 in the postnatal cortex does not directly affect GABAergic interneurons? This isoform of NRG1 plays an important role in the guidance of interneurons during embryonic development, in a process that is also dependent on ErbB4 function (Flames et al., 2004), and so interneurons are in principle "geared up" to respond to this signal. One possibility is that binding of type I NRG1 to interneurons requires an additional partner that is not present in the postnatal cortex. Alternatively, the formation of inhibitory synapses might be exclusively dependent on a membrane-bound NRG1 isoform. Consistent with this idea, expression of the diffusible type I NRG1 isoform is very low in postnatal cerebral cortex (Fazzari et al., 2010), which suggests that this protein is unlikely to play a major role in the normal process of synaptogenesis. At any rate, these various pieces of evidence suggest that different isoforms of NRG1 play different roles at different stages of development and in the adult, and so the temporal and spatial regulation of their expression might be key for activation of specific signaling pathways.

The beauty of the approach followed by Yin et al. (2013) is that their genetic model allowed them to address two additional questions related to the key issue of the temporal regulation of NRG1 expression. First, they asked whether the timing of overexpression influences the behavioral outcome. To this end, they treated their transgenic mice with doxycycline during the first 8 postnatal weeks, which effectively delayed the abnormal rise in NRG1 levels until 11 weeks of age. They found that overexpression of type I NRG1 in young adult mice is sufficient to induce excitatory synaptic defects and equivalent behavioral deficits to those found in mice with continuous overexpression of NRG1 since the first postnatal days. These results demonstrate that the effect of NRG1 overexpression is not necessarily dependent on an early role of this protein during development.

Second, they investigate the reversibility of the behavioral deficits elicited by the overexpression of NRG1 through the restoration of its normal levels. In brief, Yin et al. (2013) treated transgenic mice displaying behavioral deficits due to overexpression of type I NRG1 with doxycycline, which effectively turned off the ectopic expression of this protein. Surprisingly, Yin et al. (2013) found that bringing back type I NRG1 to their endogenous levels in adult mice restores normal synaptic function in glutamatergic axon terminals and rescues the behavioral abnormalities (Figure 1). These results are conceptually important, because they add further support to the idea that therapeutic interventions in the adult brain may restore synaptic function, as previously suggested for animal models of fragile X and Rett syndromes (Dölen et al., 2007; Guy et al., 2007).

In sum, the results of the experiments carried out by Yin et al. (2013) indicate that overexpression of type I NRG1 may cause schizophrenia-related behavioral deficits in mice. It should be noted, however, that both increased and decreased NRG1 signaling has been reported in patients with schizophrenia (Bertram et al., 2007; Hashimoto et al., 2004; Petryshen et al., 2005). In addition, increased expression of the CYT-1 isoform of the ERBB4 receptor in humans carrying polymorphisms in this gene causes a counterintuitive downregulation of this signaling pathway (Law et al., 2012). Thus, it is conceivable that function of NRG1 signaling in the cerebral cortex depends on a delicate balance in the expression of the different NRG1 isoforms and their receptors. An alteration of their normal expression patterns, in any direction, may disturb cortical functioning and lead to similar behavioral abnormalities. Understanding the precise function of each of the different isoforms of NRG1 and their receptors may shed light into this process. The development of new animal models based on these discoveries, and their subsequent analysis, should also fuel discoveries in this area and unravel potential therapeutic targets.

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