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Amy Geddes
University of Wollongong

Xu-Feng Huang
University of Wollongong, xhuang@uow.edu.au

Kelly A. Newell
University of Wollongong, knewell@uow.edu.au

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Schizophrenia is a debilitating neurodevelopmental psychiatric disorder. Both the N-methyl-D-aspartate receptor (NMDAR) and neuregulin1 (NRG1) are key molecules involved in normal brain development that have been linked to schizophrenia pathology and aetiology. The NR2 proteins are critical structural and functional subunits of the NMDAR and are developmentally and spatially regulated. Altered NR2 gene and protein expression has been found in human post-mortem schizophrenia brain tissue together with changes in NRG1 and its receptor ErbB4. The NR2 subunits and ErbB4 share a common anchoring domain on the postsynaptic density and therefore a disruption to either of these molecules may influence the functioning of the other. It has been shown that NRG1 signalling can affect NMDAR levels and function, particularly phosphorylation of the NR2 subunits. However little is known about the possible effects of NMDAR dysfunction on NRG1 signalling, which is important with regards to schizophrenia aetiology as numerous risk factors for the disorder can alter NMDAR functioning during early brain development. This review focuses on the role of the NMDA receptor subunits and NRG1 signalling in schizophrenia and proposes a mechanism by which a disruption to the NMDAR, particularly via altering the balance of NR2 subunits during early development, could influence NRG1 signalling.

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Reciprocal signalling between NR2 subunits of the NMDA receptor and Neuregulin1 and their role in schizophrenia

Amy E Dawson[a,b], Kelly A Newell[a,b], Xu-Feng Huang[a,b,*]

a: Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, NSW 2522, Australia
b: Schizophrenia Research Institute, Darlinghurst, 2010, NSW, Australia

***** Corresponding author: Professor Xu-Feng Huang, University of Wollongong, School of Health Sciences, Northfields Ave, Wollongong, NSW 2522, Australia. Fax +61 2 4221 4300. Email xhuang@uow.edu.au.

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Abstract

Schizophrenia is a debilitating neurodevelopmental psychiatric disorder. Both the N-methyl-D-aspartate receptor (NMDAR) and neuregulin1 (NRG1) are key molecules involved in normal brain development that have been linked to schizophrenia pathology and aetiology. The NR2 proteins are critical structural and functional subunits of the NMDAR and are developmentally and spatially regulated. Altered NR2 gene and protein expression has been found in human post-mortem schizophrenia brain tissue together with changes in NRG1 and its receptor ErbB4. The NR2 subunits and ErbB4 share a common anchoring domain on the postsynaptic density and therefore a disruption to either of these molecules may influence the functioning of the other. It has been shown that NRG1 signalling can affect NMDAR levels and function, particularly phosphorylation of the NR2 subunits. However not much is known about the possible effects of NMDAR dysfunction on NRG1 signalling, which is important with regards to schizophrenia aetiology as numerous risk factors for the disorder can alter NMDAR functioning during early brain development. This review focuses on the role of the NMDA receptor subunits and NRG1 signalling in schizophrenia and proposes a mechanism by which a disruption to the NMDAR, particularly via the altering the balance of NR2 subunits during early development, could influence NRG1 signalling.

Section: Disease-Related Neuroscience

Keywords: NMDA receptor; NR2 subunit; Neuregulin1; Schizophrenia; ErbB4

Abbreviations:

NMDA-R	N-methyl-D-aspartate receptor
NR2A	2A subunit of the NMDA receptor
NR2B	2B subunit of the NMDA receptor
NRG1	Neuregulin 1
PSD-95	Post-synaptic density protein 95
ED	Embryonic day
PN	Postnatal day
PPI	Prepulse inhibition
PCP	Phencyclidine
TM	Transmembrane
EGF	Epidermal growth factor
TACE	Tumor necrosis factor-α converting enzyme
BACE	β-site of amyloid precursor protein cleaving enzyme
ErbB4	v-erb-a erythroblastic leukemia viral oncogene homolog 4
SNP	Single nucleotide polymorphism
DLPFC	Dorsolateral prefrontal cortex
PDZ	Post-synaptic density 95, Drosophila disc large tumor suppressor, Zonula occludens-1
Pyk2	Proline-rich tyrosine kinase 2
Fyn	Proto-oncogene tyrosine-protein kinase Fyn

1. Introduction

Schizophrenia is a complex mental disorder that is thought to arise from an interaction of genetics and environment, with its origins in early neurodevelopment. A considerable amount of research has focused on the role of the ionotropic glutamate receptor, N-methyl-D-aspartate (NMDAR), in the pathogenesis of schizophrenia, since the discovery several decades ago that antagonists of this receptor, such as phencyclidine (PCP) and ketamine, mimic symptoms of the disorder in humans and exacerbate symptoms in schizophrenia patients (Olney et al., 1999). Not only do these NMDAR antagonists induce schizophrenia-like symptoms in humans, they have also been shown to induce schizophrenia-like symptoms in animals, as well as neural circuitry changes reminiscent of schizophrenia. The ability of these NMDA receptor antagonists to induce this schizophrenia-like phenotype in humans and animals supports the concept that schizophrenia may be the result of reduced or abnormal functioning of the NMDAR.

Schizophrenia is widely believed to result from disruptions early in brain development. The NMDAR, which plays a crucial role in brain development (du Bois and Huang, 2007), can be affected during early brain development by various factors including drug abuse during pregnancy, maternal stress or malnutrition, neonatal anaesthetic agents and gene mutations, which have all been associated with or suggested to increase the risk of developing schizophrenia (Ikonomidou et al., 2001, Abel et al., 2008, Shen et al., 2008). Various animal studies have been conducted using perinatal NMDAR antagonist treatment to further investigate the possible underlying neurobiology of schizophrenia (For review see du Bois and Huang, 2007). Long-term behavioural effects such as enhanced locomotion (Facchinetti et al., 1993,

Harris et al., 2003), impairments in cognitive function as examined by spatial working memory, cognitive set-shifting, and other learning tests (Sircar, 2003, Andersen and Pouzet, 2004, Fredriksson et al., 2004, Stefani and Moghaddam, 2005) and deficits in prepulse inhibition (Harris et al., 2003) are observed in adult rats and mice following perinatal NMDAR antagonist treatment. These behavioural changes mimic certain aspects of human schizophrenia and support the involvement of the glutamatergic system in schizophrenia-like symptoms. In addition to these behavioural changes, animals treated with NMDAR antagonists during the perinatal period have shown alterations in the brain which are similar to features of post-mortem schizophrenia tissue (Wilson et al., 1998, Wang et al., 2001, Sircar and Soliman, 2003, Wang et al., 2008, du Bois et al., 2009). As well as the many proposed environmental factors that increase the risk of schizophrenia, various family, twin and adoption studies have provided strong support for genetic involvement in the pathogenesis of the disorder (Pulver, 2000, Maynard et al., 2001), however the inheritance appears to be in a complex polygenetic and non-Mendelian manner (Risch and Baron, 1984). Genetic linkage analyses have identified several loci that are associated with the disorder (Berry et al, 2003) further suggesting that no single genetic mechanism can be responsible for the aetiology of schizophrenia. More likely, it may be that a range of interacting genetic and environmental factors contribute to an increased vulnerability of developing the disorder (Maynard et al., 2001, Lewis and Levitt, 2002).

Neuregulin 1 (NRG1) has been identified as one of the leading candidates for a schizophrenia susceptibility gene (Stefansson et al., 2002, Williams et al., 2003, Bakker et al., 2004, Fukui et al., 2006, Munafo et al., 2006). NRG1 signalling is important for early neural development as well as the regulation of neurotransmission, especially glutamatergic and GABAergic signalling, providing further evidence for an

involvement in the pathophysiology of schizophrenia (Mei and Xiong, 2008). The NR2 subunits of the NMDAR and the ErbB family of receptors for NRG1 share a common anchoring protein in the post synaptic density (Garcia et al., 2000, Lin et al., 2004) and thus it is expected that with this structural connection a disturbance to either signalling pathway would produce alterations of the other. This review will focus on the role of NMDARs and NRG1 in schizophrenia, with a particular focus on the interaction between the NR2 subunits and NRG1 during early development. This review will present evidence supporting the reciprocal nature of the NR2/NRG1 interaction and the consequences for schizophrenia aetiology.

2. NMDA Receptors: Structure, Function and Distribution

NMDARs are composed of a heterometric assembly of subunits where at least one obligatory NR1 subunit combines with distinct arrangements of NR2 and/or NR3 subunits. To add to the complexity of these receptors, there are eight different splice variants of the gene encoding for the NR1 subunit and six other genes that encode for different forms of the NR2 (NR2A, NR2B, NR2C, NR2D) and NR3 (NR3A, NR3B) subunits, producing a multitude of different NMDAR's (Paoletti and Neyton, 2007). This formation of NMDAR's gives rise to a diverse range of functional and pharmacological properties (Sheng et al., 1994, Flint et al., 1997, Kristiansen et al., 2007).

The extracellular N-terminal domain of the NR2 subunits contains the binding site for glutamate, the primary activator of the NMDAR, while the NR1 subunit binds glycine, a co-activator. Inside the cell, the C-terminal region of the NR2 subunits is anchored to PDZ (Post-synaptic density 95, Drosophila disc large tumor suppressor, Zonula occludens-1)-containing scaffold proteins in the postsynaptic density (PSD) including PSD-95, synapse associated protein (SAP)102, PSD-93 and SAP97, with

PSD-95 being the most studied (Kornau et al., 1995, Muller et al., 1996, Niethammer et al., 1996, Bassand et al., 1999, Sans et al., 2000, Chen et al., 2006, Sato et al., 2008, Cousins et al., 2009). These proteins form the DLG (disks large homolog) subfamily of the membrane-associated guanylate kinases (MAGUKs), which have three PDZ domains, a src homology 3 (SH3) domain and a guanylate kinase domain (GUK) (Montgomery et al., 2004). Due to their close positioning to the postsynaptic membrane these proteins are involved in critical processes during synaptogenesis and synapse maturation including localisation, trafficking and organisation of many membranous and cellular proteins (Sheng and Hoogenraad, 2007, Elias et al., 2008, Howard et al., 2010).

The NR2 subunits of the NMDA-R are developmentally and spatially regulated, providing an important level of receptor regulation. (Herin and Aizenman, 2004, Mueller and Meador-Woodruff, 2004). Animal studies have revealed that the NR2B and NR2D subunits emerge early in development with NR2B and NR2D mRNA present prenatally in the rodent brain, as early as embryonic day 14, while the NR2A and NR2C mRNAs are not detectable until at or after birth (Watanabe et al., 1992, Monyer et al., 1994, Sheng et al., 1994). Similarly NR2B protein is already widely and strongly expressed by birth with very little expression of NR2A and NR2C protein at postnatal day zero (Portera-Cailliau et al., 1996, Wenzel et al., 1997, Sans et al., 2000, Liu et al., 2004). The expression pattern of the PSD and SAP proteins mirrors that of the NR2 subunits with the SAP102 and SAP97 proteins present early in development and involved in synaptogenesis while PSD-95 and PSD-93 emerge later during synapse maturation (Sans et al., 2000, Elias et al., 2008, Howard et al., 2010). There has been evidence to suggest that certain NR2 subunits preferentially associate with one or another of the DLG proteins for example, one

study has shown that NR2A has a slight but not complete preference for PSD-95 and NR2B a preference for SAP102 (Sans et al., 2000). On the other hand it has been found that the number of binding domains for PSD-95 on the NR2B subunit is more than that of the NR2A subunit and thus suggested that NR2B may be more closely associated to PSD-95 (Cousins et al., 2009).

Throughout postnatal development, expression of NR2A mRNA and protein increases to a peak in all brain areas at around the third postnatal week and then a slight reduction in the adult rodent brain (Monyer et al., 1994, Sheng et al., 1994, Zhong et al., 1995, Wenzel et al., 1997, Guilarte and McGlothan, 1998, Ritter et al., 2002). There is a pronounced increase in NR2B expression, particularly protein levels, during the first week in most brain regions (Monyer et al., 1994, Wenzel et al., 1997, Ritter et al., 2002). However there appears to be conflicting results of NR2B expression from this point until adulthood with some studies showing cortical NR2B expression remains constant from early development to adulthood (Sheng et al., 1994, Zhong et al., 1995, Portera-Cailliau et al., 1996) while others have found a decrease in NR2B levels, between PN21 and adulthood (Monyer et al., 1994, Wenzel et al., 1997). There has also been a reported decrease in NR2B mRNA levels in the hippocampus between PN5 to PN35 with an opposing increase occurring in NR2A levels (Guilarte and McGlothan, 1998, Ritter et al., 2002). The most prominent feature of NR2B expression during development is the confinement to forebrain areas in adulthood, opposing that of the NR2A subunit which is increasingly expressed in all brain regions throughout development (Watanabe et al., 1992, Portera-Cailliau et al., 1996, Wenzel et al., 1997). Forebrain areas, in particular the prefrontal cortex, striatum and hippocampus, are closely associated with schizophrenia pathology as the

neural circuits contained within and between these regions play a central role in eliciting the positive, negative and cognitive symptoms that are experienced by patients with the disorder (Heimer, 2000). Therefore this regional expression of NR2 subunits, may play an important role in schizophrenia symptoms.

The expression of NR2C mRNA and protein in the rodent brain is fairly weak until the third postnatal week when there is a dramatic increase in the cerebellum and thalamus (Monyer et al., 1994, Zhong et al., 1995, Wenzel et al., 1997). This increase in NR2C levels correlates with a decrease in NR2B levels in the cerebellum at this time suggesting a switch in the subunit expression (Monyer et al., 1994). Compared to the other subunits NR2D expression peaks earlier in development, around PN7, but is restricted to regions such as the midbrain and thalamus (Monyer et al., 1994).

A similar pattern of NR2 mRNA and protein expression has been observed in a study of the human foetal brain, where NR2B is the predominant subunit expressed in all of the cortical layers as early as gestational week 8 with transient increases at week 11, 13 and 19 (Ritter et al., 2001). Moreover, there is a decline in NR2B mRNA in the human hippocampus from its peak expression during the neonatal stage of life to adulthood, whereas NR2A mRNA expression appears to remain consistent throughout the life stages (Law et al., 2003). Interestingly, the adult levels of NR2A and NR2B mRNA in each subregion of the hippocampus are quite similar, with the exception of the dentate gyrus where NR2B mRNA levels remain significantly higher than NR2A at each point in development (Law et al., 2003).

The developmental expression of the NR1 subunit has also been considered with most studies indicating a slight increase from embryonic stages until the third postnatal week, similar to the NR2 subunits, and then a plateau effect to adulthood (Watanabe et al., 1992, Monyer et al., 1994, Sheng et al., 1994). However one study showed that the NR1 splice variants without the 5' insert and with or without both the 3' inserts had a much higher expression in the cortex and hippocampus throughout development, while in the cerebellum, the NR1 splice variant without the 5' insert dominated early in development but with the 5' insert and without the 3' insert was highest at adulthood (Zhong et al., 1995). Therefore, contrary to popular belief, there may also be some developmental and spatial regulation of the splice variants of the NR1 subunit.

3. NMDA Receptor subunits and Schizophrenia

Several studies have found alterations in the level of NR1 and NR2 mRNA and protein, as well as the proteins in the PSD, in schizophrenia subjects in comparison to control subjects (See Table 1). For example, NR1 mRNA appears to be decreased in the hippocampus and thalamus and increased in the occipital and anterior cingulate cortices (Gao et al., 2000, Ibrahim et al., 2000, Dracheva et al., 2001, Kristiansen et al., 2006). However these results vary in terms of the specificity of the probe used to detect the mRNA with some studies using pan probes while others are specific to certain splice variants of NR1. There are alterations in NR2 subunit mRNA in the prefrontal cortex, including an increase in NR2D and a reduction in NR2A and NR2C levels (Akbarian et al., 1996, Beneyto and Meador-woodruff, 2008). NR2B subunit mRNA has been found to be increased in the hippocampus (Gao et al., 2000) while NR2B subunit protein has been found to be selectively upregulated in the

superior temporal cortex (Grimwood et al., 1999). These findings show significant changes however there are also studies showing no changes in the same brain regions (See Table 1). Clinton and colleagues have found that the use of an elderly versus a younger cohort of samples can influence direction of change in the subunits. The PSD and SAP proteins appear to be particularly vulnerable to age with opposite changes in both the prefrontal cortex and thalamus depending on whether the cohort is elderly or younger aged (Clinton et al., 2003, Clinton and Meador-Woodruff, 2004, Clinton et al., 2006). Therefore due to the inconsistent findings, which could be attributed to differences in tissue cohorts and methodologies, these results must be interpreted with caution.

Furthermore alterations in associated transporter and regulatory proteins of the NMDAR subunits has been reported in schizophrenia, particularly associated with the NR2B subunit (Kristiansen et al., 2010). For example, molecules in the NR2B trafficking complex are dysregulated in schizophrenia patients, with an increase in CASK and mLin7C mRNA in the prefrontal cortex and an increase in mLin7A and APBA mRNA and decrease in CASK and mLin7C protein in the anterior cingulate cortex (Kristiansen et al., 2010).

TABLE 1 is about here.

While human post-mortem data supports that NMDARs and their subunits are altered in schizophrenia, whether they be increased or decreased in brain regional specific manner, animal studies have and will continue to assist in unravelling the functional and developmental significance of those alterations in schizophrenia patients. There are a number of animal studies that have examined the role of a non-

specific NMDAR blockade on behaviour and neurochemical alterations in the rodent, however, there is currently limited animal data available with regard to a blockade of the specific subunits. A small number of behavioural studies have been conducted in adult rodents using various NR2 antagonists, primarily NR2A and NR2B, or genetically modified mice. Studies have shown that Ro 25-6981, an NR2B antagonist, produced hyperlocomor activity (20mg/kg), disrupted prepulse inhibition (PPI) (5-20mg/kg), an increase in a measure of anxiety-like behaviour (10mg/kg) and an impairment in the acquisition of fear memory (10mg/kg) in adult rats, which are well recognised as schizophrenia-like behaviours in the rodent (Chaperon et al., 2003, Mathur et al., 2009). However there have also been reports of a lack of behavioural changes in rodents treated similarly (Kosowski and Liljequist, 2004, Mathur et al., 2009). Adult rats treated with a more potent and selective NR2B antagonist, Ro 63-1908, displayed increased locomotor activity (10-30mg/kg) and impaired response inhibition (1-10mg/kg) but no disruption to PPI (1-10mg/kg) (Higgins et al., 2003). A recent study has shown that there are deficits in both spatial and non-spatial memory following a forebrain-specific deletion of NR2B, whereas hippocampal-restricted deletion of NR2B induces a selective working memory deficit (von Engelhardt et al., 2008). Mice with a complete knockout of NR2B die shortly after birth and therefore cannot undergo behavioural analysis.

On the other hand, blockade of the NR2A subunit with NVP-AAM077 has been reported to reduce locomotor activity (5-10mg/kg) and lack a disruption of PPI (5-20mg/kg) (Chaperon et al., 2003), findings supported by NR2A knockout mice (Spooren et al, 2004). In another study NR2A knockout mice also exhibited reduced anxiety and depression-like behaviour (Boyce-Rustay and Holmes, 2006). Although

these studies show behavioural abnormalities that mirror schizophrenia symptoms with NR2B antagonists in adult animals, although they are not to the same extent as those observed with non-selective NMDAR antagonists. Due to the expression pattern of the NR2 subunits in early development and the relevance of the developmental neurobiology of schizophrenia, NR2 antagonist treatment may need to be further examined in a perinatal model in order to verify its relevance to the etiology of schizophrenia.

Anastasio and colleagues (2009) have investigated the behavioural and cellular effects of perinatal NR2A and NR2B antagonism. They reported enhanced apoptosis following perinatal (PN7, 9 and 11) NR2A antagonist treatment (10 and 20mg/kg PEAQX) in addition to a locomotor sensitisation effect to a phencyclidine (PCP) challenge, while perinatal NR2B antagonist treatment (1 or 5mg/kg ifenprodil) did not produce elevated apoptosis or a locomotor sensitisation to PCP (Anastasio et al., 2009). The NR2B antagonist (ifenprodil) used, is not as potent or specific to the NR2B subunit as Ro 63-1908 (Zhou et al., 1999) in which doses of up to 30mg/kg have been used in adult rodents. Therefore, this dose of ifenprodil may not be high enough to see any behavioural effects and hence there remains the possibility that NR2B-induced apoptosis does occur. While the apoptotic effect might be related to the NR2A-containing NMDAR's, this may leave an imbalance of NR2 subunits (possibly via a responsive upregulation of NR2A subunits and hence a relative decrease in NR2B) which may cause behavioural and neurochemical abnormalities reminiscent of schizophrenia.

Changes in PSD-95 and NMDAR subunit expression have been observed following NMDAR antagonist treatment. One study found that acute treatment with the non-competitive antagonist PCP, increased the expression of membrane NR2B protein and decreased the level of NR2B protein in the endoplasmic reticulum on PN7 in the frontal cortex of the rat brain (Anastasio and Johnson, 2008). This was suggested to be due to an increase in the trafficking of the NR2B subunit to the membrane. This short-term upregulation of NR2B was accompanied by an increase in the level of PSD-95 but not the NR2A subunit (Anastasio and Johnson, 2008). On the other hand subchronic treatment with PCP resulted in an elevated membrane and endoplasmic reticulum level of NR2A but not NR2B or PSD-95 which has been attributed to an increase in synthesis of NR2A protein (Anastasio and Johnson, 2008). Following an 8 hour NMDAR blockade an increase in the insertion of new NR2A subunit containing NMDA receptors has also been found in hippocampal cell cultures, however no change in the NR2B subunit (von Engelhardt et al., 2009). Another study found that NR2A mRNA was significantly increased in the striatum, cortex and hippocampus 4 hours following MK-801 treatment to rats on PN7 (Wilson et al., 1998). It has been hypothesised that this imbalance of the NMDA-R subunits, particularly the disproportionate NR2A/NR2B ratio, after treatment with NMDAR antagonists causes an acceleration of the normal NMDAR development processes (Wilson et al., 1998). This disproportionate development of the NMDAR subunits and the consequent alteration in NMDAR function (Kristiansen et al., 2007) could trigger a cascade of modifications to other molecules such as those in the NRG1/ErbB4 pathway and hence, render the brain more susceptible to schizophrenia onset later in life.

4. NRG1/ErbB4: Structure, Function and Distribution

NRG1 belongs to a family of genes encoding for growth/differentiation factors that exert their effects on various organs, including the brain, by activating the ErbB tyrosine kinase receptors (Garcia et al., 2000). The NRG1 gene transcripts can be classed as six types and comprise over 31 different isoforms of NRG1 in total due to the presence of multiple promoter regions and alternative splicing of the gene sequence (Falls, 2003, Harrison and Law, 2006, Mei and Xiong, 2008).

NRG1 is synthesised in a membrane-bound form, often referred to as pro-NRG1, a precursor to the mature NRG1 signalling protein that is generated from the proteolytic cleavage at the extracellular transmembrane (TM) region . The release of the soluble extracellular portion (inclusive of the epidermal growth factor (EGF) domain) of NRG1 occurs in most isoforms, with the exception of type III which remains in contact with the cell as both the N-terminal and C-terminal of the protein are located intracellularly (For review see Mei and Xiong, 2008). The cleavage is catalysed by the proteases tumor necrosis factor-α converting enzyme (TACE), β-site of amyloid precursor protein cleaving enzyme (BACE) and meltrin β, and leads to the diffusion of mature NRG1 into the extracellular space for functional purposes (Hu et al., 2006, Yokozeki et al., 2007, Mei and Xiong, 2008).

The pattern of expression of NRG1 mRNA and protein in the rodent CNS during development varies between the different isoforms. Type I mRNA is predominant during early embryogenesis whereas type II and III mRNA are not detected until midgestation but display a broader expression pattern in the brain (Meyer et al., 1997). Similarly NRG1 type I protein is expressed early in embryonic development with a strong distribution already present in the cortical plate, piriform

cortex, septum, putamen and basal telencephalon by embryonic day 17 (Pinkas-Kramarski et al., 1994). Later in development the distribution of NRG1 transcripts and protein is widespread throughout the rodent brain, mirroring that seen in the adult human brain (Law et al., 2004), however the expression remains in a distinct nuclei and cortical layer specific manner (Meyer and Birchmeier, 1994, Pinkas-Kramarski et al., 1994).This differential expression indicates that each isoform may have a distinct functional role during development (Meyer et al., 10997). ErbB4 is the predominant receptor for NRG1 in neurons (Bjarnadottir et al., 2007) with emerging evidence showing a preferential location on parvalbumin-containing GABAergic interneurons (Fazzari et al., 2010). Alternative splicing of ErbB4 transcripts is known to generate at least four isoforms made up of different combinations of juxtamembrane (JMa and JMb) and intracellular regions (CYT1 and CYT2), which consequently are coupled to distinct signalling pathways inside the cell (For review see Mei and Xiong, 2008). The activation of ErbB receptors by NRG1 results in the formation of homodimers or heterodimers of the various receptor subtypes (ErbB1-4) and the phosphorylation of the intracellular kinase domain. These phosphorylated tyrosine residues are protein binding sites for downstream signalling molecules and enzymes that can have varying effects on the regulation of transcriptional and translational factors in the cell (Mei and Xiong, 2008).

5. NRG1/ErbB4 and Schizophrenia

Genetic linkage studies in several populations have provided strong evidence of NRG1 as a susceptibility gene for schizophrenia (Stefansson et al., 2002, Williams et al., 2003, Bakker et al., 2004, Fukui et al., 2006, Munafo et al., 2006) with multiple associated single nucleotide polymorphisms (SNP's) located in the 5` and 3` regions

of the NRG1 gene (Mei and Xiong, 2008). Additionally, researchers have found altered levels of NRG1 subtypes in post-mortem brain tissue of schizophrenia patients (See Table 1). In particular, upregulation of NRG1 type I mRNA has been observed in the dorsolateral prefrontal cortex and hippocampus (Hashimoto et al., 2004, Law et al., 2006) and elevated NRG1 and ErbB4 protein levels have been found in the prefrontal cortex of schizophrenia patients (Chong et al., 2008). Furthermore, an association between a SNP in the at-risk haplotype of NRG1 and altered NRG1 transcript expression in the brain has been reported (Law et al., 2006).

An association between the ErbB4 gene and schizophrenia has also been reported. The identification of single SNP's in the non-coding, intronic region of the candidate gene and the increased disease risk for individuals who carry these SNP's has lead researchers to investigate the possible functional consequences of these genetic variations (Norton et al., 2006, Silberberg et al., 2006, Law et al., 2007). Indeed it has been shown that certain splice-variants of ErbB4 mRNA's are altered in the brains of schizophrenia patients (Law et al., 2007). CYT-1 and JM-a isoforms of ErbB4 are increased in the dorsolateral prefrontal cortex (DLPFC) compared to controls and interestingly a positive correlation has been found between the abnormal expression of these isoforms and the various risk SNP's for schizophrenia (Silberberg et al., 2006, Law et al., 2007).

Several genetic knockout animal studies have also highlighted the potential role of NRG1 in brain and behavioural abnormalities that are related to schizophrenia. Results have revealed that TM-domain NRG1 heterozygous mutant mice display hyperlocomotion, increased aggressive and exploratory behaviours, social interaction deficits and have impaired PPI compared to wild type mice when tested at adulthood (Stefansson et al., 2002, Karl et al., 2007, O'Tuathaigh et al., 2007, O'Tuathaigh et al.,

2008). Chen and colleagues (2008) recently reported that adult mutant mice heterozygous for type III NRG1 also show disrupted PPI as well as impairments in working memory tasks, but no change in locomotor activities. These behavioural alterations are similar to those observed with NMDAR hypofunction in rodents and are also representative of schizophrenia symptomology. While these animal models support the possibility that genetic mutations in NRG1 could contribute to schizophrenia symptomology, as stated by Li and colleagues (2007), mutations in NRG1 genes are only likely to account for a fraction of schizophrenia cases. In other cases, primary dysfunction in other genes/molecules (eg glutamate) may lead to disrupted development of the NRG1 system and subsequently schizophrenia symptomology.

6. NR2 and NRG1 interactions

The NR2 subunits of the NMDAR and the ErbB4 receptor share a common anchoring region on the PDZ2 (Post synaptic density 95, Drosophila disc large tumor suppressor, and Zonula occludens-1) domain of PSD-95 and other molecules in the PSD and through this commonality it is thought that interactions occur (Garcia et al., 2000) (Fig. 1).

FIGURE 1 is about here.

There have been various reports of NMDAR modulation by NRG1 signalling. Firstly, it has been shown that NRG1 EGF-domain heterozygous mutant mice display a reduction in MK-801 binding in the prefrontal cortex, suggestive of reduced NMDAR expression (Stefansson et al., 2002). Other studies have found that NRG1

stimulation in the prefrontal cortex enhances the association of ErbB4 with both PSD-95 and the NR1 subunit in schizophrenia patients and causes a reduction in NR2A phosphorylation and NMDAR activation and an increase in NMDAR internalisation (Gu et al., 2005, Hahn et al., 2006). Highlighting a regional difference in signalling pathways, the disruption of NRG1-mediated ErbB4 stimulation in the hippocampus, also results in the loss of NMDARs (Li et al., 2007). Application of NRG1 to cultured hippocampal neurons does not affect the mRNA expression levels of the NR2 subunits (Okada and Corfas, 2004) however, it has been found that NRG1 signalling in the hippocampus enhances phosphorylation of the NR2B subunit, concurrent with ErbB4 phosphorylation and the activation of Fyn and Pyk2 (proline-rich tyrosine kinase 2), two non-receptor tyrosine kinases thought to be involved in the downstream amplification of NRG1 signalling (Bjarnadottir et al., 2007). NR2B was also found to be hypophosphorylated in the hippocampus of TM-domain $NRG1^{+/-}$ mutant mice, as well as in $ErbB4^{+/-}$ mutant mice, which can lead to reduced channel opening and functioning of the NMDAR, but the hypophosphorylation was reversed with clozapine treatment (Moghaddam, 2003, Bjarnadottir et al., 2007). Interestingly, clozapine has also been shown to increase NR2B-containing NMDAR currents in the nucleus accumbens (Wittmann et al., 2005). From these findings it has been speculated that attenuated NRG1 signalling in the hippocampus may cause abnormal modulation of NMDAR function through the altered regulation of NR2B phosphorylation and hence, contribute to the pathophysiology of schizophrenia (Bjarnadottir et al., 2007).

Adding further complications to the regional specificity of NRG1 signalling, NR2C mRNA expression has been found to be decreased in a population of schizophrenia patients with a NRG1 polymorphism in the cerebellar molecular layer

of the right hemisphere and vermis (Schmitt et al., 2010). Supporting this finding, the NRG1-β isoform has been shown to upregulate the expression of NR2C mRNA in cultured cerebellar slices (Ozaki et al., 1997). Together these findings reveal the differential effects that NRG1 signalling has on the NMDAR subunits in the different brain regions, however there is still work to be done in order to further understand this complex signalling system.

While it has been shown that the NRG1-ErbB4 signalling pathway can affect glutamatergic function, the reverse may also be true and the activity of NRG1 could be influenced by NMDAR functioning. The link between the NR2 subunits and the PSD provides support for a disruption to the NRG1/ErbB4 system following an NMDAR insult, as ErbB4 is also physically linked to PSD proteins. Results have shown that PSD-95 and ErbB4 are capable of forming a ternary complex in neurons which considerably increases tyrosine phosphorylation of the ErbB4 receptor and enhances NRG1-induced ErbB4 signalling (Huang et al., 2000). It is therefore likely that this regulation of the NRG1/ErbB4 pathway by PSD-95 could be affected by disruptions to NMDAR functioning, or more specifically the NR2 subunits, and play a major role in schizophrenia pathophysiology. Recent data from our laboratory has shown that perinatal PCP treatment can produce long-term alterations in NRG1 and ErbB4 protein expression (duBois, personal communication). In addition, a recent article by Feng and colleagues 2010 found that chronic treatment with the specific NMDAR antagonist MK-801 in adult rats produced an increase in NRG1 and ErbB4 protein expression in the prefrontal cortex and hippocampus, mirroring that observed in schizophrenia (Feng et al., 2010). Therefore regulation of NRG1/ErbB4 signalling via the NMDAR is possible, however the exact mechanism of how this occurs remains unknown and whether there is region or subunit specificity also remains

elusive. Furthermore, a blockade of the NMDAR has the ability to produce functional deficits in GABAergic interneurons in certain cortical areas and the hippocampus (Li et al., 2002, Braun et al., 2007, Homayoun and Moghaddam, 2007) which similarly has been noted in schizophrenia pathology (Wassef et al., 2003). More specifically it has been shown that the loss of parvalbumin and GAD67 in cortical interneurons following NMDA antagonism can be attributed to the NR2A subunit (Kinney et al., 2006). As mentioned previously these interneurons are the preferential location of ErbB4 receptors and so it is highly probable that alterations to NMDAR functioning will affect ErbB4.

While animal studies have provided evidence for NMDAR/NRG1 interactions, further investigation of NR2B, NR2A and NRG1 alterations and interactions, especially in key regions such as the prefrontal cortex and hippocampus, in patients with schizophrenia is necessary to be able to relate any animal study or cell culture findings to what is happening in the disease state. As can be seen from Table 1, while changes in the NR2 subunits, PSD-associated proteins, ErbB4 and NRG1 have been reported in schizophrenia post-mortem brain tissue, there are still many gaps in the literature, particularly involving NRG1 and ErbB4, and many brain regions that have not yet been studied. From the studies to date, few have been able to draw any conclusions about possible NMDA/NRG1 interactions in the schizophrenia brain and there is a large amount of variation between the studies with regards to the specificity of the brain region examined, the methodologies used and the population and demographic data within these studies. Moreover, in addition to examining the mRNA and protein levels of these molecules, the signalling alterations, functional changes and correlations between these factors need to be examined in order to piece together more of the puzzle.

7. Conclusion

Existing knowledge has shown that both the NR2 subunits of the NMDAR and associated proteins in the PSD are spatially and temporally regulated throughout development and there is a physical link between both the NR2 subunits and ErbB4, and PSD proteins. Furthermore NR2 and NRG1 are functionally connected, with alterations to NRG1 stimulation of ErbB4 affecting NMDAR channel properties and subunit specific phosphorylation. However more research is required into whether this interaction is reciprocal, fixed, dynamic, brain region specific, isoform specific, only in some cell types and particularly whether it is altered in disorders such as schizophrenia. A further understanding of the mechanisms by which the molecules in these pathways are regulated would also help to gain insight into their possible role in schizophrenia pathophysiology.

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Figure Legend

Figure 1. Interaction between NR2B and NRG1 via PSD-95.

Both the NR2B subunit of the NMDA-R and the ErbB4-R are linked to the PDZ2 domain of PSD-95. It has been found that alterations of NRG1 signalling can affect NMDA-R function and particularly that of the NR2B subunit, possibly via this physical link between NR2B and the NRG1 receptor, ErbB4. We propose that the relationship between NR2B and NRG1 is reciprocal and that a dysfunction of NR2 subunits may influence NRG1 signalling. As both NR2B and NRG1 signalling pathways are involved in schizophrenia pathology, studying this interaction could provide further knowledge of the mechanism of their involvement in schizophrenia and possible interventions.

Abbreviations:- NMDA-R: N-methyl-D-aspartate receptor, NR2B: subunit 2B of the NMDA-R, NR1: subunit 1 of the NMDA-R, NRG1: Neuregulin1, ErbB4: v-erb-a erythroblastic leukemia viral oncogene homolog 4, PSD-95: post-synaptic density protein 95, PDZ2: Post-synaptic density 95, Drosophila disc large tumor suppressor, Zonula occludens-1, Fyn: Proto-oncogene tyrosine-protein kinase Fyn.

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