Available online at www.sciencedirect.com
ScienceDirect
Schizophrenia Research 99 (2008) 341 – 349
SCHIZOPHRENIA RESEARCH
www.elsevier.com/locate/schres

Increased levels of serotonin$_{2A}$ receptors and serotonin transporter in the CNS of neuregulin 1 hypomorphic/mutant mice

Brian Dean [a,e,f,h,*], Tim Karl [b,c], Geoffrey Pavey [a], Simone Boer [a,g], Liesl Duffy [b,c], Elizabeth Scarr [a,d]

[a] *The Rebecca L. Cooper Research Laboratories, The Mental Health Research Institute, Parkville, Victoria*
[b] *Schizophrenia Research Institute, Sydney, NSW*
[c] *Neuroscience Research Program, Garvan Institute of Medical Research, Sydney, NSW*
[d] *Centre for Neuroscience, The University of Melbourne, Parkville, Victoria*
[e] *Department of Psychiatry, The University of Melbourne, Parkville, Victoria*
[f] *Department of Pathology, The University of Melbourne, Parkville, Victoria*
[g] *Department of Clinical and Biomedical Sciences (Geelong), The University of Melbourne, Parkville, Victoria*
[h] *The Department of Psychological Medicine, Monash University, Clayton, Victoria, Australia*

Received 12 July 2007; received in revised form 25 September 2007; accepted 18 October 2007
Available online 28 November 2007

Abstract

Changes in neuregulin 1 expression have been reported in the CNS from subjects with schizophrenia. As neuregulin 1 is important in cortical development we postulated that changes in neuregulin 1 expression may contribute towards changes in cholinergic, glutamatergic and serotonergic markers that are well documented in the CNS of subjects with that disorder. To begin to test this hypothesis, we used *in situ* radioligand binding to measure levels of muscarinic M1/M4 receptors, the kainate receptor, the NMDA receptor, the serotonin 2A receptor, the serotonin 1A receptor and the serotonin transporter in the CNS from heterozygous transmembrane domain neuregulin 1 mutant mice. The major outcomes from these studies was the demonstration of an overall increase in levels of the serotonin 2A receptor ($F=11.3$, d.f.$=3,1,72$, $p=0.0012$) and serotonin transporter ($F=5.00$, d.f.$=1,3,72$, $p<0.05$) in the mutant mice. Levels of the other receptors did not vary in the mutant mice compared to their wild type-like litter mates. These data are the first evidence to suggest that *NRG1* gene expression may be involved in regulating the development of the serotonergic system in the mammalian CNS.

Keywords: Neuregulin; Schizophrenia; Serotonin receptors; Serotonin transporter; Muscarinic receptors; Glutamate receptors

1. Introduction

Numerous genetic studies support the hypothesis that variation in the neuregulin 1 (*NRG1*) gene is associated with an increased susceptibility for schizophrenia (Stefansson et al., 2002; Stefansson et al., 2003; Williams et al., 2003; Bakker et al., 2004; Hall et al., 2004;Yang et al., 2003; Tang et al., 2004; Li et al., 2004; Zhao et al., 2004). This posit is further supported by the finding that the expression of type 1 *NRG1* is increased in dorsolateral prefrontal cortex (Hashimoto et al., 2004) and hippocampus (Law et al., 2006) from subjects with the disorder. Up to 15 isoforms NRG1 exist as a result of

* Corresponding author. The Rebecca L. Cooper Research Laboratories, The Mental Health Research Institute, Parkville, Victoria, 3052, Australia. Tel.: +1 613 9389 2940; fax: +1 613 9387 5061.
E-mail address: anddali@unimelb.edu.au (B. Dean).

0920-9964/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.schres.2007.10.013

alternative splicing and multiple promoters (Harrison and Law, 2006) with postmortem CNS studies failing to show changes in mRNA for either type II / type III (Hashimoto et al., 2004) or type II to IV (Law et al., 2006) in schizophrenia. It has also been reported that cortical levels of total NRG1 protein are either not changed (Hahn et al., 2006) or decreased (Bertram et al., 2007) in subjects with schizophrenia. Hence it appears that CNS region and transcript-specific changes in *NRG1*, which may not always manifest as a change total NRG1 protein, may contribute to the pathology of schizophrenia.

It has long been accepted that abnormal cortical functioning is present in subjects with schizophrenia (Weinberger, 1988). This is particularly relevant to *NRG1*, which plays a role in cortical development (Lopez-Bendito et al., 2006). Extending this argument, there have been replicated findings of a changed cortical molecular cytoarchitecture in subjects with schizophrenia; these changes include important components of the glutamatergic, cholinergic and serotonergic pathways (Meltzer, 1987; Dean, 2000; Raedler et al., 2007). Given the role of NRG1 in cortical development, it seems possible that changes in *NRG1* expression in the CNS from subjects with schizophrenia could be contributing to the abnormalities in the glutamatergic, cholinergic and serotonergic pathways that have been observed in the CNS of subjects with the disorder. To begin to address this issue, we have determined whether there are changes in important components of the glutamatergic, cholinergic and serotonergic systems in the CNS of heterozygous transmembrane domain neuregulin 1 mutant (*Nrg1*$^{+/-}$) mice.

2. Materials and methods

2.1. Materials

[^{3}H]pirenzepine, [^{3}H]kainate, [^{3}H]MK-801, [^{3}H]ketanserin and [^{3}H]citalopram were obtained from New England Nuclear. [^{3}H]8OH-DPAT and [^{3}H]microscales were obtained from Amersham. Paroxetine was kindly donated by GlaxoSmithKline. All other chemicals were obtained from Sigma.

2.2. Tissue collection

Frozen CNS from 5-6 months old male mutant mice *Nrg1*$^{+/-}$mice on a C57BL/6j background (17th backcross generation- heterozygous breeding) and wild type-like (WT) littermates were obtained from the colony maintained at the biological testing facility of the Garvan Institute of Medical Research under enriched environmental conditions (Karl et al., 2007).

For each radioligand binding measured; five frozen sections (3 total binding; 2 non-specific binding [NSB]) were cut from CNS of 10 *Nrg1*$^{+/-}$and 10 WT mice, approximately 1.5 mm anterior to bregma.

The binding of [^{3}H]pirenzepine to muscarinic M1/M4 receptors (Dean et al., 1996a), [^{3}H]kainate to kainate receptors (Scarr et al., 2005), [^{3}H]MK-801 to NMDA receptors (Scarr et al., 2005), [^{3}H]ketanserin to serotonin (5HT) 2A receptors (Dean and Hayes, 1996), [^{3}H]8OH-DPAT to 5HT1AR (Dean et al., 1999b) and [^{3}H] citalopram to the 5HT transporter (SERT) (Dean et al., 1999b) in mouse CNS was measured as described previously. A more comprehensive methodology is supplied as supplementary material. Importantly, the levels of radioligand binding on the resulting autoradiographs, which were in the form of phosphoimages, could be measured by comparison to the intensity of the blocks of radioactivity on the [^{3}H]microscales that were opposed to the same phosphoimage. These comparisons were achieved using the AIS image analysis software, with results being expressed as dpm mg−1 estimated wet weight tissue equivalents (ETE) and then converted to fmol mg−1 ETE. In this way, radioligand binding was measured using a single point saturation analysis, which gives a good approximation of the density of radioligand binding sites in tissue sections.

Statistically significant variation in radioligand binding was identified by two-way ANOVA with *Nrg1* status (WT vs. *Nrg1*$^{+/-}$mice) and CNS regions as variables. For [^{3}H]ketanserin binding, each layer of cortical radioligand binding was treated as a discrete CNS region. Bonferroni posthoc tests were used to identify the specific differences in radioligand binding that contributed to any global variance in binding. Differences were regarded as statistically significant if $p<.05$.

3. Results

Analyses of [^{3}H]pirenzepine binding across the cortex and striatum showed no significant variation in levels of binding of this radioligand (Fig. 1A) and therefore an integrated measure was taken across the entire cortex and striatum. By contrast, whilst the binding of [^{3}H]kainate, [^{3}H]MK-801, [^{3}H]ketanserin, ^{3}H]citalopram and [^{3}H]8OH-DPAT (Fig. 1B-F) was homogeneous across the striatum, and was therefore taken as an integrated measure across that region, all these radioligands showed layered binding in the cortex. For [^{3}H]kainate and [^{3}H]8OH-DPAT, a comparison with cresyl violet stained sections showed that the distinct outer

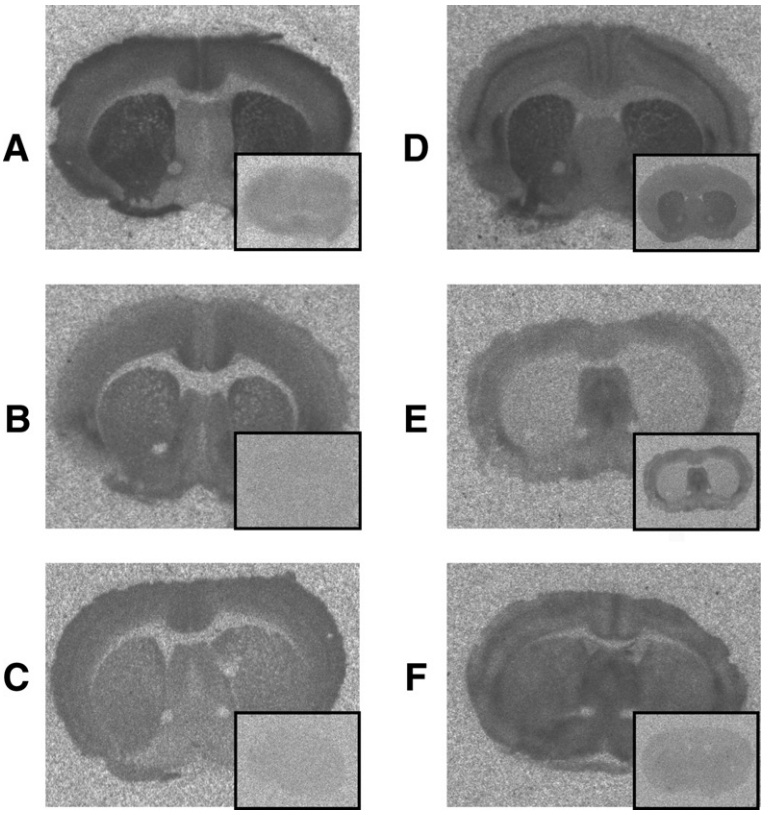

Fig. 1. Typical autoradiographs showing the binding of [^{3}H]pirenzepine (A), [^{3}H]kainate (B), [^{3}H]MK-801 (C), [^{3}H]ketanserin (D), [^{3}H]8OH-DPAT (E) and [^{3}H]citalopram (F) to CNS from wild type-like *Nrg1*$^{\pm}$ and wild type-like mice; the binding of each radioligand in the presence of the appropriate displacing agent (non-specific binding) is shown as an insert to each image.

binding layer (Layer 1) was over laminae I to III and inner binding layer (Layer 2) was over laminae IV to VI. For [^{3}H]MK-801, [^{3}H]citalopram and [^{3}H]ketanserin three distinct layers of radio ligand binding were detected in the mouse cortex with radioligand binding Layer 1 included cortical laminae I and III, Layer 2 included cortical laminae IV whilst Layer 3 included laminae V and VI. Subsequently, the binding of [^{3}H[kainate, [^{3}H]8OH-DPAT, [^{3}H]MK-801, [^{3}H]citalopram and [^{3}H]ketanserin across each binding layer was measured in both WT and *Nrg1*$^{+/-}$mice.

3.1. Muscarinic Receptors

There was significant variance in levels of [^{3}H]pirenzepine binding between CNS regions ($F=272$, d.f.$=1,1,36$, $p<0.0001$) but not with *Nrg1* status ($F=0.01$, d.f.$=1,1,36$, $p=0.91$) and there was no interaction between the variables ($F=0.54$, d.f.$=1,1,36$, $p=0.46$) (Table 1). Post-hoc test analysis showed that the variation between regions was due to lower [^{3}H]pirenzepine binding in the cortex compared to the striatum in both WT ($p<0.001$) and *Nrg1*$^{+/-}$($p<0.001$) mice.

Table 1
Levels (mean±SEM) of [^{3}H]pirenzepine, [^{3}H]kainate and [^{3}H]ketanserin binding in the cortex and striatum from *Nrg1*$^{+/-}$mice (KO) and wild type-like (WT) mice

	Cortex						Striatum	
Layer	1		2		3			
Radioligand	WT$^{\psi}$	*Nrg1*$^{+/-\psi}$	WT$^{\psi}$	*Nrg1*$^{+/-\psi}$	WT$^{\psi}$	*Nrg1*$^{+/-\psi}$	WT$^{\psi}$	*Nrg1*$^{+/-\psi}$
[^{3}H]pirenzepine	277±5.0	270±5.7					393±9.9	398±7.9
[^{3}H]kainate	75±2.7	82±3.2	83±2.3	86±3.6			83±2.3	86±3.4
[^{3}H]MK-801	239±7.2	235±8.9	203±5.6	195±5.3	136±3.7	142±5.8	110±3.3	110±4.1
[^{3}H]citalopram	44±2.9	50±2.9	71±5.0	79±3.7	54±3.8	62±2.5	69±2.3	70±2.2

Abbreviations: $^{\psi}$= fmol / mg estimated tissue equivalents.

3.2. Glutamatergic Receptors

There was no significant variance in the levels of [^{3}H] MK-801 binding with *Nrg1* status (F=0.14, d.f.=1,3,72, p=0.15) but radioligand binding did vary between CNS regions (F=3.81, d.f.=1,3,72, p<0.0001); there was no interaction between the variables (F=0.60, d.f.=3,3,72, p=0.64) (Table 1). The variation of [^{3}H]MK-801 between CNS regions reflected differences in levels of radioligand binding which had the rank order of Layer 1>Layer 2>Layer 3>striatum in both the *Nrg1*$^{+/-}$and WT mice.

There was no significant variance in the levels of [^{3}H]kainate binding with *Nrg1* status (F=3.14, d.f.= 1,1,36, p=0.08) or CNS region (F=3.81, d.f.=1,1,36, p=0.06) and there was no interaction between the variables (F=0.60, d.f.=1,1,36, p=0.29).

3.3. Serotonergic markers

There was significant variance in the levels of [^{3}H] ketanserin binding with *Nrg1* status (F=11.3, d.f.= 3,1,72, p=0.0012) and CNS region (F=170, d.f.= 3,1,72, p<0.0001) but there was no significant interaction between the variables (F=0.10, d.f.=3,1,72, p=0.96). Posthoc tests failed to detect significant differences in [^{3}H]ketanserin binding within any single CNS region studied (Fig. 2A). The explanation for these findings is that there are non-significant increases in [^{3}H] ketanserin binding in all regions of the CNS from *Nrg1*$^{+/-}$ mice compared to that in WT mice (percentage increase in binding layers in the cortex: Layer 1=26%, Layer 2=18%, Layer 3=9%; the striatum=18%), the sum of these changes resulting in the significant region-wide increase in [^{3}H]ketanserin binding. For WT mice there were significant differences in [^{3}H]ketanserin binding between all CNS regions (Layer 2>striatum>Layer 3>Layer 1) but for *Nrg1*$^{+/-}$levels of [^{3}H]ketanserin binding did not differ between Layer 2 and the striatum (Layer 2=striatum>Layer 3>Layer 1).

There was no significant variation in [^{3}H]8OH-DPAT binding between the *Nrg1*$^{+/-}$and WT mice (F=0.77, d.f.= 1,2,54, p=0.38) but levels of radioligand binding did vary between CNS regions (F=851, d.f.=2,1,54, p<0.0001); there were no interaction between the variables (F=0.39, d.f.=2,2,54, p=0.68) (Table 1). The rank order of [^{3}H] 8OH-DPAT binding being Layer 2>Layer 1>striatum.

There was a significant variation in [^{3}H]citalopram binding between *Nrg1*$^{+/-}$and WT mice (F=5.00, d.f.= 1,3,72, p<0.05) and CNS region (F=29.01, d.f.= 3,1,72, p<0.0001) and there was no interaction between the variables (F=0.38, d.f.=3,3,72, p=0.76). Post hoc analysis failed to show any significant differences in

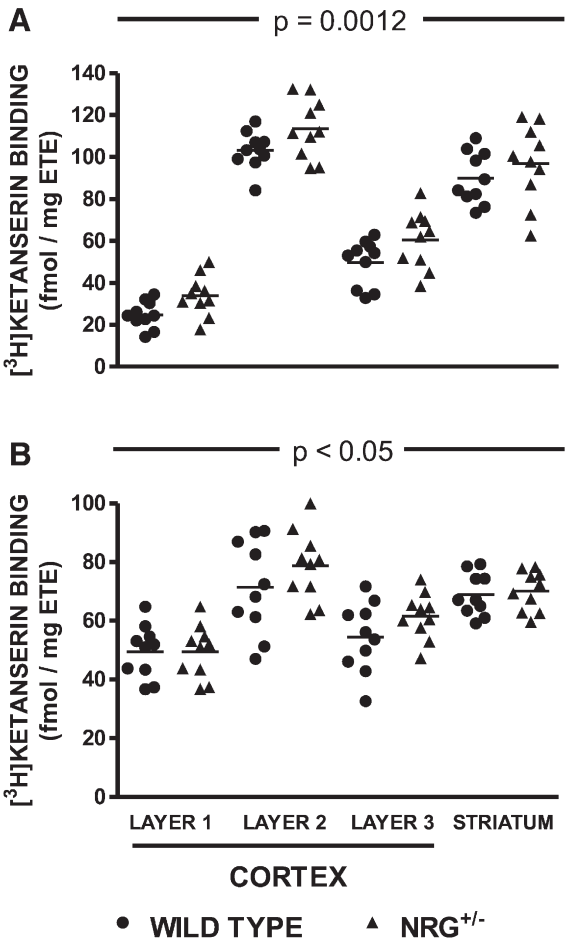

Fig. 2. Levels (mean±SEM: fmol/mg ETE) of (A) $5HT_{2A}R$ and (B) SERT in the CNS of *Nrg1*$^{+/-}$mice.

[^{3}H]citalopram binding in any individual CNS region and therefore the CNS region-wide change in radioligand binding was reflecting the summed effect of non-significant increase in [^{3}H]citalopram binding in each CNS region in the *Nrg1*$^{+/-}$mice (Fig. 2B). The variation in [^{3}H]citalopram binding across regions were complex; binding was significantly lower in Layer 1 compared to Layer 2 and the striatum in both *Nrg1*$^{+/-}$ and WT mice (p from <0.01 to <0.001). By contrast, levels of radioligand binding were significantly lower in Layer 3 compared to Layer 2 in the WT (p<0.05) but not in the *Nrg1*$^{+/-}$mice.

4. Discussion

The hypothesis tested in this study was that changes in the molecular cytoarchitecture of key markers in the glutamatergic, cholinergic and serotonergic pathways, which have been shown to be altered in the cortex of

subjects with schizophrenia, may also be affected in the cortex of the heterozygous *Nrg1*$^{+/-}$mouse. This study has subsequently shown that there are omnibus increases in the levels of [^{3}H]ketanserin to the 5HT2AR and [^{3}H]citalopram to SERT, but not [^{3}H] 8OH-DPAT binding to the 5HT1AR, in the *Nrg1*$^{+/-}$ mouse. Hence our data suggests that there are subtle, selective changes in the serotonergic pathways in the *Nrg1*$^{+/-}$mouse. This supports the concept that development of serotonergic pathways in the mammalian cortex may be, in part, regulated by *NRG1*.

The increase in 5-HT$_{2A}$R was not uniform throughout the cortex, with mean levels of binding increasing by 26% in Layer 1, 18% in Layer 3 and 9% in Layer 2. Indeed, using a focused comparison, the increase in [^{3}H] ketanserin binding in Layer 1 of the *Nrg1*$^{+/-}$mice is statistically significant (unpaired t-test: $p=0.022$). Significantly, changes in cortical 5HT$_{2A}$ receptors have been widely reported in schizophrenia (Dean, 2003) and therefore our findings raise the possibility changes in the 5HT$_{2A}$ receptors may be in some way related to changes in *NRG1* expression in subjects with the disorder.

This study has also identified a generalised increase in levels of SERT in the *Nrg1*$^{+/-}$mouse. These data support the view that *NRG1* may have a complex role in regulating the serotonergic pathways. In the cortex our data suggests that, unlike for SERT, changes in 5HT$_{2A}$R are not uniform across the cortex of the *Nrg1*$^{+/-}$mouse. Significantly, changes in SERT have been reported in the CNS of subjects with schizophrenia (Dean et al., 1995, 1996b, 1999b; Hernandez and Sokolov, 1997) raising the possibility that changes *NRG1* may be influence the development of the cortical serotonergic systems in schizophrenia as well as in the *Nrg*$^{\pm}$ mouse.

By contrast to our data on serotonergic markers, we failed to show any significant differences in levels of [^{3}H]pirenzepine binding to the muscarinic M1/M4R, [^{3}H]MK-801 binding to the NMDAR or [^{3}H]kainate binding to kainate receptor in the cortex or striatum from *Nrg1*$^{+/-}$mice. Thus, our data would not support the hypothesis that changes in *NRG1* expression has a role in mediating the decreased level of muscarinic M1/M4 or kainate receptors that are widely reported in subjects with schizophrenia (Dean et al., 1996a,b; Crook et al., 2000; Crook et al., 2001; Dean et al., 2002; Zavitsanou et al., 2004; Deng and Huang, 2005; Porter et al., 1997; Scarr et al., 2005; Ibrahim et al., 2000; Meador-Woodruff et al., 2001; Garey et al., 2006) in this mouse model.

Unlike this study, it has been previously reported that *Nrg1*$^{+/-}$mice have decreased levels of cortical NMDA receptors (Stefansson et al., 2002). There are two main reasons for such discrepant results. First, the levels of NMDA receptors were measured using two different methodologies (*in situ* radioligand binding and particulate membrane binding). In addition, environment has been shown to affect NMDA receptor sub-unit expression(Guilarte et al., 2003) and therefore the differences in levels of cortical NMDA receptors reported could have resulted from maintaining mice in different housing conditions, the animal in this study being maintained in an enriched environment (Karl et al., 2007).

It is notable that, at the level of radioligand binding, it is most commonly reported that levels of NMDA receptors are increased in the CNS from subjects with schizophrenia (Table 2). However, there is no convincing data to show changes in radioligand binding to the NMDA receptor in studies limited to Brodmann's area 9 or 10, suggesting that changes in NMDA receptors may have a degree of regional specificity. The NMDA receptor is a heteromeric complex that incorporates subunits from three families of proteins, the NR1, NR2 and NR3 subunits (Paoletti and Neyton, 2007). Studies examining the expression of these subunits at the level of protein or mRNA predominantly suggest that subunit expression is unchanged in schizophrenia (Table 2). However, there are reports of both increased and decreased gene expression of different subunits in different regions.

Focusing on radioligand binding, it would seem that findings from this and a previous showing decreased cortical NMDA receptors in the *Nrg1*$^{+/-}$mice (Stefansson et al., 2002) can both be accommodated by the findings in postmortem CNS from subjects with schizophrenia. However, given the findings on the NMDA receptor in schizophrenia it must be concluded that any interactions between NRG1 and the NMDA receptor that are associated with the disorder are also complex. Adding to the complexity of findings on the relationships between *NRG1*, the NMDA receptor and schizophrenia are two recent findings in rat and human CNS suggesting that NRG1 may be able to act to alter the activity of NMDA receptors directly (Gu et al., 2005; Hahn et al., 2006), without affecting receptor expression. Moreover, it has been suggested that the mechanisms by which *NRG1* can affect NMDA receptor activity are altered in the CNS of subjects with schizophrenia (Hahn et al., 2006). Clearly, a better understanding of the relationship between *NRG1* and NMDA receptor activity and / or expression is required to fully understand the potential role of this nexus in the pathology of schizophrenia. Further study on how levels of NMDA receptors may be affected by changes in *NRG1* expression completed in *Nrg1*$^{+/-}$mice may be invaluable in resolving this conundrum.

Table 2
A Summary of Findings on Levels of the NMDA receptor in the CNS of subjects with schizophrenia

Methodology	CNS region	Outcome	Reference
Radioligand Binding			
[^{3}H]MK-801	Frontal Cortex	↑ 8.5%	Kornhuber et al. (1989)
	Striatum	↑ 44%	
	Striatum	No Change	Noga et al. (1997)
	Cortex – Brodmann's area 24	↑ 44% only in laminae II & III	Zavitsanou et al. (2002)
	Cortex – Brodmann's area 9	No Change	Scarr et al. (2005)
[^{3}H]TCP	Cortex – Brodmann's area 11	↑ 26%	Simpson et al. (1991)
	Brodmann's area 10	No Change	
	Cortex – Brodmann's Area 9	No Change	Dean et al. (1999a)
[^{3}H]glycine	Prefrontal Cortex	No Change	Ishimaru et al. (1994)
	Precentral Cortex	↑ 82%- Premotor area	
	Temporal Cortex	No Change	
	Parietal Cortex	Depending on region: No Change to ↑ 107%	
	Occipital Cortex	↑ 48%	
[^{3}H]L-689,560	Striatum (Putamen only)	↑ 35%	paricio-Legarza et al. (1998)
	Cortex – Brodmann's area 22	↑ 15%	Nudmamud and Reynolds, (2001)
	Brodmann's area 10	No Change	
	Cortex – Brodmann's area 22	No Change	Nudmamud-Thanoi and Reynolds, (2004)
	Cortex – Brodmann's area 22	↑ 27%	Grimwood et al. (1999)
	Cortex – Brodmann's area 6	No Change	
Western blotting			
	Dorsolateral Prefrontal Cortex	No Change	Kristiansen et al. (2006)
	Anterior Cingulate Cortex	↑ 31% NR1^{C2} subunit but No Change in NR1$^{C2\prime}$, NR2A, NR2B, NR2C or NR2D sub-units	
	Cortex – Brodmann's area 22	No Change NR1	Nudmamud-Thanoi and Reynolds, (2004)
	Cortex – Brodmann's areas 11, 12, 45 and 47	No Change NR1	Toro and Deakin, (2005)
mRNA			
	Prefrontal Cortex	↑ 53% NR2D but No Change in NR1, NR2A or NR2D	Akbarian et al. (1996)
	Parieto-temporal Cortex	No Change	
	Cortex – Brodmann's area 22	↓ 30% in NR1 Schizophrenia with Dementia No Change in Schizophrenia without Dementia	Humphries et al. (1996)
	Frontal Cortex	↓ 80% NR1 in Antipsychotic-Free Subjects Only	Sokolov (1998)
	Cortex – Brodmann's area 46	↑ 164% NR1 but No Change in NR2A or NR2B	Dracheva et al. (2001)
	Cortex – Brodmann's area 17	↑ 268% NR1, ↑ 93% NR2A but No Change in NR2B	
	Cortex – Brodmann's area 46	No Change NR2B	Martucci et al. (2006)
	Dorsolateral Prefrontal Cortex	No Change NR1^{C2} or NR1$^{C2\prime}$ sub-unit	Kristiansen et al. (2006)
	Dorsolateral Prefrontal Cortex	↑ 32% NR3A	Mueller and Meador-Woodruff, (2004)
	Inferior Temporal Cortex	No Change	

In conclusion, the main finding of this study is that there are increased levels of the 5HT$_{2A}$ receptor and SERT in the CNS of the *Nrg1*$^{+/-}$mouse. Our data is therefore the first to suggest that *NRG1* may play a role in regulating the serotonergic systems of the CNS; a system recognised as being affected by the pathology of schizophrenia (Meltzer, 1987). Such findings strongly support the argument that further study of NRG1, both in human CNS and knockout animals, will inform on the neurobiological abnormalities that underpin the disorder (Harrison and Law, 2006).

Role of funding source

Funding for this study was provided by NHMRC, The Australian Rotary Health Research Fund and The Rebecca L. Cooper Medical Research Foundation. None of the funding bodies had any further role in the study design; in the collection, analysis and interpretation of data, in the writing of the report or in the decision to submit the paper for publication.

None of the authors have any actual or potential conflict of interests relating to this submission.

Contributors

Author Brian Dean was involved in the design of the study, undertook data analysis and wrote the manuscript. Author Tim Karl was involved in the study design, oversaw the breeding of the mice used in this study and contributed to writing the submitted manuscript. Authors Geoffrey Pavey and Simone Boer completed radioligand binding assays, analysed data from these assays and contributed to the writing of the submitted manuscript. Author Liesl Duffey bred and reared the mice used in the study and was involved in the writing of the submitted manuscript. Author Elizabeth Scarr was involved in the design of the study and in editing all drafts of the manuscript. All authors have contributed and approved the final manuscript.

Conflict of interest

None of the authors have any actual or potential conflict of interests relating to this submission.

Acknowledgements

BD is a NHMRC Senior Research Fellowship (# 400016) and ES is the Ronald Griffiths Schizophrenia Research Fellow and holds a NARSAD Young Investigator Award. TK is supported by the Schizophrenia Research Institute (SRI) utilizing infrastructure funding from NSW Health, the Sylvia and Charles Viertel Charitable Foundation, a Bill Ritchie Postdoctoral Research Fellowship and the National Alliance for Research on Schizophrenia and Depression (Young Investigator Award). SB is funded through the ARHRF with and Ian Scott Scholarship. The study was also supported by NHMRC Project Grant # 114253, NIMH Grant # MH069691-01A1, The Rebecca L. Cooper Medical Research Foundation and The Woods Family Trust.

References

Akbarian, S., Sucher, N.J., Bradley, D., Tafazzoli, A., Trinh, D., Hetrick, W.P., Potkin, S.G., Sandman, C.A., Bunney W.E., Jr., Jones, E.G., 1996. Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. J. Neurosci. 16, 19–30.

Bakker, S.C., Hoogendoorn, M.L., Selten, J.P., Verduijn, W., Pearson, P.L., Sinke, R.J., Kahn, R.S., 2004. Neuregulin 1: genetic support for schizophrenia subtypes. Mol. Psychiatry 9, 1061–1063.

Bertram, I., Bernstein, H.G., Lendeckel, U., Bukowska, A., Dobrowolny, H., Keilhoff, G., Kanakis, D., Mawrin, C., Bielau, H., Falkai, P., Bogerts, B., 2007. Immunohistochemical evidence for impaired neuregulin-1 signaling in the prefrontal cortex in schizophrenia and in unipolar depression. Ann N Y Acad Sci. 1096, 147–156.

Crook, J.M., Tomaskovic-Crook, E., Copolov, D.L., Dean, B., 2000. Decreased muscarinic receptor binding in subjects with schizophrenia: a study of the human hippocampal formation. Biol. Psychiatry 48, 381–388.

Crook, J.M., Tomaskovic-Crook, E., Copolov, D.L., Dean, B., 2001. Low muscarinic receptor binding in prefrontal cortex from subjects with schizophrenia: a study of Brodmann's areas 8, 9, 10, and 46 and the effects of neuroleptic drug treatment. Am. J. Psychiatr. 158, 918–925.

Dean, B., 2000. Signal transmission, rather than reception, is the underlying neurochemical abnormality in schizophrenia. Aust. N. Z. J. Psychiatry. 34, 560–569.

Dean, B., 2003. The cortical serotonin2A receptor and the pathology of schizophrenia: a likely accomplice. J. Neurochem. 85, 1–13.

Dean, B., Opeskin, K., Pavey, G., Naylor, L., Hill, C., Keks, N., Copolov, D.L., 1995. [3H]paroxetine binding is altered in the hippocampus but not the frontal cortex or caudate nucleus from subjects with schizophrenia. J. Neurochem. 64, 1197–1202.

Dean, B., Hayes, W., 1996. Decreased frontal cortical serotonin2A receptors in schizophrenia. Schizophr. Res. 21, 133–139.

Dean, B., Crook, J.M., Opeskin, K., Hill, C., Keks, N., Copolov, D.L., 1996a. The density of muscarinic M1 receptors is decreased in the caudate-putamen of subjects with schizophrenia. Mol. Psychiatry. 1, 54–58.

Dean, B., Hayes, W., Opeskin, K., Naylor, L., Pavey, G., Hill, C., Keks, N., Copolov, D.L., 1996b. Serotonin2 receptors and the serotonin transporter in the schizophrenic brain. Behav. Brain Res. 73, 169–175.

Dean, B., Hussain, T., Hayes, W., Scarr, E., Kitsoulis, S., Hill, C., Opeskin, K., Copolov, D.L., 1999a. Changes in serotonin2A and GABA(A) receptors in schizophrenia: studies on the human dorsolateral prefrontal cortex. J. Neurochem. 72, 1593–1599.

Dean, B., Tomaskovic-Crook, E., Opeskin, K., Keks, N., Copolov, D., 1999b. No change in the density of the serotonin1A receptor, the serotonin4 receptor or the serotonin transporter in the dorsolateral prefrontal cortex from subjects with schizophrenia. Neurochem. Int. 34, 109–115.

Dean, B., McLeod, M., Keriakous, D., McKenzie, J., Scarr, E., 2002. Decreased muscarinic(1) receptors in the dorsolateral prefrontal cortex of subjects with schizophrenia. Mol. Psychiatry. 7, 1083–1091.

Deng, C., Huang, X.F., 2005. Decreased density of muscarinic receptors in the superior temporal gyrusin schizophrenia. J. Neurosci. Res. 81, 883–890.

Dracheva, S., Marras, S.A., Elhakem, S.L., Kramer, F.R., Davis, K.L., Haroutunian, V., 2001. N-methyl-D-aspartic acid receptor expression in the dorsolateral prefrontal cortex of elderly patients with schizophrenia. Am. J. Psychiatry. 158, 1400–1410.

Garey, L.J., Von Bussmann, K.A., Hirsch, S.R., 2006. Decreased numerical density of kainate receptor-positive neurons in the orbitofrontal cortex of chronic schizophrenics. Exp. Brain Res.

Grimwood, S., Slater, P., Deakin, J.F., Hutson, P.H., 1999. NR2B-containing NMDA receptors are up-regulated in temporal cortex in schizophrenia. Neuroreport. 10, 461–465.

Gu, Z., Jiang, Q., Fu, A.K., Ip, N.Y., Yan, Z., 2005. Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. J. Neurosci. 25, 4974–4984.

Guilarte, T.R., Toscano, C.D., McGlothan, J.L., Weaver, S.A., 2003. Environmental enrichment reverses cognitive and molecular deficits induced by developmental lead exposure. Ann. Neurol. 53, 50–56.

Hahn, C.G., Wang, H.Y., Cho, D.S., Talbot, K., Gur, R.E., Berrettini, W.H., Bakshi, K., Kamins, J., Borgmann-Winter, K.E., Siegel, S.J., Gallop, R.J., Arnold, S.E., 2006. Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. Nat. Med. 12, 824–828.

Hall, D., Gogos, J.A., Karayiorgou, M., 2004. The contribution of three strong candidate schizophrenia susceptibility genes in demographically distinct populations. Genes Brain Behav. 3, 240–248.

Harrison, P.J., Law, A.J., 2006. Neuregulin 1 and schizophrenia: genetics, gene expression, and neurobiology. Biol. Psychiatry. 60, 132–140.

Hashimoto, R., Straub, R.E., Weickert, C.S., Hyde, T.M., Kleinman, J.E., Weinberger, D.R., 2004. Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. Mol. Psychiatry 9, 299–307.

Hernandez, I., Sokolov, B.P., 1997. Abnormal expression of serotonin transporter mRNA in the frontal and temporal cortex of schizophrenics. Mol. Psychiatry 2, 57–64.

Humphries, C., Mortimer, A., Hirsch, S., de, B.J., 1996. NMDA receptor mRNA correlation with antemortem cognitive impairment in schizophrenia. Neuroreport. 7, 2051–2055.

Ibrahim, H.M., Hogg A.J., Jr., Healy, D.J., Haroutunian, V., Davis, K.L., Meador-Woodruff, J.H., 2000. Ionotropic glutamate receptor binding and subunit mRNA expression in thalamic nuclei in schizophrenia. Am. J. Psychiatry. 157, 1811–1823.

Ishimaru, M., Kurumaji, A., Toru, M., 1994. Increases in strychnine-insensitive glycine binding sites in cerebral cortex of chronic schizophrenics: evidence for glutamate hypothesis. Biol. Psychiatry. 35, 84–95.

Karl, T., Duffy, L., Scimone, A., Harvey, R.P., Schofield, P.R., 2007. Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. Genes Brain Behav.

Kornhuber, J., Mack-Burkhardt, F., Riederer, P., Hebenstreit, G.F., Reynolds, G.P., Andrews, H.B., Beckmann, H., 1989. [3H]MK-801 binding sites in postmortem brain regions of schizophrenic patients. J. Neural. Transm. 77, 231–236.

Kristiansen, L.V., Beneyto, M., Haroutunian, V., Meador-Woodruff, J.H., 2006. Changes in NMDA receptor subunits and interacting PSD proteins in dorsolateral prefrontal and anterior cingulate cortex indicate abnormal regional expression in schizophrenia. Mol. Psychiatry. 11, 737–47, 705.

Law, A.J., Lipska, B.K., Weickert, C.S., Hyde, T.M., Straub, R.E., Hashimoto, R., Harrison, P.J., Kleinman, J.E., Weinberger, D.R., 2006. Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5¢ SNPs associated with the disease. Proc. Natl. Acad. Sci. U. S. A. 103, 6747–6752.

Li, T., Stefansson, H., Gudfinnsson, E., Cai, G., Liu, X., Murray, R.M., Steinthorsdottir, V., Januel, D., Gudnadottir, V.G., Petursson, H., Ingason, A., Gulcher, J.R., Stefansson, K., Collier, D.A., 2004. Identification of a novel neuregulin 1 at-risk haplotype in Han schizophrenia Chinese patients, but no association with the Icelandic/Scottish risk haplotype. Mol. Psychiatry 9, 698–704.

Lopez-Bendito, G., Cautinat, A., Sanchez, J.A., Bielle, F., Flames, N., Garratt, A.N., Talmage, D.A., Role, L.W., Charnay, P., Marin, O., Garel, S., 2006. Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. Cell. 125, 127–142.

Martucci, L., Wong, A.H., De L., V., Likhodi, O., Wong, G.W., King, N., Kennedy, J.L., 2006. N-methyl-D-aspartate receptor NR2B subunit gene GRIN2B in schizophrenia and bipolar disorder: Polymorphisms and mRNA levels. Schizophr Res. 84, 214–221.

Meador-Woodruff, J.H., Davis, K.L., Haroutunian, V., 2001. Abnormal kainate receptor expression in prefrontal cortex in schizophrenia. Neuropsychopharmacology. 24, 545–552.

Meltzer, H.Y., 1987. Biological studies in schizophrenia. Schizophr Bull. 13, 77–111.

Mueller, H.T., Meador-Woodruff, J.H., 2004. NR3A NMDA receptor subunit mRNA expression in schizophrenia, depression and bipolar disorder. Schizophr Res. 71, 361–370.

Noga, J.T., Hyde, T.M., Herman, M.M., Spurney, C.F., Bigelow, L.B., Weinberger, D.R., Kleinman, J.E., 1997. Glutamate receptors in the postmortem striatum of schizophrenic, suicide, and control brains. Synapse. 27, 168–176.

Nudmamud, S., Reynolds, G.P., 2001. Increased density of glutamate/N-methyl-D-aspartate receptors in superior temporal cortex in schizophrenia. Neurosci. Lett. 304, 9–12.

Nudmamud-Thanoi, S., Reynolds, G.P., 2004. The NR1 subunit of the glutamate/NMDA receptor in the superior temporal cortex in schizophrenia and affective disorders. Neurosci. Lett. 372, 173–177.

Paoletti, P., Neyton, J., 2007. NMDA receptor subunits: function and pharmacology. Curr. Opin. Pharmacol. 7, 39–47.

paricio-Legarza, M.I., Davis, B., Hutson, P.H., Reynolds, G.P., 1998. Increased density of glutamate/N-methyl-D-aspartate receptors in putamen from schizophrenic patients. Neurosci. Lett. 241, 143–146.

Porter, R.H., Eastwood, S.L., Harrison, P.J., 1997. Distribution of kainate receptor subunit mRNAs in human hippocampus, neocortex and cerebellum, and bilateral reduction of hippocampal GluR6 and KA2 transcripts in schizophrenia. Brain Res. 751, 217–231.

Raedler, T.J., Bymaster, F.P., Tandon, R., Copolov, D., Dean, B., 2007. Towards a muscarinic hypothesis of schizophrenia. Mol. Psychiatry 12, 232–246.

Scarr, E., Beneyto, M., Meador-Woodruff, J.H., Deans, B., 2005. Cortical glutamatergic markers in schizophrenia. Neuropsychopharmacology. 30, 1521–1531.

Simpson, M.D., Slater, P., Royston, M.C., Deakin, J.F., 1991. Alterations in phencyclidine and sigma binding sites in schizophrenic brains. Effects of disease process and neuroleptic medication. Schizophr. Res. 6, 41–48.

Sokolov, B.P., 1998. Expression of NMDAR1, GluR1, GluR7, and KA1 glutamate receptor mRNAs is decreased in frontal cortex of "neuroleptic-free" schizophrenics: evidence on reversible up-regulation by typical neuroleptics. J. Neurochem. 71, 2454–2464.

Stefansson, H., Sarginson, J., Kong, A., Yates, P., Steinthorsdottir, V., Gudfinnsson, E., Gunnarsdottir, S., Walker, N., Petursson, H., Crombie, C., Ingason, A., Gulcher, J.R., Stefansson, K., St, C.D., 2003. Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. Am. J. Hum. Genet. 72, 83–87.

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., Brynjolfsson, J., Gunnarsdottir, S., Ivarsson, O., Chou, T.T., Hjaltason, O., Birgisdottir, B., Jonsson, H., Gudnadottir, V.G., Gudmundsdottir, E., Bjornsson, A., Ingvarsson, B., Ingason, A., Sigfusson, S., Hardardottir, H., Harvey, R.P., Lai, D., Zhou, M., Brunner, D., Mutel, V., Gonzalo, A., Lemke, G., Sainz, J., Johannesson, G., Andresson, T., Gudbjartsson, D., Manolescu, A., Frigge, M.L., Gurney, M.E., Kong, A., Gulcher, J.R., Petursson, H., Stefansson, K., 2002. Neuregulin 1 and susceptibility to schizophrenia. Am. J. Hum. Genet. 71, 877–892.

Tang, J.X., Chen, W.Y., He, G., Zhou, J., Gu, N.F., Feng, G.Y., He, L., 2004. Polymorphisms within 5¢ end of the Neuregulin 1 gene are genetically associated with schizophrenia in the Chinese population. Mol. Psychiatry 9, 11–12.

Toro, C., Deakin, J.F., 2005. NMDA receptor subunit NRI and postsynaptic protein PSD-95 in hippocampus and orbitofrontal cortex in schizophrenia and mood disorder. Schizophr Res. 80, 323–330.

Weinberger, D.R., 1988. Schizophrenia and the frontal lobe. Trends Neurosci. 11, 367–370.

Williams, N.M., Preece, A., Spurlock, G., Norton, N., Williams, H.J., Zammit, S., O'Donovan, M.C., Owen, M.J., 2003. Support for genetic variation in neuregulin 1 and susceptibility to schizophrenia. Mol. Psychiatry. 8, 485–487.

Yang, J.Z., Si, T.M., Ruan, Y., Ling, Y.S., Han, Y.H., Wang, X.L., Zhou, M., Zhang, H.Y., Kong, Q.M., Liu, C., Zhang, D.R., Yu, Y.Q., Liu, S.Z., Ju, G.Z., Shu, L., Ma, D.L., Zhang, D., 2003. Association study of neuregulin 1 gene with schizophrenia. Mol. Psychiatry. 8, 706–709.

Zavitsanou, K., Katsifis, A., Filomena, M., Xu-Feng, H., 2004. Investigation of M1/M4 muscarinic receptors in the anterior

cingulate cortex in schizophrenia, bipolar disorder, and major depression disorder. Neuropsychopharmacology. 29, 619–625.
Zavitsanou, K., Ward, P.B., Huang, X.F., 2002. Selective alterations in ionotropic glutamate receptors in the anterior cingulate cortex in schizophrenia. Neuropsychopharmacology. 27, 826–833.
Zhao, X., Shi, Y., Tang, J., Tang, R., Yu, L., Gu, N., Feng, G., Zhu, S., Liu, H., Xing, Y., Zhao, S., Sang, H., Guan, Y., St, C.D., He, L., 2004. A case control and family based association study of the neuregulin1 gene and schizophrenia. J. Med. Genet. 41, 31–34.