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Excitatory and inhibitory neurotransmission is chronically altered following perinatal NMDA receptor blockade

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

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

Chao Deng
University of Wollongong, chao@uow.edu.au

Teresa M. Du Bois
University of Wollongong, teresadb@uow.edu.au

Mei Han
University of Wollongong, mei@uow.edu.au

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Abstract

N-methyl-d-aspartate (NMDA) receptor blockade in rodents induces behavioural and neurochemical changes reminiscent of schizophrenia symptoms and pathology. To examine how NMDA receptor blockade affects glutamatergic and GABAergic pathways when administered during early brain development, [3H]MK-801 and [3H]muscimol binding to NMDA and GABAA receptors was examined at four time-points following injections of phencyclidine (PCP) or saline on postnatal days (PN)7, 9 and 11. [3H]MK-801 binding was significantly increased in PCP-treated rats in the thalamus from PN18 to PN96, in the prefrontal and anterior cingulate cortices at PN32, and in the hippocampus at PN96. In a similar manner, [3H]muscimol binding was increased in PCP-treated rats in the thalamus and hippocampus from PN18 to PN96, and in the prefrontal and anterior cingulate cortices at PN32. Glutamatergic and GABAergic transmission is therefore chronically altered by this treatment, which has relevance to disease processes that may be involved in schizophrenia.

Keywords

Excitatory, inhibitory, neurotransmission, chronically, altered, following, perinatal, NMDA, receptor, blockade

Disciplines

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TITLE PAGE

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Authors:	Teresa Marie du Bois[a,b], Chao Deng[a,b], Mei Han[a,b], Kelly Anne Newell[a,b] and Xu-Feng Huang[a,b*]
	a: Schizophrenia Research Institute (SRI), Sydney, Australia b: Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia
*Corresponding author:	Professor Xu-Feng Huang University of Wollongong, School of Health Sciences, Northfields Avenue, NSW, 2522, Australia Phone: +61 2 42214300 Fax: +61 2 42214096 Email: xhuang@uow.edu.au

ABSTRACT

N-methyl-D-aspartate (NMDA) receptor blockade in rodents induces behavioural and neurochemical changes reminiscent of schizophrenia symptoms and pathology. To examine how NMDA receptor blockade affects glutamatergic and GABAergic pathways when administered during early brain development, [^{3}H]MK-801 and [^{3}H]muscimol binding to NMDA and $GABA_A$ receptors was examined at four time-points following injections of phencyclidine (PCP) or saline on postnatal days (PN)7, 9 and 11. [^{3}H]MK-801 binding was significantly increased in PCP-treated rats in the thalamus from PN18 to PN96, in the prefrontal and anterior cingulate cortices at PN32, and in the hippocampus at PN96. In a similar manner, $GABA_A$ receptor binding was increased in PCP-treated rats in the thalamus and hippocampus from PN18 to PN96, and in the prefrontal and anterior cingulate cortices at PN32. Glutamatergic and GABAergic transmission is therefore chronically altered by this treatment, which has relevance to disease processes that may be involved in schizophrenia.

Key words: brain development, $GABA_A$ receptor, NMDA receptor, phencyclidine, schizophrenia

1. INTRODUCTION

Glutamate, predominantly through its actions on *N*-methyl-D-aspartate (NMDA) receptors, plays a key role in brain developmental processes such as neuronal migration, synaptogenesis and synaptic plasticity (McDonald and Johnston 1990; Pearce et al. 1987). The timing of expression of glutamate receptors appears to be crucial for normal brain development, since changes in the activity of glutamate receptors may interfere with these processes (Ritter et al. 2001; Ritter et al. 2002).

During development in the rat, particularly postnatal days (PN)7–14, the brain is highly sensitive to the toxic effects of modulation of the NMDA receptor (Haberny et al. 2002), which may relate to the increased expression of specific NMDA receptor subunits (Mitani et al. 1998). Given the important role of the NMDA receptor in brain developmental processes, antagonism of this receptor could have severe long-term detrimental effects such as reduced and/or non-functional neuronal connections (Behar et al. 1999).

In rodents, treatment with NMDA receptor antagonists such as phencyclidine (PCP) and MK-801 during the perinatal period induces neuronal apoptosis (Fredriksson et al. 2004; Hansen et al. 2004; Harris et al. 2003; Ikonomidou et al. 1999; Wang et al. 2001). In the long-term, perinatal NMDA receptor blockade impairs cognitive function (Andersen and Pouzet 2004; Sircar 2003; Stefani and Moghaddam 2005; Wang et al. 2001; Wiley et al. 2003), sensorimotor gating (Harris et al. 2003; Wang et al. 2001; Wang et al. 2003) and locomotor activity (du Bois et al. 2008b; Facchinetti et al. 1993; Wang et al. 2001), which are particularly relevant to schizophrenia symptoms and pathology. Abnormal expression of neurotransmitter receptors

including dopamine D2 (du Bois et al. 2008a; Sircar and Soliman 2003) and NMDA (Harris et al. 2003; Wang et al. 2001; Wilson et al. 1998) is a further long-term consequence of perinatal NMDA receptor blockade. Recently it was shown that perinatal PCP treatment caused a selective loss of parvalbumin-containing (GABAergic) neurons in the cortex of rats at adulthood (Wang et al. 2007).

Hypofunction of NMDA receptors has been implicated in schizophrenia, since NMDA receptor anatgonists such as PCP and ketamine mimic schizophrenia symptoms so well in healthy individuals and strongly exacerbate symptoms in schizophrenia patients (Javitt and Zukin 1991). However, post mortem studies of the binding site of the NMDA receptor have revealed inconsistent results. Increased [^{3}H]MK-801 binding has been reported in the anterior and posterior cingulate cortices (Newell et al. 2005; Zavitsanou et al. 2002), superior temporal gyrus (Nudmamud and Reynolds 2001) and putamen (Kornhuber et al. 1989) of schizophrenia patients versus controls, while binding was reported as unchanged in other areas including the frontal and entorhinal cortices, hippocampus and amygdala (Kornhuber et al. 1989). Conversely, decreased NMDA receptor binding has been observed in the hippocampus (Dean et al. 1999), using [^{3}H]TCP.

Findings also point to a deficit in GABAergic transmission in schizophrenia. Decreased GAD_{67}, a marker for GABAergic terminals, has been found in the prefrontal cortex (Akbarian et al. 1995; Guidotti et al. 2000; Volk et al. 2000). Reductions of GABAergic interneurons have been found in anterior cingulate and prefrontal cortices and hippocampus (Benes et al. 1998; Benes et al. 1991). Moreover, reductions of parvalbumin-containing interneurons have been reported in superficial

layers of the prefrontal cortex (Beasley and Reynolds 1997; Reynolds et al. 2002). Deficits in GABA synthesis and release may therefore contribute to the observed increase in [^{3}H]muscimol binding in several brain regions (Benes et al. 1992; Benes et al. 1996; Deng and Huang 2006; Newell et al. 2007).

Along with perinatal PCP treatment of rodents, another developmental model of schizophrenia (prenatal methylazoxymethanol acetate (MAM) administration) has shown decreased parvalbumin-containing interneurons (Lodge et al. 2008), suggesting that common targets and pathways contribute to the schizophrenia-like pathology in these animal models. The fact that these models alter the expression of parvalbumin-containing (GABAergic) neurons has important construct validity, as disinhibition of pyramidal neurons from reduced GABAergic activity is thought to contribute to schizophrenia pathology (Lisman et al. 2008). To further investigate alterations to key pathways implicated in schizophrenia, we investigated NMDA and $GABA_A$ receptor binding in various brain regions of rats at several time-points following perinatal PCP exposure, to determine if interruption to NMDA receptor function early in development can have persistent long-term consequences on brain function. We hypothesised that changes in NMDA receptor expression would tightly influence expression of $GABA_A$ receptors in brain regions examined.

2. EXPERIMENTAL PROCEDURES

2.1 Animals

Timed pregnant Sprague-Dawley rats were obtained at gestation day 14 from the Animal Resource Centre (Perth, WA, Australia). They were housed individually under a 12:12-hour light-dark cycle in a temperature controlled environment. Food

and water was freely available. Day of birth was considered postnatal day (PN)0. Pups were sexed on PN7 and litters were assigned to PCP or saline groups (5 litters/group). Male pups were kept in the litters until weaning but were not treated. Pups were weaned at PN24-28 and were then housed in pairs. Female rats were used because the literature shows that treating female rat pups on PN7, 9 and 11 with PCP produces apoptosis and alterations in sensorimotor gating, motor activity and working memory (Wang et al. 2001; Wiley et al. 2003), all of which have particular relevance to schizophrenia symptoms and pathology. This study was approved by the Animal Ethics Committee of the University of Wollongong, and procedures complied with the Australian Code of Practice for the Care and Use of Animal for Scientific Purposes, which conforms to International Guiding Principles for Biomedical Research Involving Animals. All efforts were made to minimize numbers of animals used and their suffering.

2.2 Perinatal PCP treatment

On PN7, 9 and 11, pups were given a subcutaneous injection of 10 mg/kg phencyclidine hydrochloride (Sigma, Castle Hill, NSW, Australia) or 0.9% NaCl at a volume of 1 ml/kg. Six rats from each group (PCP and control) were sacrificed at four different time-points (24 hr, 1 week, 3 weeks or 12 weeks) following their last injection for biochemical analyses, representing juvenile (PN12 and 18), adolescent (PN32) and adult (PN96) ages.

2.3 Histology

Rats were sacrificed by carbon dioxide asphyxiation and brains were removed and frozen in liquid nitrogen immediately after extraction. Brain tissue was kept at -80°C

until sectioning. Brains were cut into 14 µm coronal sections with a cryostat at -18°C, and were mounted on Polysine™ microscope slides. Sections were collected at levels approximately corresponding to bregma 3.70 and -5.20 mm, with the aid of a standard rat brain atlas (Paxinos and Watson 1986). Tissue sections were stored at -20°C until further use.

2.4 NMDA receptor binding

The method for NMDA receptor binding was taken from Newell et al. (2005). Sections were incubated at room temperature for 2.5 hr in 30 mM *N*-2-hydroxyethyl piperazine-*N*′-2-ethanesulphonic acid (HEPES) buffer, pH 7.5, containing 100 mM glycine, 100 mM glutamate, 1 mM ethylenediaminetetraacetic acid (EDTA) and 20 nM [^{3}H]MK-801 (specific activity 17.1 Ci/mmol, Perkin Elmer, Boston, Massachusetts, USA). Nonspecific binding was determined by incubating adjacent sections with [^{3}H]MK-801 in the presence of 20 mM MK-801. Following incubation, sections were washed twice for 20 min each at 0ºC in 30 mM HEPES containing 1 mM EDTA (pH 7.5). Autoradiographic images for NMDA receptor binding were taken using a Beta-Imager™ camera (BioSpace, Paris, France). Sections were scanned for 3.5 hr at a high-resolution setting. A series of sections with known amount of radioactivity were used as standards in all scans. Quantitative analysis of these images was performed using the program β-Image Plus (version 4, BioSpace).

2.5 $GABA_A$ receptor binding

The method for $GABA_A$ receptor binding followed that of Newell et al. (2007). Sections were pre-incubated three times for 5 min at 4ºC in 50 mM Tris-citrate (pH 7). Sections were then incubated for 45 min at 4ºC in the same buffer containing 3 nM

[^{3}H]muscimol (specific activity 29.5 Ci/mmol; PerkinElmer, USA). Non-specific binding was determined by the addition of 100 μM GABA to adjacent sections. Following incubation, sections were rinsed four times for 2 s in 4ºC buffer and dried in a stream of cool air. Sections were opposed to Kodak BioMax MR film with Amersham microscales for 12 weeks. Autoradiographs were developed using Kodak GBX developer and fixed with Kodak GBX fixer. Autoradiographic images were captured and analysed using a computer-assisted image analysis system, Multi-Analyst, connected to a GS-690 Imaging Densitometer (Bio-Rad, California).

2.6 Quantification

A set of sections from each age and treatment group were stained with cresyl violet for confirmation of anatomical structures. Specific binding was calculated by subtracting non-specific binding from total binding. Non-specific binding was less than 30% of total binding. The data presented is the average of three brain sections from each of the six rats per group. Areas quantified included the prefrontal and anterior cingulate cortices, the ventral thalamus and hippocampus. Sub-regions of the hippocampus (CA1-3) were quantified, but no differences were found so the mean of the sub-regions was used in the results.

2.7 Statistical Analyses

Statistical analyses were performed using SPSS (version 15.0, SPSS Inc., Chicago). Two-way (age x treatment) ANOVAs were used to analyse NMDA and $GABA_A$ receptor binding among individual brain regions. Unpaired two-tailed t-tests were used to compare binding across developmental time-points and for comparing binding in PCP and control groups at individual time-points. Pearson's correlations were used

to examine the relationship between NMDA and $GABA_A$ receptor binding at individual time-points in overall groups. Data is expressed as means ± S.E. Significance was set at $p<0.05$. The measurement of radioligand binding signals is presented in fmoles/mg of tissue equivalent.

3. RESULTS

3.1 NMDA receptor binding across developmental time-points

Prefrontal cortex

In the prefrontal cortex, there were significant main effects of age ($F_{3, 34} = 107.35$, $p<0.001$) and treatment ($F_{1, 34} = 23.20$, $p<0.001$), as well as interaction between the two factors ($F_{3, 34} = 4.23$, $p<0.01$). NMDA receptor binding significantly increased with age in the control group from PN12 to PN96 ($p<0.01$). In the PCP group binding also progressively increased but this was only significant from PN12 to PN18 ($p<0.001$). In terms of treatment effects, NMDA receptor binding was significantly increased in PCP-treated rats at PN18 (18%, $p<0.001$) and 32 (16%, $p<0.05$) compared to controls (Fig. 1A). At PN96 there was no change compared to controls.

Anterior cingulate cortex

The anterior cingulate cortex also showed significant main effects of age ($F_{3, 37} = 191.29$, $p<0.001$) and treatment ($F_{1, 37} = 25.21$, $p<0.001$), as well as interaction between the two factors ($F_{3, 37} = 28.81$, $p<0.001$). In terms of age differences, NMDA receptor binding progressively increased significantly until PN96 in the control group ($p<0.05$). Conversely, in the PCP group, binding significantly increased until PN32 ($p<0.001$) and then declined at PN96 ($p<0.01$). Perinatal PCP treatment had the effect

of significantly increasing NMDA receptor binding (Fig. 1B) at PN32 only (37%, $p<0.001$; Fig. 2A, B) compared to controls.

Thalamus

In the thalamus there were significant main effects of age ($F_{3, 40} = 263.7$, $p<0.001$) and treatment ($F_{1, 40} =95.0$, $p<0.001$), as well as interaction between the two factors ($F_{3, 40} = 12.7$, $p<0.001$). The control group displayed a temporal pattern whereby binding increased from PN12 to PN18 ($p<0.001$) then increased slightly more from PN32 to PN96 ($p<0.05$). The PCP-treated group also showed a large increase in binding from PN12 to PN18 ($p<0.001$), but this was then followed by a further increase at PN32 ($p<0.01$) with no change at PN96. In terms of treatment effects, there were increases in NMDA receptor binding at PN18 (12%), PN32 (29%) and PN96 (22%) compared to the control group (p's <0.001; Fig. 1C).

Hippocampus

In the hippocampus there was a main effect of age ($F_{3,40} = 283.52$, $p<0.001$) but not treatment ($F_{1,40} = 0.186$, $p=0.669$), although there was significant interaction between the two factors ($F_{3,40} = 17.78$, $p<0.001$). Control rats showed an increase in binding up until PN32 ($p<0.01$) and PCP rats showed progressive increases in binding until PN96, significant at all points ($p<0.001$) except from PN18 to PN32. Perinatal PCP treatment increased NMDA receptor binding by 14% at adulthood only, compared to controls ($p<0.001$; Fig 1D).

3.2 $GABA_A$ receptor binding across developmental time-points

Prefrontal cortex

In the PFC, there were significant main effects of age ($F_{3,38}$ = 66.7; p<0.001) and treatment ($F_{1,38}$ = 18.1; p<0.001) on $GABA_A$ receptor binding, as well as significant interaction between the two factors ($F_{3,38}$ = 11.7; p<0.001). In terms of age effects, the control group generally showed increased binding until PN96 (p<0.05). In the PCP group, binding increased with age from PN18 to PN32 (p<0.05) then remained unchanged. In terms of treatment effects, PCP-treated rats showed increased $GABA_A$ receptor binding levels compared to controls at PN32 (43%; p<0.001), while there were no differences at the other time-points (Fig. 1E).

Anterior cingulate cortex

In the ACC, there was a significant main effect of age ($F_{3,39}$ = 30.5; p<0.001) but not treatment, although there was a significant interaction between the two factors ($F_{3,39}$ = 15.0; p<0.001). When looking at the temporal pattern of $GABA_A$ receptor binding, control rats showed increased binding levels at PN18 (p<0.05), followed by a further increase at PN96 (p<0.01). In PCP-treated rats, $GABA_A$ receptor binding increased with age until PN32 (p<0.05), after which it significantly declined (p<0.001). Similar to the prefrontal cortex, $GABA_A$ receptor binding levels were significantly elevated (59%; p<0.001; Fig. 2C, D) in PCP-treated rats compared to controls at PN32, while there were no significant differences at other time-points (Fig. 1F).

Thalamus

In the thalamus, there were significant main effects of age ($F_{3,34}$ = 70.0; p<0.001) and treatment ($F_{1,34}$ = 44.8; p<0.001) on $GABA_A$ receptor binding, as well as significant interaction between the two factors ($F_{3,34}$ = 6.5; p<0.001). In terms of age effects, the control group showed no changes in the level of $GABA_A$ receptor binding until PN96

when levels significantly declined (p<0.001). Conversely, in PCP-treated rats, binding was significantly increased at PN18 and 32 compared PN12 (p<0.01), and binding was significantly lower at PN96 than all other ages (p<0.001). In terms of treatment effects, $GABA_A$ receptor binding was significantly elevated (27-47%; p<0.01) in PCP-treated rats compared to controls at all ages from PN18 onwards (Fig 1G).

Hippocampus

In the hippocampus, there were significant main effects of age ($F_{3,34}$ = 79.5; p<0.001) and treatment ($F_{1,34}$ = 28.9; p<0.001) on $GABA_A$ receptor binding, as well as significant interaction between the two factors ($F_{3,34}$ = 4.8; p<0.01). When looking at the temporal pattern of $GABA_A$ receptor binding, control rats showed a reduction in binding at PN32 (p<0.05) and PN96 (p<0.001) compared to earlier time-points. PCP-treated rats showed a different pattern of binding, with an increase at PN18 (p<0.01), followed by reductions at PN32 and PN96 (p<0.001). Similar to what was observed in the thalamus, the PCP-treated group showed increased $GABA_A$ receptor binding compared to controls (22-45%; p<0.05) at all time-points from PN18 onwards (Fig 1H).

3.3 The relationship between NMDA and $GABA_A$ receptor binding in brain regions examined

$GABA_A$ receptor binding levels were highly correlated with NMDA receptor binding levels in the brain regions and time-points that both NMDA and $GABA_A$ receptor binding were changed. There were significant positive correlations between NMDA and $GABA_A$ receptor binding at PN32 in the prefrontal (r=0.876, p<0.001) and anterior cingulate (r=0.934, p<0.001) cortices (Fig. 3A, B). In the thalamus, there was

a significant positive correlation between NMDA and $GABA_A$ receptor binding at PN18 (r=0.614, p<0.05), 32 (r=0.751, p<0.01) and 96 (r=0.829, p<0.01; Fig. 3C). Finally, in the hippocampus, there was a significant positive correlation between NMDA and $GABA_A$ receptor binding at PN96 (r=0.919, p<0.001; Fig3D).

4. DISCUSSION

The present study examined how NMDA and $GABA_A$ receptor binding are altered during the course of brain development following perinatal PCP treatment. We found that the pattern of change of these two systems was remarkably similar and highly correlated in all regions examined, highlighting the close relationship between the two systems. In PCP-treated rats, the thalamus and hippocampus both showed increased NMDA and $GABA_A$ receptor binding levels compared to controls, which were still evident at adulthood. The prefrontal and anterior cingulate cortices also showed increased binding of these two receptors, mainly at adolescence. Control rats displayed developmental patterns of [^{3}H]MK-801 and [^{3}H]muscimol binding that are consistent with previous reports from the literature (Facchinetti et al. 1993; Insel et al. 1990; Morin et al. 1989; Xia and Haddad 1992).

Perinatal administration of NMDA receptor antagonists is known to induce massive increases in neuronal apoptosis (Fredriksson et al. 2004; Hansen et al. 2004; Harris et al. 2003; Ikonomidou et al. 1999; Wang et al. 2001). In fact, using corticostriatal slices from PN7 rats, Wang et al. (2005) showed that PCP-induced apoptosis required upregualtion of NR1 and NR2A polypeptides. Co-incubation of PCP with an antisense oligodeoxynucleotide specific to the NR1 and NR2A mRNAs prevented both PCP-induced NR1 and NR2A upregulation and cortical DNA fragmentation. We

did not observe any NMDA receptor binding changes at PN12. It is possible that the concomitant effects of apoptosis and NMDA receptor upregulation balance out any overall change in binding 24hrs after treatment cessation. Alternatively, changes in receptor expression levels may require more time to become evident at the protein level.

Wang et al. (2001) reported increased expression of NR1 one day after cessation of perinatal PCP treatment in the basal forebrain and not the hippocampus. In addition, an acute study where rats were treated with MK-801 on PN7 showed that expression of the obligatory NMDA receptor subunit, NR1, was increased in the ventral part of the hippocampus in adulthood (Harris *et al.* 2003). These results are consistent with our binding results that show NMDA receptor upregualtion in cortical regions shortly following treatment and upregualtion of binding in the hippocampus in adulthood. No studies on the effects of perinatal NMDA receptor blockade on [^{3}H]muscimol binding are available in the literature for comparison. It is unclear why the prefrontal and anterior cingulate cortices only showed changes at adolescence while the thalamus and hippocampus were affected at all time-points from PN18 onwards. The varying response of different brain regions to PCP treatment may be due to innate differences in organisation and connectivity (Benes 2000), as well as differences in temporal expression of NMDA and $GABA_A$ receptors. The observation that certain regions are affected only at adolescence, and that this time-point shows the greatest magnitude of change suggests an increased vulnerability of the brain to further adverse impacts at this developmental stage.

An explanation for the sustained increase in NMDA and $GABA_A$ receptor binding seen in PCP-treated rats is that perinatal NMDA receptor blockade could lead to a loss of or improper wiring of glutamatergic neurons and a decrease in glutamatergic transmission. This would be expected to cause a compensatory upregulation of NMDA receptors. Decreased glutamatergic activation of NMDA receptors, as well as AMPA and kainate receptors on GABAeric interneurons would be expected to reduce activity of those cells, thereby decreasing GABA release and causing compensatory increase in $GABA_A$ receptor levels. This could explain the strong positive correlation between NMDA and $GABA_A$ receptor binding observed in the present study. Investigation of AMPA and kainate receptor densities following perinatal PCP treatment would help to clarify the contribution of glutamate levels to PCP-induced pathology.

Alternatively, blockade of NMDA receptors during the critical period of receptor sensitivity could by some unknown mechanism, cause an upregulation of NMDA receptors which never returns to normal. This blockade could also selectively destroy specific GABAergic neuronal populations, and therefore a decrease in the number of GABA-producing cells may lead to an upregulation of $GABA_A$ receptors. In support of this, Wang et al. (2007) found that perinatal PCP treatment resulted in the selective loss of parvalbumin-containing interneurons in cortical brain regions in early adulthood. However, the widespread upregulation of $GABA_A$ in the present study suggests that perhaps a combination of both deficits in glutamatergic transmission and loss of specific neuronal populations contribute to increased binding of both NMDA and $GABA_A$ receptors.

A loss of or decreased stimulation GABAegic cells would presumably lead to disinhibition and over-excitation of pyramidal neurons. In the prefrontal cortex, a reduction in GABAergic transmission would reduce inhibitory inputs to pyramidal neurons, resulting in less filtering of noise, which could contribute to cognitive deficits seen in this model (Andersen and Pouzet 2004; Wang et al. 2001; Wiley et al. 2003). Similarly, in schizophrenia, disinhibition of pyramidal neurons through a loss of GABAergic outputs is thought to contribute to symptoms of the disorder (Lisman et al. 2008). In the hippocampus, disinhibition of pyramidal neurons could elevate activity of ventral tegmental area (VTA) neurons. This elevation of VTA activity has been shown to occur in another schizophrenia animal model which has deficits in hippocampal interneurons caused by prenatal methylazoxymethanol acetate (MAM) administration, and can be reversed by inactivating the subiculum (Lodge and Grace 2007). This is consistent with our previous findings of elevated tyrosine hydroxylase mRNA expression in the VTA, and decreased D2 receptor binding in striatum following perinatal PCP treatment, which we suggest reflects increased dopamine output from the VTA (du Bois et al. 2008a).

Our finding that perinatal blockade of NMDA receptors may lead to long-term deficits in glutamatergic and GABAergic neurotransmission is relevant to schizophrenia pathology and treatment. Interestingly, co-administering agents that enhance GABAergic transmission with antipsychotic drugs provide more effective therapy to schizophrenia patients than antipsychotics alone (Casey et al. 2003; Wassef et al. 2000), including in treatment-resistant patients (Dursun and Deakin 2001; Tiihonen et al. 2003). Moreover, agents which enhance NMDA receptor channel activity such as agonists of the glycine binding site (eg. D-cycloserine, glycine and D-

serine), and sarcosine, a glycine transport inhibitor, have had good success in treating when co-administered with antipsychotic drugs (Coyle and Tsai 2004; Lane et al. 2006; Tsai et al. 2004). In addition, it has been recently shown that an mGlu2/3 agonist is effective alone in treating symptoms (Patil et al. 2007), and part of the effect may be related to enhancement of postsynaptic NMDA receptor function (Tyszkiewicz et al. 2004). Recently, Andersen and Pouzet (2004) showed that cognitive impairments in the Morris water maze task resulting from perinatal PCP treatment of Sprague-Dawley rats could be reversed with chronic D-serine treatment, which further supports the idea of NMDA receptor hypofunction in this model and has good predictive validity for schizophrenia.

In summary, this study has shown that blockade of NMDA receptors persistently affects NMDA and $GABA_A$ receptor binding levels during the course of brain development. It would appear that the binding changes reflect deficits in excitatory and inhibitory neurotransmission, consistent with what has been reported by others in this model. This could result in the disinihibition of pyramidal neurons, which would have flow on effects such as causing a hyperdopaminergic state. Further studies examining these possibilities are required before any firm conclusions can be made. The perinatal NMDA receptor antagonist treatment model therefore represents some of the possible disease processes that may be involved in psychiatric disorders such as schizophrenia.

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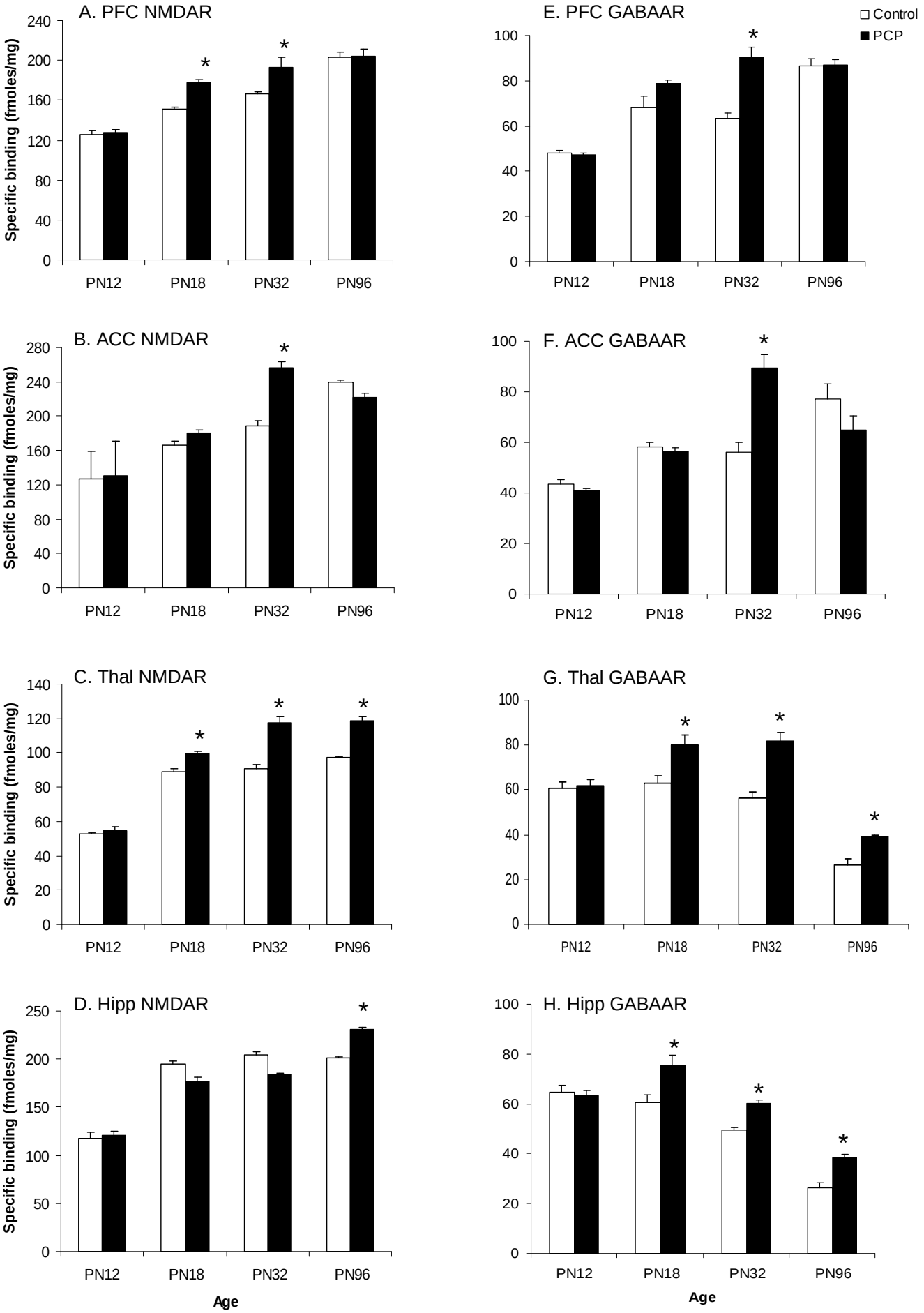
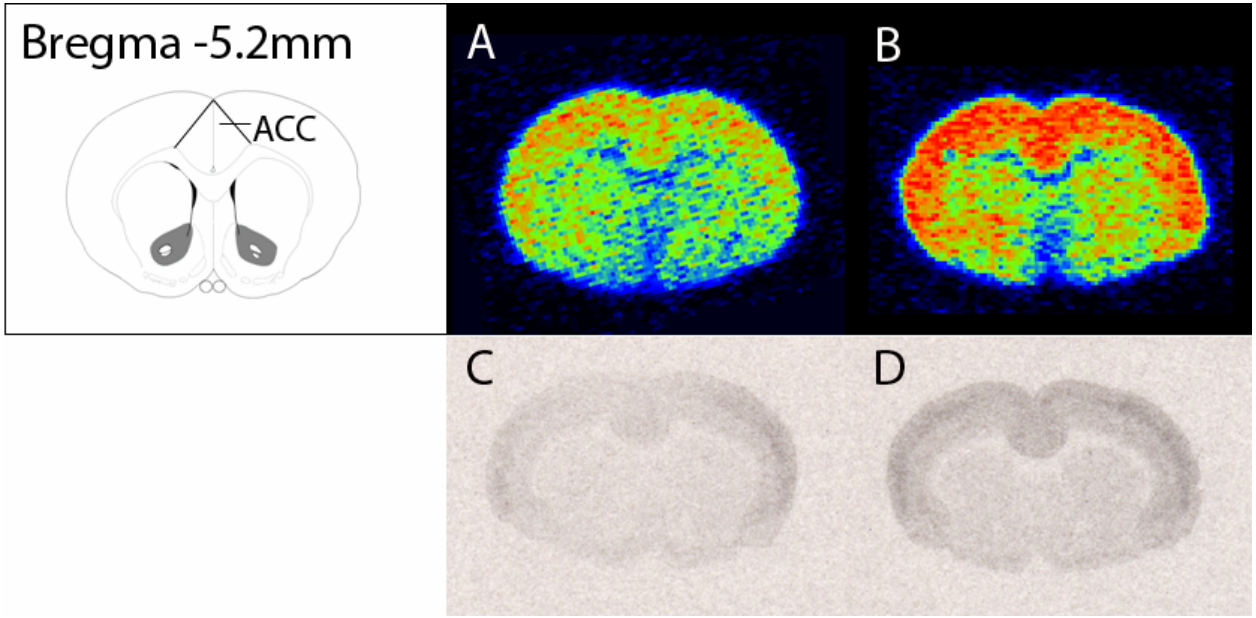
Figure legends:

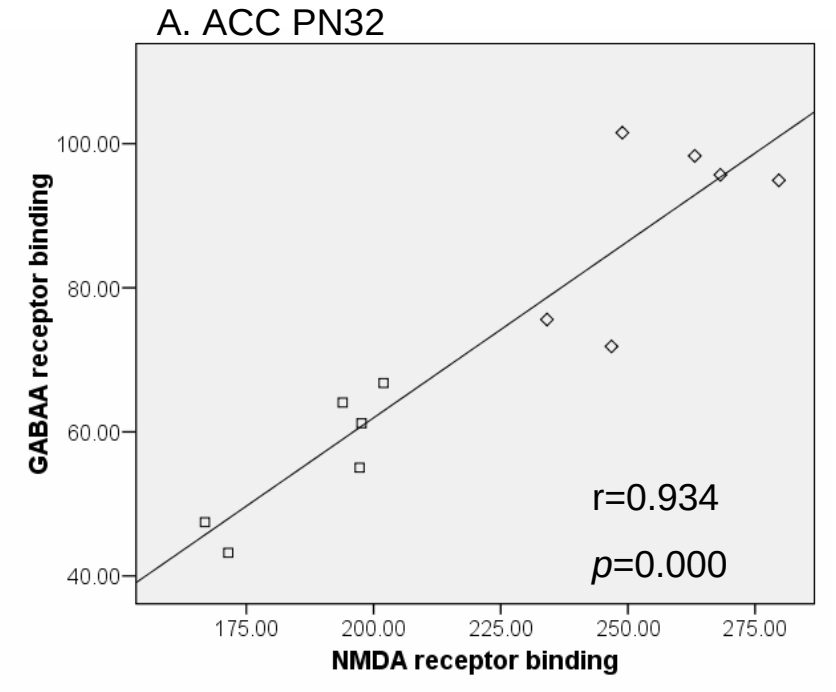
Figure 1. NMDA receptor binding (A-D) and $GABA_A$ receptor binding (E-H) in PCP-treated and control rats across developmental time-points. Abbreviations: ACC, anterior cingulate cortex; GABAAR, $GABA_A$ receptor; Hipp, hippocampus; NMDAR, NMDA receptor; PCP, phencyclidine; PFC, prefrontal cortex; PN, postnatal day, Thal, thalamus. *p<0.05 vs. control at individual time-point.

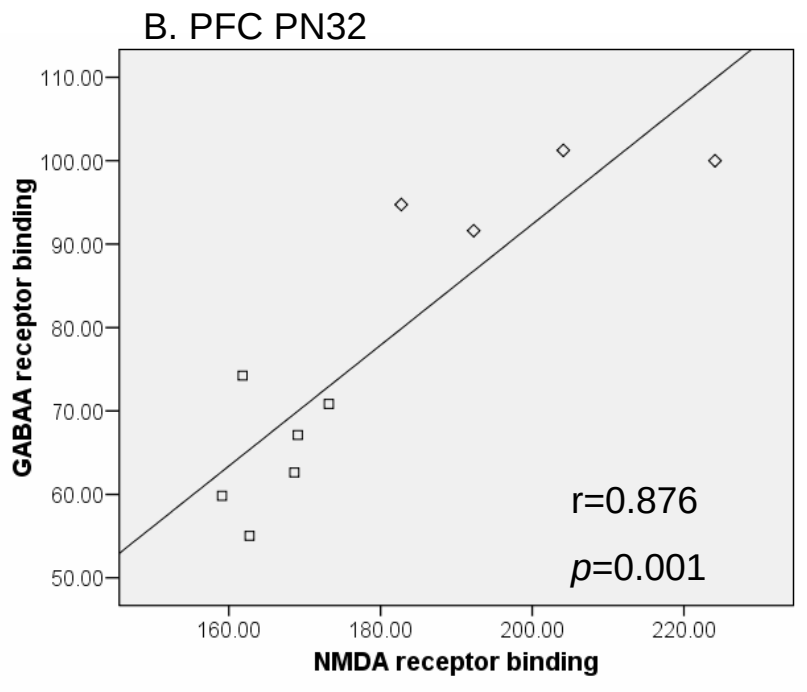
Figure 2. NMDA receptor binding is shown in the anterior cingulate cortex of control (A) and PCP-treated (B) groups at PN32. Similarly, $GABA_A$ receptor binding is shown in the anterior cingulate cortex control (C) and PCP-treated (D) groups at PN32, where the greatest magnitude of change in NMDA and $GABA_A$ receptor binding was observed. Abbreviations: ACC, anterior cingulate cortex; PCP, phencyclidine; PN, postnatal day.

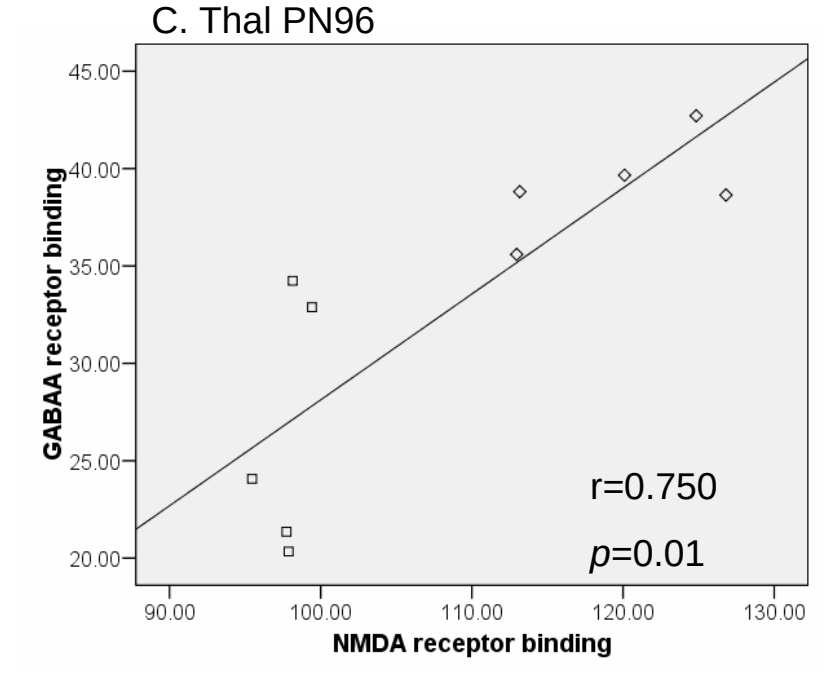
Figure 3. Correlation between $GABA_A$ and NMDA receptor binding in the anterior cingulate and prefrontal cortices at PN32 (A and B) and in the thalamus and hippocampus at PN96 (C and D). Control rats are indicated by the square markers and PCP-treated rats by the diamond markers. Abbreviations: ACC, anterior cingulate cortex; Hipp, hippocampus; PFC, prefrontal cortex; PN, postnatal day; Thal, thalamus.

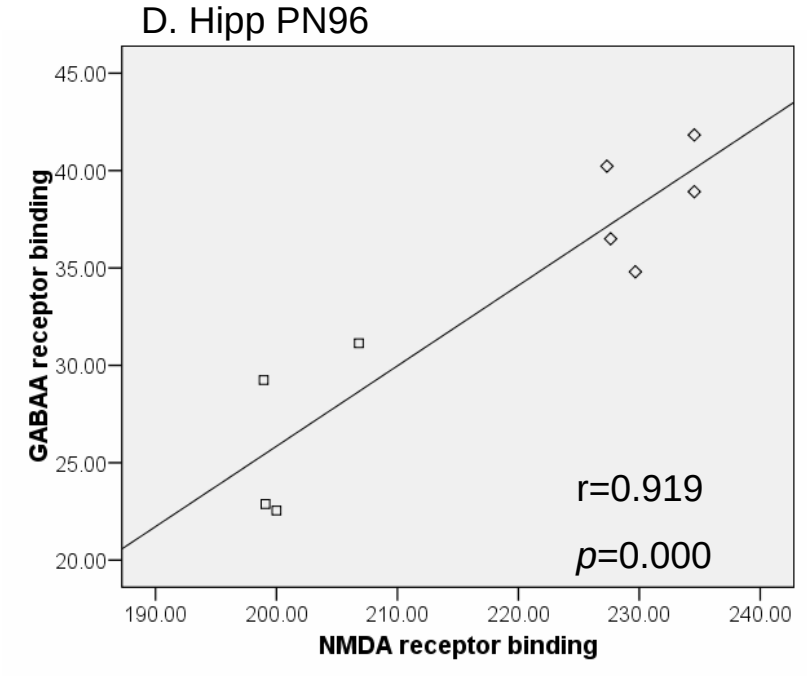

A. PFC NMDAR
B. ACC NMDAR
C. Thal NMDAR
D. Hipp NMDAR
E. PFC GABAAR
F. ACC GABAAR
G. Thal GABAAR
H. Hipp GABAAR
Control
PCP
Specific binding (fmoles/mg)
PN12
PN18
PN32
PN96
Age
*


Bregma -5.2mm
ACC
A
B
C
D


A. ACC PN32
GABAA receptor binding
NMDA receptor binding
r=0.934
p=0.000


B. PFC PN32
GABAA receptor binding
NMDA receptor binding
r=0.876
p=0.001


C. Thal PN96
GABAA receptor binding
NMDA receptor binding
r=0.750
p=0.01


D. Hipp PN96
GABAA receptor binding
NMDA receptor binding
r=0.919
p=0.000

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Contributors

Xu-Feng Huang, Chao Deng and Teresa du Bois designed the study and wrote the protocol. Teresa du Bois, Mei Han and Chao Deng performed the animal experiments. Teresa du Bois, Kelly Newell and Mei Han performed the receptor autoradiography experiments. Teresa du Bois performed the quantification and statistical analyses and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflict of interest.

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