# NEUROTRANSMITTER RECEPTOR BINDING IN THE POSTERIOR CINGULATE CORTEX IN SCHIZOPHRENIA AND IN THE PHENCYCLIDINE MOUSE MODEL

## AN EXPLORATION OF THE NMDA HYPOFUNCTION HYPOTHESIS OF SCHIZOPHRENIA

A thesis submitted in fulfillment of the
requirements for the award of the degree

DOCTOR OF PHILOSOPHY

From

SCHOOL OF HEALTH SCIENCES
UNIVERSITY OF WOLLONGONG

By

Kelly Newell

2007

# CERTIFICATION

I, Kelly Newell, declare that this thesis, submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the School of Health Sciences, University of Wollongong, is entirely my own work unless otherwise referenced or acknowledged. This manuscript has not been submitted for qualifications at any other academic institution.

Kelly Newell

May 2007

# ACKNOWLEDGEMENTS

I would like to sincerely thank several people, without whose assistance and guidance this research project and thesis would not have been possible.

To my supervisors Professor Xu-Feng Huang and Dr Katerina Zavitsanou, I would like to thank you for your encouragement, guidance and support. In particular, thanks to Xu-Feng for your support in the preparation of my published papers and my thesis, and for your continuous support throughout this period of study. Your motivational support and encouragement was greatly appreciated, and this thesis would not have been possible without it. I thank you for your commitment to help see this project through to its final completion, and your wise guidance during its development. Finally, I thank you for providing me with the opportunity to work with a talented team of researchers.

This work was supported by the St. George Foundation, and the Neuroscience Institute of Schizophrenia and Allied Disorders (NISAD) utilizing infrastructure funding from NSW Health. I am sincerely grateful to NISAD and the St George Foundation for giving me the financial ability to conduct this research. I am very proud to be a part of NISAD.

Thank you to Ms Mei Han for your help sacrificing the mice in the animal studies.

To Associate Professor Ken Russel and Professor David Griffiths, School of Mathematics and Applied Statistics, University of Wollongong, for suggestions regarding the statistical analyses.

Post-mortem brain tissue was obtained from the Tissue Resource Center, which is supported by the University of Sydney, NISAD, National Institute of Alcohol Abuse and Alcoholism and NSW Department of Health. Thank you for providing the brain tissue and clinical and demographic information regarding the schizophrenia and control post-mortem brain tissue.

To my family and friends for your continual support and understanding throughout this period of study. Special thanks to my sister Karen, and fellow student Teresa for enthusiastically reading my final thesis and providing editorial advice.

Finally, special thanks to my husband Stephen, who provided tremendous support throughout my PhD and for his continual faith in me over the duration of this study.

# PUBLICATIONS

The following publications and presentations have arisen directly from the work conducted for this thesis.

## Publications in Refereed Journals

**Newell, K.A.,** Zavitsanou, K., and Huang, X.F. Short and long-term changes in NMDA receptor binding in mouse brain following chronic phencyclidine treatment. Journal of Neural Transmission, In Press.

**Newell, K.A.,** Zavitsanou, K., and Huang, X.F. Opposing short and long-term effects on muscarinic M1/4 receptor binding following chronic phencyclidine treatment. Journal of Neuroscience Research, 85: 1358-1363, 2007.

**Newell, K.A.,** Zavitsanou, K., Kum-Jew, S. and Huang, X.F. Alterations of muscarinic and GABA receptor binding in the posterior cingulate cortex in schizophrenia. Progress in Neuropsychopharmacology & Biological Psychiatry, 31: 225-233, 2007.

**Newell, K.A.,** Deng, C., and Huang, X.F. Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. Experimental Brain Research, 172 (4): 556-560, 2006.

**Newell, K.A.,** Zavitsanou, K., and Huang, X.F. Ionotropic glutamate receptor binding in the posterior cingulate cortex in schizophrenia patients. NeuroReport, 16(12): 1363-1367, 2005.

## Publications in Conference Proceedings

**Newell, K.A.** Zavitsanou, and Huang X.F. The posterior cingulate cortex: a site of altered neural circuitry in schizophrenia and in NMDA hypofunction. Schizophrenia Bulletin, 33(2): 321, 2007.

**Newell, K.A.,** Zavitsanou, K., and Huang, X.F. NMDA and muscarinic M1/4 receptor binding density is decreased 2 weeks after, but not immediately after chronic PCP treatment. Proceedings of the Australian Neuroscience Society, the 26th Annual Meeting. 17: 105, Sydney 2006.

**Newell, K.A.,** and Huang, X.F. Increased NMDA receptor density in the posterior cingulate cortex in schizophrenia. Proceedings of the Australian Neuroscience Society, the 25th Annual Meeting. 16:124, Perth 2005.

**Newell, K.A.,** Zavitsanou, K., and Huang, X.F. Alterations in the serotonin and cannabinoid systems in the posterior cingulate cortex in schizophrenia. Proceedings of the Australian Neuroscience Society, the 24th Annual Meeting. 15:138, Melbourne 2004.

**Newell, K.A.,** Klose, B., Zavitsanou, K., Han, M., and Huang, X.F. Effects of clozapine and haloperidol on motor activity in the chronic PCP mouse model. 7th Biennial Australasian Schizophrenia Conference. 7:115, Sydney, 2002.

## Additional Publications

The following publications have arisen from other projects I have been involved in throughout my doctoral studies.

Han, M., **Newell, K.A**., Zavitsanou, K., Deng, C., and Huang, X.F. Effects of antipsychotic medication on muscarinic M1 receptor mRNA expression in the rat brain. Journal of Neuroscience Research. Submitted.

Han, M., Deng, C., Burne, T.H.J., **Newell, K.A.,** and Huang, X.F. Olanzapine-induced weight gain is related to a reduction in histamine H1 receptor mRNA expression in the rat hypothalamus. Schizophrenia Research. Submitted.

Deng, C., Han, M., **Newell, K.A.,** and Huang, X.F. No changes in cannabinoid CB1 receptor binding density in the superior temporal gyrus in schizophrenia. Schizophrenia Bulletin, 33(2): 308, 2007.

Han, M., Deng, C., **Newell, K.A.,** and Huang, X.F. Histamine mRNA expression is decreased in the rat hypothalamus following olanzapine treatment. Schizophrenia Bulletin, 33(2): 317, 2007.

Huang, X.F., du Bois, T., Hsu, C., Eftimovska, J., Tan, Y.Y., Zavitsanou, K., **Newell, K.A.,** and Deng, C. NMDA receptor hypofunction during early brain development: relevance to schizophrenia. Schizophrenia Bulletin, 33(2): 317, 2007.

Zavitsanou, K., Nguyen, V., **Newell, K.,** Ballantyne, P., and Huang, X.F. Increased [$^{3}$H]MK801 binding in the cingulate cortex of the rat after a single injection of phencyclidine. Proceedings of the Australian Neuroscience Society, the 26$^{th}$ Annual Meeting. 17: 102, Sydney, 2006.

Han, M. Zavitsanou, K. **Newell, K.,** and Huang, X.F. Increased CB1 mRNA in the cortical areas of mice prone to diet-induced obesity. Proceedings of the Australian Neuroscience Society, the 24$^{th}$ Annual Meeting. 15:113, Melbourne 2004.

Huang, X.F., Han, M., **Newell, K.,** and Zavitsanou, K. A low level of Y1 and Y5 gene expression may contribute to the prevention of chronic high energy diet-induced obesity. Proceedings of the Australian Neuroscience Society, the 23$^{rd}$ Annual Meeting. 14:228, Adelaide 2003.

# Abstract

Schizophrenia is a severe psychiatric disorder with no clear cause. Recent evidence suggests that N-methyl-D-aspartate (NMDA) receptor hypofunction may underlie the pathogenesis of schizophrenia. The posterior cingulate cortex (PCC) has been shown to be the most susceptible brain region to damage caused by NMDA hypofunction in rodents. This suggests that the PCC may play an important role in schizophrenia pathology. However, studies examining neurotransmitter balance in the PCC in schizophrenia have until now been neglected. Furthermore, the long-term consequences of NMDA hypofunction on neurotransmitter balance in animal models have not been studied. The aims of this study were to investigate neurotransmitter receptor binding profiles in the PCC in schizophrenia, while also examining the effects of chronic phencyclidine (PCP; an NMDA antagonist) treatment on neurotransmitter receptor binding in mouse brain in the long-term following treatment. To achieve these aims, the study was divided into two parts.

In experimental part A, PCC sections from 10 schizophrenia and 11 control subjects matched for age, gender and post-mortem interval were obtained from the Tissue Resource Center, Sydney. Using quantitative autoradiography, the density of several neurotransmitter receptors was examined. The results demonstrated specific alterations in neurotransmitter receptors in the PCC in schizophrenia. Specifically, increased NMDA, gamma-aminobutyric acid A ($GABA_A$) and cannabinoid 1 (CB1) receptor density was found in this region, along with reduced muscarinic 1/4 (M1/4) and serotonin 2A (5-hydroxy-tryptamine, $5HT_{2A}$) receptor density. No changes were found in $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate or M2/4 receptor density in the PCC in schizophrenia subjects compared to controls. These

results have shown for the first time that there are specific neurotransmitter imbalances in this region, and it is possible that these changes stem from NMDA hypofunction.

In experimental part B, mice were treated chronically (14 days) with PCP. Using quantitative autoradiography the density of several receptors was examined in the short (1hr and 24hr) and long-term (14 days) following chronic PCP treatment. In addition, clozapine and haloperidol were tested for their ability to prevent the PCP-induced alterations in neurotransmitter receptor density. The results showed opposing effects of PCP treatment on neurotransmitter receptor density in the short compared to the long-term. While there were limited increases in NMDA receptor density in the short-term, there were widespread reductions in NMDA receptor density in the long-term following chronic PCP treatment. Muscarinic M1/4 receptor binding, which was increased in the short-term, showed reductions in the long-term in the limbic system, caudate-putamen and cortex, but not in the thalamus in which no change was found. Clozapine and haloperidol treatments were both unable to prevent the PCP-induced long-term changes in receptor density.

In conclusion, this study has provided new information regarding neurotransmitter alterations in the PCC in schizophrenia and in mouse brain in the long-term following chronic PCP treatment. These findings may assist not only in understanding the pathology of schizophrenia, but also for designing new pharmacological treatments for this disease.

# Table of Contents

# List of Figures

# List of Tables

# List of Abbreviations

Abbreviations used throughout this thesis are defined below.

| Abbreviations | Definition |
|---|---|
| 5-HT | 5-hydroxy-tryptamine |
| Acb | Nucleus accumbens |
| ACC | Anterior cingulate cortex |
| AMPA | α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid |
| APD | Antipsychotic drug |
| BSA | Bovine serum albumin |
| CNS | Central nervous system |
| CPu | Caudate-putamen |
| D-AP5 | D-2-amino-5-phosphnopentanoate |
| DSM | Diagnostic and Statistical Manual of Mental Disorders |
| EDTA | Ethylenediamine teraacetic acid |
| GABA | Gamma-aminobutyric acid |
| GAD | Glutamic acid decarboxylase |
| GAT 1 | GABA transporter 1 |
| HEPES | N-(2-Hydroxyethyl) piperazine-N`-2-ethane sulfonic acid |
| LSD | Lysergic acid diethylamide |
| LV | Lateroventral |
| MRI | Magnetic resonance imaging |
| NHMRC | National Health and Medical Research Council |
| NMDA | N-methyl-D-aspartate |
| PCC | Posterior cingulate cortex |
| PCP | Phencyclidine |
| PET | Positron emission tomography |
| RSC | Retrosplenial cortex |
| $\Delta^9$-THC | $\Delta^9$-tetrahydrocannabinol |