Developmental Neurobiology Panel
Summary and Update of Collaborative Research Program

Details of all projects forming part of the DNP Collaborative Research Program.

Title	Investigators/ Site	Technique(s)	Target(s)	Tissue Requested
Expression of synaptic markers in the prefrontal cortex in schizophrenia	Fung S, Weickert CS POWMRI	primers and ABI probes (gene expression changes) western blotting (protein) in situ hybridization and immunohistochemistry (anatomical expression patterns)	VAMP, GAP43, VGLUT1, VGAT, complexin 1, complexin 2	10ug of total RNA (with SRL) 2ug/ul aliquots (total ~20ug) protein
Changes in Alternate BDNF Transcript Expression in the Human Prefrontal Cortex in Patients with Schizophrenia	Wong J, Weickert CS POWMRI	quantitative real-time PCR on an ABI 7900HT using gene and splice variant specific primers and taqman probes (mRNA expression)	Brain derived neurotrophic factor (BDNF) and its alternate splice variants I-IX, II-IX, IV-IX, VI-IX and pan	total RNA of 10 µg (the same aliquot used in SRL Aug 2008)
Changes in trkB Expression in the Prefrontal Cortex in Patients with Schizophrenia	Wong J, Weickert CS POWMRI	Western blotting	TrkB and its alternate splice variants which include the trkB-full length, trkB TK- and trkB-SHC	2ug/ul aliquots (total ~46ug) (1 aliquot protein)
Neuronal PAS domain protein 3 (NPAS3) and fibroblast growth factor receptor 1 (FGFR1) expression in the DLPFC of patients with schizophrenia	Duncan C, Weickert CS POWMRI	qPCR to quantify mRNA expression and Western blot analysis and optical density measurments to quantify protein expression	NPAS3 FGFR1	2 plates of the 40 allocated for total RNA 2 allotments of protein homogenates (40 µg each)
GABAA receptor α-subunit mRNA expression in DLPFC of patients with schizophrenia	Duncan C, Weickert CS POWMRI	qPCR to quantify the expression of α-subunit mRNAs	GABAA receptor subunits α1, α2, α3, α4, α5 (GABRA1, GABRA2, GABRA3, GABRA4, GABRA5)	within the 5 plates (10 µg) of total RNA allocated to SRL/POWMRI
GABAA receptor α-subunit protein expression in DLPFC of patients with schizophrenia.	Duncan C, Weickert CS POWMRI	Western blot analysis and optical density measurments to quantify the expression of α-subunit mRNAs	GABAA receptor α1 subunit (GABRA1) GABAA receptor α2 subunit (GABRA2)	One allotment of protein (40 µg)

Expression of the glucocorticoid receptor (GR) and other stress-responsive genes in the DLPFC of patients with schizophrenia	Sinclair D, Weickert CS, Fullerton J POWMRI	quantitive real-time PCR to quantify gene expression of the above targets in the DLPFC of individuals with schizophrenia	NR3C1 alpha variant (GRα) (Taqman probe ID Hs00230818_m1) NR3C1 beta variant (GRβ) (Taqman probe ID Hs00354508_m1) NR3C1 gamma variant (GRγ) (Taqman primer/probe by design) RGS4 (Taqman probe ID Hs00194501_m1) VDR (Taqman probe ID Hs01045840_m1)	within the 5 plates (10 µg) of total RNA allocated to SRL/POWMRI
Tumour necrosis factor death receptor pathways in schizophrenia	Catts V, Weickert CS POWMRI	quantitative RT-PCR using commercially available TaqMan primers.	APRIL FAS ligand (CD95) FAS receptor c-FLIP BID	within the 5 plates (10 µg) of total RNA allocated to SRL/POWMRI
The mechanisms by which DNA variants in the NRG1 gene lead to altered transcriptional regulation of NRG1 isoforms in schizophrenia brains	Fullerton J, Kwok J, Weickert CS, Sivagnanasundaram S, Schofield P POWMRI	deep targeted resequencing 2-3kb upstream of transcription start sites. Q RT-PCR (with Murray Cairns) Putative regulatory regions investigated using sequence conversion, cloned and assessed for functionality via luciferase assay Electrophoretic Mobility Shift Assay using nuclear protein extracts	NRG1 isoform I promoter NRG1 isoform II & IV promoter NRG1 isoform I promoter	1µg DNA
Gene and miRNA expression in the dorsolateral prefrontal cortex (BA46) in schizophrenia	Cairns M, Tooney P, Beveridge N, Carroll A, Gardiner E, Santarelli D. Newcastle	Gene and miRNA expression analysed using human HT12 gene expression arrays & miRNA sentrix array matrix (Illumina). Validation of gene and miRNA expression using quantitative RT-PCR. Analysis of 3´UTR polymorphisms by resequencing 3'UTR segments of NRG1 cDNA.		full cohort of RNA (schizophrenia and controls) from the BA46
Brain abundant signal protein 1 (BASP1) in brain development and schizophrenia pathogenesis	Sivagnanasundaram S, Weickert CS POWMRI	mRNA transcripts will be measured in the TRC cohort of 37 cases and 37 controls using quantitative PCR technique.	BASP1	10µg of total RNA, which is part of the total request for SRL

Markers of migration and GABAergic interneurons in schizophrenia pathogenesis	Sivagnanasundaram S, Fung S, Weickert CS POWMRI	mRNA transcripts will be measured in the TRC cohort of 37 cases and 37 controls using quantitative PCR technique. Protein levels will be measured using Western Blot approach and/or ELISA.	Doublecortin Neuron navigator 1 Parvalbumin Somatostatin Cholecystokinin Calbindin Calretinin Neuropeptide Y Vasointestinal polypeptide	10µg of total RNA, which is part of the total request for SRL 2µg/ul aliquots (total ~ 20µg)
Quantitative assessment of neuregulin 1 and ErbB4 in schizophrenia dorsolateral prefrontal cortex	Sivagnanasundaram S, Fullerton J, Weickert CS POWMRI	mRNA transcripts will be measured in the TRC cohort of 37 cases and 37 controls using quantitative PCR technique. Protein levels will be measured using Western Blot approach and/or ELISA.	Nrg1 Type I alpha + beta Nrg1 Type II alpha + beta Nrg1 Type III Nrg1 Type IV	10µg of total RNA, which is part of the total request for SRL 2µg/ul aliquots (total ~ 20µg)
Changes in BDNF protein and trkB mRNA Expression in the Prefrontal Cortex in Patients with Schizophrenia	Wong J, Weickert CS POWMRI	Changes in protein expression will be analysed. Western blotting will be conducted using antibodies for our specific proteins of interest. Global changes in mRNA expression will be analysed by quantitative real-time PCR on an ABI 7900HT using gene and splice variant specific primers and taqman probes.	Protein: BDNF mRNA: TrkB -full length and trkB TK-	from previously supplied aliquots
Metabotropic glutamate receptor 5 (mGluR5) in schizophrenia pathogenesis	Newell K, Huang XF Wollongong	mGluR5 binding density will be measured using receptor binding autoradiography Homer will be measured using Western Blot	mGluR5 Homer	4 slides per case (part of the 20-25 batch sent out). One allotment of protein will be needed for the Homer detection
mGlu2/3 receptor binding in the PFC of schizophrenia patients and controls	Frank E, Huang XF Wollongong	In brain slides mGlu2/3 - binding density will be measured using receptor binding autoradiography - functional binding will be measured via a [^{35}S]GTPyS binding assay	mGlu2/3 mGlu2	8 slides per case (part of the 20-25 batch sent out).

NR2b receptor binding density and mRNA expression in the prefrontal cortex of schizophrenia subjects	Huang XF, Newell K, Deng C, Frank E Wollongong	Both NR2b protein and mRNA expression will be analysed using: - Radioligand binding assay using [3H] Ifenprodil. - In situ hybridisation to detect mRNA at the intracellular carboxy terminal where NR2b is anchored to PSD95.	Nr2B	6 slides per case (part of the 20-25 batch sent out).
Cannabinoid (CB1) receptor density and functionality in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia.	Dalton V, Nguyen V, Zavitsanou K ANSTO	In vitro autoradiography and agonist stimulated autoradiography	Cannabinoid CB1 receptor protein Cannabinoid CB1 receptor functionality	48 trial slides 8 slides from each subject
Investigation of GABAA receptor density in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia.	Zavitsanou K Dalton V \ANSTO	In vitro autoradiography	GABAA receptors	4 slides from each subject
Markers of GABAergic interneurons in schizophrenia pathogenesis	Fung S, Sivagnanasundaram S, Weickert CS	mRNA transcripts will be localised using in situ hybridisation	Somatostatin	14μm tissue sections from the entire TRC cohort (2 sections per case)
NR2B receptor levels and phosphorylation in the prefrontal cortex of schizophrenia subjects	Dawson A, Newell K, Huang XF	Both NR2B protein levels and phosphorylation will be analysed using the method of western blot.	NR2B	Two allotments of protein
Changes in ESR1 and ESR1 mRNA and Protein Expression in the Prefrontal Cortex in Patients with Schizophrenia	Wong J, Weickert CS	Global mRNA expression by quantitative real-time PCR on an ABI 7900HT using gene and splice variant specific primers and taqman probes Western blotting will be conducted using antibodies for our specific proteins of interest.	mRNA: Wild-type ESR1, Delta 7 ESR1 Protein: Wild-type ER alpha receptor, Delta 7 ER alpha receptor	10 μg RNA from previously supplied 1 aliquot protein (~46ug)
Neuronal PAS domain protein 3 (NPAS3) laminar localisation in the human DLPFC and expression in patients with schizophrenia	Duncan C, Weickert CS	in situ hybridisation to localise and quantify mRNA expression of NPAS3 – at both the 5' region and 3' region	NPAS3	2 in situ sets = 2 slides per case from the fresh frozen tissue sections for each in situ
Expression of GAP43 in the prefrontal cortex in schizophrenia	Fung S, Weickert CS	in situ hybridization to determine anatomical expression patterns of GAP43.	GAP43 CPLX-1	4-6 x 14μm DLPFC sections per case

Investigation of the role of ubiquitin protein system in schizophrenia	Fernandez-Enright F, Huang XF	protein array and Western Blot techniques	Ubiquitin proteins For ubiquitin E1: UCHL1 ubiquitin E2: E2D1 and E2D3 ubiquitin E3 ligase: Itch, Mdm2,cbl and PIAS	allotment of protein (2µg/µl aliquot)
Investigation of GABAA receptor density in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia.	Dalton V, Verdurand M, Nguyen V, Zavitsanou K ANSTO	In vitro autoradiography	GABAA receptors	3 slides from each subject
Tumour necrosis factor death receptor pathways in schizophrenia – Mark II	Catts V	quantitative RT-PCR using commercially available TaqMan primers.	Lamin A/C PSD-95 Spinophilin	within the aliquots of total RNA sample already allocated to the SRL