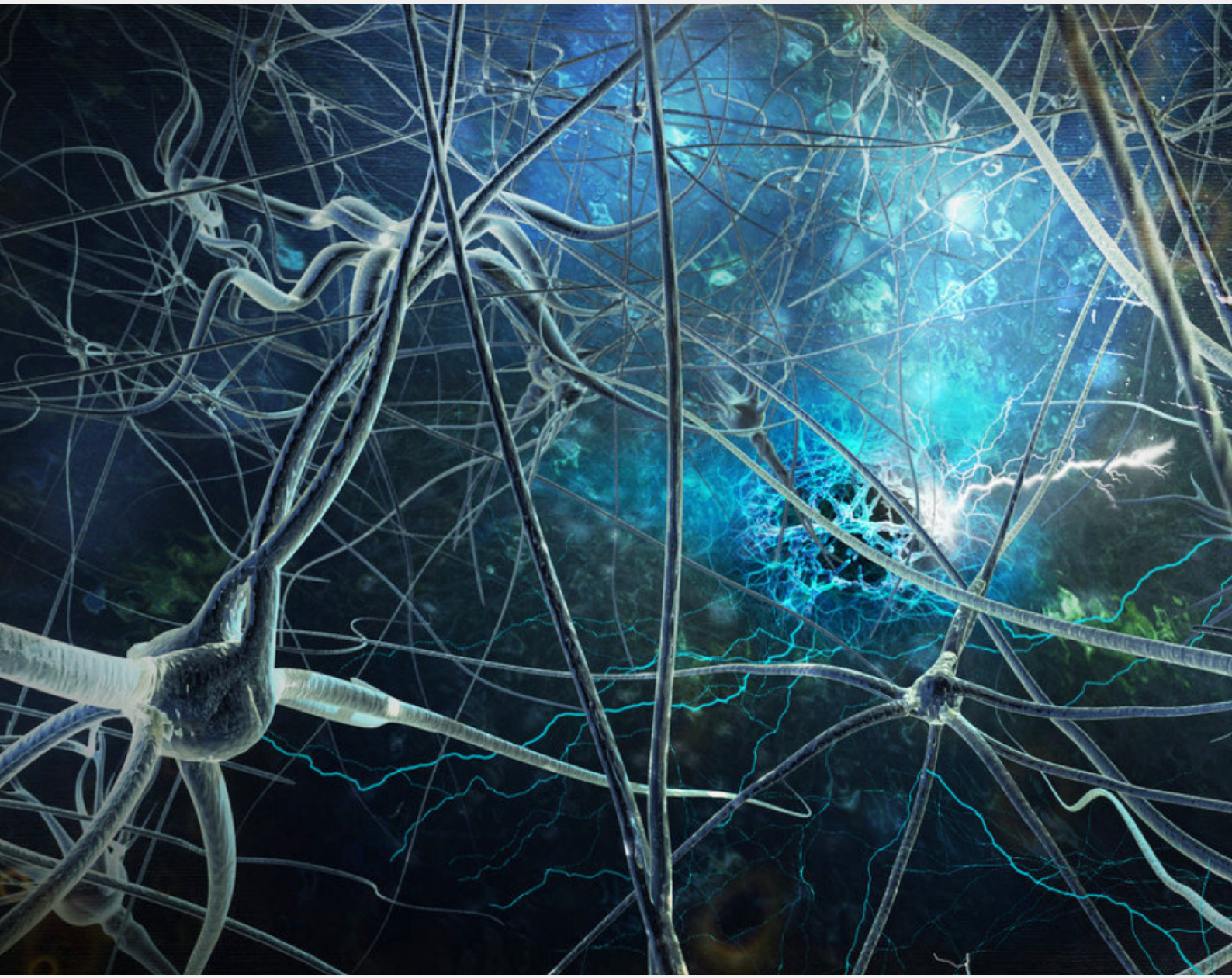
Biological Psychiatry
AUSTRALIA



6th Annual Scientific Meeting, Newcastle

Abstract book

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Table of Contents

Thursday 13 October			
Time	**Event**	**Speaker**	**Presentation Title**
8:00 – 9:00	Registrations open		
8:50 – 9:00	Opening Address	A/Prof Paul Tooney, Chair, Local organising committee	
9:00 – 10:00	7th Aubrey Lewis Lecture Chair: Lauren Harms	Dr Andrew Zalesky	Neuroimaging and Connectomics in Psychiatry
10:00 – 10:30	Data Blitz 1 Chair: Duncan Sinclair	Asad Ali	Altered steroidogenesis in fetal brain and placenta in a Developmental Vitamin D deficient fetal rat
		Tim Karl	The Effects of Acute CBD in a Mouse Model for Fragile X Syndrome
		Chantel Fitzsimmons	The integration of RNA sequencing and exome sequencing highlights the functional significance of rare mutations in patients with Schizophrenia
		Joshua R Atkins	Characterisation of 500 whole-genomes; a pilot study into personalised approaches into schizophrenia
		Brian Dean	Evidence for impaired glucose metabolism in the striatum from subjects with schizophrenia
10:30 – 11:00	Morning tea		
11:00 – 12:30	Symposium 1: We are what we eat: Regulation of brain function and behaviour by nutrition and the gut microbiome Chair: Zoltan Sarnyai	Margaret Morris	Impact of the western diet on brain, gut and behaviour
		Zoltan Sarnyai	Ketogenic diet in schizophrenia: From gut microbiota to brain and behaviour
		Ma-Li Wong	Inflammasome signalling, gut microbiome composition and rodent behaviour
		G Paul Amminger	The role of fatty acids and inflammation in emerging psychotic disorders by the Vienna omega-3 study.
12:30 – 1:15	Data Blitz 2 Chair: Kelly Newell	Karly Turner	Adult Vitamin D deficiency and attentional processing
		Xiaoying Cui	The impact of vitamin D on L-type calcium channel trafficking and function in developing cortex
		Sharon Hollins	Small RNA regulation of neural gene expression in response to environmental exposure
		Chenxing Liu	Pathway analysis in schizophrenia identified multiple converging pathways across three populations.
		Jee Hyun Kim	fMRI investigation of fear extinction in adolescent and adult humans
		Laura Bell	Increased expression of ATP13A4 in the prefrontal cortex of subjects with schizophrenia
		Alice Petty	Elevated Dopamine Release in the Dorsal Striatum: A New Animal Model of Schizophrenia
1:15 – 2:30	Lunch and Posters	Poster presenters to arrive at posters by 1:30	
2:30 – 3:30	ECR Session: Research career and a life – you can and should have both Chair: Paul Tooney	Nikola Bowden	
		Ben Harrison	
		Andrea Gogos	
3:30 – 4:00	Afternoon tea		
4:00 – 5:00	Keynote Lecture Chair: Susan Rossell	Dr Oliver Howes	Making sense of inflammation, stress and schizophrenia: latest brain imaging findings
6:30	Cocktail function, Customs House Hotel, 1 Bond Street, Newcastle		

Friday 14 October			
Time	**Event**	**Speaker**	**Presentation Title**
9:00 – 10:00	4th Isaac Schweitzer Lecture Chair: Pat Michie	Prof Cynthia Shannon Weickert	Neuroinflammation in Schizophrenia
10:00 – 10:15	Data Blitz 3 Chair: Karly Turner	Chiara Cristina Bortolasci	Atherogenicity index and oxidative stress biomarkers in bipolar disorder and tobacco use disorder
		Tertia Purves-Tyson	Inflammatory cytokines are elevated in the substantia nigra of a subset of people with schizophrenia
		Adrienne Grech	Stress-environmental enrichment paradigms in a two-hit model: sex-specific hippocampal changes
10:15 – 10:45	Morning tea		
10:45 – 12:15	Symposium 2: Mechanisms underpinning the progression of brain network pathology Chair: Michael Breakspear	Luca Cocchi	Distinct effects of local perturbation of peripheral versus core regions in the cortical hierarchy
		Andrew Zalesky	Progressive and accelerated white and grey matter ageing in schizophrenia
		Christine Guo	Distinct neurobiological signatures of brain connectivity in neuropsychiatric disorders
		Leonardo L. Gollo	Network effects of local perturbations in neural activity: Implications for the understanding and treatment of psychiatric disorders
12:15 – 1:00	Special Symposium: Highest ranked student abstracts Chair: Mary-Claire Hanlon	Samuel Millard	Maternal fluoxetine treatment influences anxiety- and depressive-like behaviours in offspring.
		Maria A. Di Biase	White matter connectivity disruptions in early and chronic schizophrenia
		Maximus Berger	Allostatic load is associated with positive symptoms in schizophrenia and first-episode psychosis
1:00 – 2:30	Lunch and Posters	Poster presenters to arrive at posters by 1:45	
1:15 – 1:45	Annual General Meeting		
2:30 – 4:00	Symposium 3: "Surfing brain waves" as a way of understanding normal and abnormal cognition Chairs: Ulrich Schall and Pat Michie Discussant: Michael Breakspear	Juanita Todd	Can "low-level" relevance filters reveal basic principles of learning?
		Patrick S. Cooper	Cortical dynamics underpinning effective cognitive control
		Matthew Hughes	Understanding executive control dysfunction in schizophrenia using magnetoencephalography (MEG)
		Nigel Jones	Gamma oscillations in schizophrenia – hearing the signal through the noise
4:00 – 4:15	Conference Discussant	Darryl Eyles	
4:15 – 4:25	Prizes and Awards		
4:25 – 4:30	Conference Close		
4:30 – 5:00	Afternoon Tea		

Welcome

Dear Colleagues,

On behalf of the Local Organising Committee (LOC) I wish to welcome you to the Biological Psychiatry Australia 2016 meeting in Newcastle. We are excited to be the first non-state capital city to host the annual meeting for our society.

We wish to thank our major sponsors the University of Newcastle and in particular Professor Kevin Hall (Deputy Vice Chancellor Research and Innovation) and Professor Robert Callister (Deputy Head of the Faculty of Health and Medicine). We would also like to thank and acknowledge the financial support of our gold sponsor the Preclinical Neurobiology Hub of the Centre for Brain and Mental Health Research at the University of Newcastle and our silver sponsor Otsuka/Lundbeck. Lastly we wish to thank Kate Reeve-Parker and Janssen for sponsoring the Keynote Lecture at BPA2016 and bringing Dr. Oliver Howes from Kings College London to Newcastle.

I would like to thank the other members of the BPA2016 LOC (particularly Lauren Harms) and the BPA Executive Committee, who have greatly assisted in organising our annual meeting in Newcastle. We believe that we have put together a program that highlights the diverse and stimulating biological based research conducted on psychiatric conditions by our Australian based researchers. We have also ensured that BPA continues to be an excellent platform for our PhD and early/mid-career researchers to gain experience presenting their research and interacting with the leaders in their fields. Indeed, half of the delegates at BPA2016 are students or early/mid-career researchers.

The BPA2016 LOC thanks our existing members for their continued support and welcome our new members to the society. We believe our program will continue to stimulate intellectual discussion and provide new directions for biological-based research into mental illness. We thank you for coming to our big country town on the Hunter River and hope that you enjoy the meeting.

Kind Regards,
Paul Tooney
Chair, Local Organising Committee
BPA 2016 Conference

Local Organising Committee

Chair: Paul Tooney
Lauren Harms
Susan Rossell
Mary-Claire Hanlon
Pat Michie
Ulrich Schall

Isaac Schweitzer Award Lecture: Neuroinflammation in Schizophrenia

Professor Cynthia Shannon Weickert, BA MPhil PhD, Macquarie Group Foundation Chair of Schizophrenia Research, Faculty – School of Psychiatry, University of New South Wales,
This is a joint position between Schizophrenia Research Institute, the University of New South Wales and Neuroscience Research Australia.

Friday, 14 Oct, 9:00 – 10:00 am

Prof Cyndi Shannon Weickert is dedicated to help those suffering from schizophrenia. Prof Shannon Weickert obtained a BA in Biology and Psychology from Keuka College in upstate New York. She earned a PhD in Biomedical Science from Mount Sinai Medical School in New York City. Prof Shannon Weickert spent the next 11 years in the Intramural Research program at the National Institute of Mental Health (NIMH) in Washington DC. In 2006, she became a Professor at the University of New South Wales. She is presently a CIA on an ARC Discovery Grant; a CIB on a NHMRC project grant with A/Prof T Karl of Western Sydney University; a CIC on another NHMRC project grant with Prof D Hodgson, Emeritus Prof P Michie, Dr L Harms, Prof U Schall and Dr J Todd from Newcastle University. In 2012, she became an NHMRC Senior Research Fellow and was recently promoted to Principle Research Fellow (2017-2021).

Currently, Prof Shannon Weickert leads a Molecular Neurobiology laboratory of 4 postdoctoral fellows, 10 graduate students and 5 Research Support persons. She leads a translational research program that uses insights from the molecular and cellular neuropathology of schizophrenia to identify novel treatments for people with schizophrenia and contributes to the running of clinical trials. She co-leads the clinical team with her husband, A/Prof Thomas Weickert.

Her work has broad impact beyond psychiatry including examining molecular mechanisms by which hormones and growth factors cooperate to control gene expression and experimental examination of how sex hormones impact social development in adolescence. She has made pivotal contributions to the conceptualization of schizophrenia as a neurodevelopmental disorder and is best known for her pioneering work on brain-derived neurotropic factor (BDNF) and estrogen receptor.

Recently, Prof Shannon Weickert and her team have identified that there is neuroinflammation in the brains of people with schizophrenia suggesting anti-inflammatory agents may be of therapeutic benefit to those suffering with psychosis.

Prof Shannon Weickert has a total of 177 papers often publishing in high ranking journals such as *Molecular Psychiatry*, *Archives of General Psychiatry* and *Biological Psychiatry*. She has a total of 9668 cites and an h-index of 57. In 2015 she awarded the competitive Nina Kondelos Prize by the Australian Neuroscience Society which is awarded to a female neuroscientist who is making outstanding contributions to basic or clinical research.

Aubrey Lewis Award Lecture: Neuroimaging and Connectomics in Psychiatry

Dr Andrew Zalesky, Melbourne Neuropsychiatry Centre, University of Melbourne

Thursday, 13 Oct, 9:00 – 10:00 am

Andrew Zalesky completed his PhD in the Department of Electrical and Electronic Engineering in 2006 at the University of Melbourne, Australia. Working alongside neuroscientists, he has utilised his engineering expertise in networks to understand human brain organization in health and disease. He developed some of the most widely used methods for modeling and performing statistical inference on brain networks. His methods have been implemented in leading software packages and widely used to investigate brain connectivity abnormalities in disease. He identified the first evidence of connectome pathology in schizophrenia. Andrew is supported by a fellowship from the National Health and Medical Research Council of Australia. He is based at the University of Melbourne and holds a joint appointment between the Melbourne Neuropsychiatry Centre and the Melbourne School of Engineering. He leads the Systems Neuropsychiatry Group. In 2016, Andrew co-authored the first textbook on connectomics. He has published in a diverse range of fields, including optics, statistics, neuroimaging and schizophrenia. He was awarded the Young Tall Poppy Science Award (2014). He serves on the editorial boards of NeuroImage, Brain Topography and Network Neuroscience.

Keynote Lecture: Making sense of inflammation, stress and schizophrenia: latest brain imaging findings

Dr Oliver Howes BM BCh MA MRCPsych PhD DM

Thursday, 13 Oct, 4:00 – 5:00 pm

Oliver Howes is Professor of Molecular Psychiatry at the Institute of Psychiatry, Psychology and Neuroscience, King's College, London and Programme Leader at the MRC Clinical Sciences Centre, Imperial College, London. His clinical work is as Consultant Psychiatrist at The Maudsley Hospital where, amongst other things, he runs a service for refractory psychoses.

His research interests centre on the causes and treatment of psychosis. His recent work has focused on characterising the dopaminergic system in the psychotic prodrome, the effects of antipsychotic drugs on the endocrine system, & the causes of cognitive impairment in schizophrenia. This work has been recognised through a number of awards including the Schizophrenia International Research Society Rising Star Award 2013, European Psychiatric Association Biological Psychiatry Prize (2012), the Royal Society of Medicine Psychiatry Prize (2010), Royal College of Psychiatrists research prize (2005), the British Association of Psychopharmacology Clinical Psychopharmacology Prize (2007) and awards at the International Conference of Schizophrenia Research (2006), the European College of Neuropsychopharmacology (2008). He was made an honorary associate of the European College of Neuropsychopharmacology in 2006.

Other career highlights include a stint as junior potato scrubber on a farm.

Symposium: We are what we eat: Regulation of brain function and behaviour by nutrition and the gut microbiome

Thursday, 13 Oct, 11:00 am – 12:30 pm

Chair: Zoltan Sarnyai, James Cook University

Description: It is becoming increasingly recognised that systemic changes in immune, metabolic and hormonal processes alter brain function and behaviour and may mediate the effects of a number of established risk factors for psychiatric disorders such as stress, diet and infections. The bi-directional communication between the brain and the gastrointestinal system has come to the forefront of psychiatric neuroscience research recently. The understanding of how the external and internal environment interact with the brain through alterations of the microbiome may results in novel therapeutic and prevention approaches to decrease the significant burden caused by psychiatric disorders. This symposium will highlight significant recent discoveries from leading research groups in this field. A brief introduction by the Chair on the general framework of gut microbiome-brain interactions will be followed by the description of the deleterious effects of high fat/high carbohydrate Western diet on gut microbiome and cognition (Morris) and by a study in animals of the relationship between a therapeutic ketogenic diet (high-fat, low-carbohydrate diet), changes in gut microbiota and schizophrenia-like behaviours (Sarnyai). The mechanistic links between chronic stress, changes in gut microbiome composition and immune activation will be presented in the context of mood disorders (Wong). Finally, the effects of dietary omega-3 fatty acids in the prevention of psychosis will be presented, with emphasis on the underlying anti- inflammatory processes and lipid metabolism (Amminger). The symposium will be closed by a discussion moderated by the Chair which will allow an integration of the talks presented through the contribution of the speakers and the audience.

1. Margaret Morris, University of New South Wales

Impact of the western diet on brain, gut and behaviour Recent evidence has linked excessive intake of the so-called western diet (rich in saturated fat and refined carbohydrates) with cognitive deficits in adolescents as well as adults. We have shown in rats that both high fat, and high sugar diets can impair hippocampal dependent behaviours, even after short-term exposure. Potential mechanisms underlying the cognitive deficits include neuroinflammation and reductions in brain neurotrophic factors. Another potential mechanism is diet-related changes in gut microbiota, as it is now clear that the microbiome affects behaviour through the brain-gut axis. We have shown that even intermittent exposure to an energy-dense, western diet is sufficient to shift the biota towards that seen in obese rats, with reduced microbial diversity. These findings may have relevance to people who repeatedly switch between healthy and 'junk' foods We are currently examining the impact of probiotic treatment on the behavioral and gut biota changes in rats, and will explore the molecular changes induced in the brain. Examining key underlying processes is an essential step to enable testing of novel interventions in humans to combat diet-related cognitive deficits.

2. Zoltan Sarnyai, James Cook University

Ketogenic diet in schizophrenia: From gut microbiota to brain and behaviour.
Systemic and brain abnormalities in glucose and energy metabolism have been recently identified as possible pathophysiological mechanisms in schizophrenia, raising the possibility of the use of metabolic manipulations as a novel treatment modality. We have identified that therapeutic ketogenic diet (KD), a high-fat, low-carbohydrate dietary intervention, prevents the development of schizophrenia-like

hyperactivity, stereotyped behaviour, abnormal social interaction and working memory deficits induced by acute pharmacological NMDA receptor hypofunction. Following up on these findings we showed that long term KD normalises the sensorimotor gating deficits induced by acute and chronic NMDA receptor blockade in mice. We also identified widespread rearrangements of the intestinal microbiota in KD-treated animals with and without NMDA receptor blockade. Finally, we established that KD modifies the effects of NMDA receptor hypofunction on the frontal context proteome. These results suggest that KD may influence abnormal sensorimotor gating, a robust quantitative phenotype of schizophrenia, through altering the gut microbiota and frontal cortical proteome networks that participate in neural energy metabolism, protein signalling and degradation.

3. Ma-Li Wong, South Australian Health and Medical Research Institute

Inflammasome signalling, gut microbiome composition and rodent behaviour. In rodents, genetic deficiency and pharmacological inhibition of caspase-1 ameliorates stress-induced anxiety- and depressive-like behaviours. Caspase-1 inhibition and chronic stress produced similar alterations in fecal microbiome. While minocycline treatment during chronic stress resulted in gut microbiota changes that included increased relative abundance of Akkermansia spp, Blautia spp and Lachnospiracea, which are respectively related to attenuated inflammation, rebalance of gut microbiota and microbiota changes of caspase-1 deficiency. Therefore, gut microbiota via inflammasome signaling may modulate pathways that alter brain functions, and affect depressive- and anxiety-like behaviors.

4. G Paul Amminger, Orygen and University of Melbourne

The role of fatty acids and inflammation in emerging psychotic disorders by the Vienna omega-3 study. Evidence that omega-3 polyunsaturated fatty acids (n-3 PUFAs) have neuroprotective and anti-inflammatory properties and do not have clinically relevant adverse effects make them an ideal candidate for indicated prevention in young people at risk of psychosis, in whom the use of antipsychotic medication is controversial. In the first randomised controlled trial of its kind, a 12-week intervention with n-3 PUFAs reduced both the risk of progression to psychotic disorder and psychiatric morbidity in general. Notably, the majority of the individuals from the n-3 group did not show severe functional impairment and no longer experienced attenuated psychotic symptoms at follow-up 7 years (median) after baseline. Lipid biomarkers predicted treatment response and other clinical outcomes (i.e. major depression, poor psychosocial functioning). Furthermore, we found that decreased levels of cell membrane fatty acids (i.e. nervonic acid, n-3s), as well as, a marker of inflammation (i.e. IL-12p40) may serve as biomarkers predicting conversion to psychotic disorder. To date, the classification of the early stages of psychotic disorders is based on clinical characteristics, which is of limited predictive value and there is a pressing need for further enhancement of predictive models to guide targeted intervention. The identification of biomarkers for conversion to psychosis is thus an important research issue. Our latest research highlights the relevance of lipid biology and inflammation for the prediction and prevention of psychosis and other psychiatric morbidities.

Symposium: Mechanisms underpinning the progression of brain network pathology

Friday, 14 Oct, 10:45 am – 12:15 pm

Chair: Michael Breakspear, QIMR Berghofer Medical Research Institute

Description: Overview: Advances in neuroimaging techniques have critically informed how the structure and function of macroscopic neural networks are altered in psychiatry disorders. However, the genesis and evolution of such network pathologies remain elusive. This symposium will provide an overview of the potential of using neuroimaging and computational models to understand neural mechanisms supporting the development and progression of network pathologies in mental disorders. Specifically, we will discuss basic concepts and techniques, present ways to combine experiments with modelling and discuss the impact of this approach on future psychiatric research. Symposium Learning Objectives: Functional neuroimaging combined with computational modelling is emerging as a powerful tool to understand mechanisms of brain function in psychiatric disorders. This symposium aims to showcase the potential of this multimodal approach and provide insight into how neuroimaging data may be informed, or inform, computational modelling. The symposium brings together four nationally and internationally recognized experts in the analysis of neural networks in mental disorders. Our speakers will discuss how knowledge of large-scale brain networks and computational modelling can be used to comprehend how focal pathologies may propagate to distant but functionally and/or anatomically related brain regions. Attendees will gain an understanding of neural network analyses and computational models, and how such methods can be combined.

1. Luca Cocchi, QIMR Berghofer Medical Research Institute

Distinct effects of local perturbation of peripheral versus core regions in the cortical hierarchy. Background: The neural principles supporting the emergence of functional interactions between low and high levels of the human cortical hierarchy are poorly understood. Objectives: In this talk, I will describe how we combined neuroimaging, brain stimulation, network science and computational modelling to examine the occurrence of dynamic interactions in the visual hierarchy. Methods: In separate experimental sessions, we applied continues theta burst stimulation (cTBS) to the right primary visual cortex and the frontal eye field of 23 healthy adult participants. Resting state functional neuroimaging was acquired before and immediately after the application of cTBS. Results: I will start by presenting the empirical results of our multimodal approach, showing that inhibitory TMS to the early visual cortex selectively increases feedforward interactions with extrastriate visual areas, including FEF. In contrast, inhibitory TMS to FEF decreases feedback interactions with early visual areas. I will then discuss how computational modelling can be used to gain insights into the neural mechanisms explaining large-scale brain network reconfiguration following focal neural insults. Conclusions: Our findings suggest that the opposing effects of stimulation on early visual cortex and FEF on large-scale neural dynamics reflect a fast-slow timescale hierarchy from periphery to core cortical areas. These results highlight that the intrinsic temporal organization of the human brain may explain the selective propagation of localized neural pathologies in the whole brain.

2. Andrew Zalesky, Melbourne Neuropsychiatry Centre

Progressive and accelerated white and grey matter ageing in schizophrenia. Objective: Although schizophrenia has been hypothesized to be a disorder of accelerated aging it remains unclear whether the rate of brain gray matter (GM) loss and axonal white matter (WM) deterioration with age is stable, accelerated, or diminished in schizophrenia compared to healthy individuals of the same age. Method: Gray matter volume (GMV) and fractional anisotropy (FA) was mapped from age 20 to 65 years in 326

individuals with schizophrenia or schizoaffective disorder and 197 healthy controls. Polynomial regression modelled the influence of age on GMV and FA at a whole-brain and voxel level. Between-group differences in GMV and FA were regionally localized across the lifespan using permutation testing and cluster-based inference. Results: GMV was reduced in schizophrenia at all ages, peaking at a loss of 8% in the sixth decade. Rate of GMV loss was significantly accelerated in schizophrenia up to the fourth decade and plateaued thereafter. Conversely, significant reductions in FA began to emerge in schizophrenia after age 35 and the rate of FA loss with age was constant and best modelled with a straight line in both groups. The slope of this line was 60% steeper in schizophrenia compared to controls. Conclusions: Our findings suggest that schizophrenia is characterized by an initial, rapid loss of GM that plateaus in middle life, followed by the emergence of a deficit in axonal WM integrity that progressively widens with age at a constant rate.

3. Christine Guo, QIMR Berhofer Medical Research Institute

Distinct neurobiological signatures of brain connectivity in neuropsychiatric disorders. Background: Establishing an evidence-based diagnostic system informed by the biological (dys)function of the nervous system is a major priority in psychiatry. This objective, however, is often challenged by difficulties in identifying homogeneous clinical populations. In this talk, I will discuss how we apply functional neuroimaging to uncover neurobiological signatures for Melancholia, a biological and endogenous subtype for major depressive disorder. Method: We employed naturalistic functional magnetic resonance imaging (fMRI) paradigms – resting state and free viewing of emotionally salient films – to search for neurobiological signatures of depression sub- types. fMRI data were acquired from 57 participants; 17 patients with melancholia, 17 patients with (non-melancholic) major depression and 23 matched healthy controls. Results: I will discuss the prominent loss of functional connectivity in patients with melancholia, particularly in hub regions during natural viewing, and in the posterior cingulate cortex while at rest. The default mode network showed diminished reactivity to external stimuli in melancholia, which correlated with the severity of anhedonia. Moreover, the subgenual ACC, a potential target for treating depression with deep brain stimulation (DBS), showed divergent changes between the two depression subtypes, with increased connectivity in the non-melancholic and decreased connectivity in the melancholic subsets. Conclusion: These findings reveal neurobiological changes specific to depression subtypes during ecologically valid behavioural conditions, underscoring the critical need to respect differing neurobiological processes underpinning depressive subtypes.

4. Leonardo L. Gollo, QIMR Berghofer Medical Research Institute

Network effects of local perturbations in neural activity: Implications for the understanding and treatment of psychiatric disorders. Background: Non-invasive brain stimulation techniques have been touted as viable interventions to restore brain connectivity and reduce psychiatric symptoms. However, such interventions are generally guided by slowly gathered empirical observations. There is therefore a need to better understand the neural mechanisms that support the selective propagation of local perturbations at the whole-brain level. Method: I will present a computational study that systematically assessed the impact of focal perturbations in neural activity to whole brain network dynamics. This approach maps the putative effect of local perturbation of hundreds of regions. Results: The expected effect of local excitation or inhibition of a cortical region on its functional connectivity with the rest of the brain gives rise to a predictive response function (tuning curves). Highly interconnected brain regions (hubs) showed greater resilience to focal perturbations in neural activity. Conversely, neural activity changes in sensory areas caused widespread modifications in the patterns of connectivity that such regions maintain with other brain regions. Results also suggested that, more than the anatomical architecture, endogenous neural dynamics play a key role in determining the selective propagation of focal neural changes in the brain. Conclusion: Results from this work highlights putative core neural factors determining

changes in brain networks connectivity following local changes in neural activity. Such principles may: (i) facilitate the understanding of psychiatric network pathology; (ii) allow the selective modulation of altered connectivity showed in psychiatric patients.

Symposium: "Surfing brain waves" as a way of understanding normal and abnormal cognition

Friday, 14 Oct, 2:30 – 4:00 pm

Chairs: Ulrich Schall, Discipline of Psychiatry, University of Newcastle
Pat Michie, School of Psychology, University of Newcastle
Discussant: Michael Breakspear, QIMR Berghofer Medical Research Institute

Description: Electroencephalography (EEG) has enjoyed a renaissance in recent years associated with the development of new models of the neuronal mechanisms generating the EEG signal and of brain function and new methods of analyses. When compared to the high spatial resolution derived from blood flow or blood oxygenation level-derived functional brain imaging techniques, such as PET or fMRI, EEG-based examination of brain function offers superior temporal resolution at substantially lower costs. The lower spatial resolution of EEG compared to PET and fMRI can be overcome by magneto-encephalography (MEG), which however relies on costly equipment in highly specialised settings. EEG recording equipment, on the other hand, is widely available in many clinical settings, thus facilitating the use EEG-based procedures, such as event-related potentials (ERPs) or oscillatory activity, aimed at understanding basic principles of brain function and cognitive processes. The symposium will provide four examples of how EEG and MEG-based procedures examine human brain function and how they might help to understand and perhaps better diagnose and treat neuropsychiatric disorders. Juanita Todd will present ERP data that offers new insights into basic learning principles of the human brain, Patrick Cooper will present findings derived from mapping the cortical dynamics of cognitive control whereas Matt Hughes, utilising MEG, will focus on response inhibition as a measure of impaired executive control in schizophrenia. Nigel Jones will report use of animal models to clarify the nature of the relationship between abnormal regulation of gamma oscillations and behavioural phenotypes reminiscent of schizophrenia.

1. Juanita Todd, Functional Neuroimaging Laboratory, School of Psychology, University of Newcastle

Can "low-level" relevance filters reveal basic principles of learning? The term "first impression" refers to the way in which future learning and memory can be anchored to the earliest experience in a given context. It is perhaps best known and documented in literature pertaining to how our beliefs about a person are heavily influenced by the first encounter. However, in research exploring supposed "low-level" relevance filters, we have demonstrated how a disproportionately strong influence of first impressions may be a ubiquitous property of brain function. In our research the first impression influence on brain activity was measured using auditory evoked potentials, primarily mismatch negativity (MMN). In sound sequences any form of probable regularity is readily extrapolated into an inferred pattern, even without focused attention and even during sleep. In computational biology this has been framed in Bayesian terms as reflecting the presence of an "internal model" referencing a "belief" about the most likely next-state of brain activation. This belief is weighted by precision – high when the pattern is stable, lower when there is variance. Our research shows that the initial portion of a sound sequence creates a first impression that has a lasting impact on precision estimates. These precision estimates have a lasting influence on learning rates that we infer from the speed of model updating (i.e., the readiness to change one's internal model in the face of contradictory evidence).

2. Patrick S. Cooper, Functional Neuroimaging Laboratory, School of Psychology, University of Newcastle

Cortical dynamics underpinning effective cognitive control. While the pathogenesis of mental illnesses varies widely, virtually all such disorders are linked with cognitive impairments. In particular, an impairment of cognitive control, the ability to regulate thoughts and behaviours toward context-appropriate goals, is a central hallmark of many disorders of the brain. In part, this likely arises from the

fact that extensive frontoparietal networks, that underpin cognitive control, are sensitive to damage and disruption. Changes to frontoparietal networks will ultimately influence network functioning. Therefore, characterising functional dynamics of cognitive control network may provide a common line of inquiry into cognitive impairments across a range of psychiatric disorders, regardless of the underlying pathogenesis of the illness. Here, I present recent work highlighting the utility of mapping frontoparietal network dynamics to cognitive control using electroencephalography (EEG). Over a series of studies, I show that P3 event-related potential (ERP) components can be modulated in a contextually-sensitive manner. Such ERP components correspond to cognitive demands placed upon the frontoparietal network. Additionally, oscillatory activity during cognitive control produces specific spatiotemporal signatures across multiple frequency bands (e.g., frontocentral theta, posterior alpha). These oscillatory signatures are dependent on particular structural architecture of control networks. Together, cortical dynamics measured with EEG provide tell-tale signatures of frontoparietal network functioning. These signatures are ideal candidates for characterising the functional mechanisms underpinning cognitive control processes and ultimately provide novel insight into the functioning of cognitive networks in both health and disease.

3. Matthew Hughes, Brain and Psychological Services Centre, Swinburne University of Technology

Understanding executive control dysfunction in schizophrenia using magnetoencephalography (MEG). Dysfunction of the executive system is a core feature of schizophrenia that is unrelated to psychotic symptoms, exists in non-ill relatives of patients, is not markedly improved by antipsychotic medication, and presents independently from a general intellectual impairment. Researchers are increasingly utilizing encephalographic techniques such as EEG and MEG to understand such dysfunction as they afford millisecond temporal resolution, and more importantly, allow analysis of the neural oscillatory dynamics that underpin neural communication during cognitive processing. MEG also has several advantages over EEG as it enables whole head encephalography and the neuromagnetic signal is not degraded by head tissues, facilitating source modelling. MEG also has a relatively fast set up which is important during lengthy testing sessions. In the current study we are using MEG at Swinburne (ELEKTA Neuromag TRIUX) to understand impaired response inhibition in schizophrenia using the stop-signal task, which requires participants to make frequent 'go' responses and attempt to inhibit (or 'stop') when stop-signals (tones) are occasionally presented just after go stimulus onset. In previous work, we confirmed reports that schizophrenia patients have slowed inhibition processing speed (stop-signal RT, SSRT), but further showed that this impairment may be due to an inability to engage the right inferior frontal gyrus (rIFG) that is known to be critical for efficient stopping. Hence the focus of this work is upon induced neuromagnetic activity in rIFG occurring around SSRT. Preliminary analyses (4 SZ, 4 HC) indicate that patients exhibit diminished oscillatory power at a sensor over rIFG just before estimated SSRT.

4. Nigel Jones, Department of Medicine, Melbourne Brain Centre, University of Melbourne

Gamma oscillations in schizophrenia – hearing the signal through the noise. High-frequency (gamma; 30-80Hz) oscillations are cortical brain rhythms implicated in many higher-order cognitive processes, including memory, attention, and sensory perception. These same behaviours are disrupted in schizophrenia, a psychiatric condition which itself is recognised to present with abnormal regulation of gamma oscillations. The prevalent assumption is that this represents a causal relationship: abnormal regulation of gamma oscillations directly leads to disturbed behaviour and symptoms, but definitive evidence of this is scarce, and it remains plausible that these are epiphenomena. Over the past 8 years, we have been attempting to clarify this relationship using genetic and pharmacological animal models, which allow detailed examination of oscillations without the limitations and confounds associated with human studies. In this presentation, I will discuss how NMDAr antagonism influences the regulation of different types of gamma oscillations, and attempt to link these oscillation changes to abnormal behaviours reminiscent of

endophenotypes of schizophrenia. I will describe the impact of current and preclinical antipsychotics, and explore how different cell types can influence these oscillatory patterns and accompanying behaviours. I will conclude by identifying areas to focus on in future investigations.

Special Symposium: Highest rated student abstracts

Friday, 14 Oct, 12:15 – 1:00 pm

1. MATERNAL FLUOXETINE TREATMENT INFLUENCES ANXIETY- AND DEPRESSIVE-LIKE BEHAVIOURS IN OFFSPRING.

Authors: Samuel Millard, Connor Mackay, Marta Ramos, Rebecca Webby, Jeremy S. Lum, Francesca Fernandez, Kelly A. Newell

Affiliations: University of Wollongong, Illawarra Health and Medical Research Institute (IHMRI)

Background: Approximately 10% of pregnant women are prescribed antidepressant drugs, most commonly the selective serotonin reuptake inhibitor, Fluoxetine. Fluoxetine crosses the placenta and is excreted in breast milk raising concerns regarding the long-term consequences of infant exposure. Human studies suggest that Fluoxetine treatment during pregnancy may increase the risk to offspring of developing neurobehavioural abnormalities. Human studies however, are confounded by the difficulty of separating the effects of maternal depression and innate genetic vulnerability from the effects of Fluoxetine exposure.
Method: The aim of this study was to evaluate the effects of maternal Fluoxetine treatment on offspring behaviours of relevance to neurodevelopmental and psychiatric disorders, using a rodent model of depression. Sprague-Dawley (SD; healthy model) and Wistar-Kyoto (WKY; depression model) pregnant rats were treated with Fluoxetine (10mg/kg/day) or vehicle, from gestational day 0 until postnatal day 14 (~5 weeks in total). Once offspring reached adolescence (~5 weeks of age), anxiety-like and depressive-like behaviours were assessed using the elevated plus maze (EPM) and forced swim test (FST) (n=10-14/group).
Results: WKY rats showed a depressive- and anxiety-like behavioural phenotype compared to SD rats, as evidenced by significantly increased time spent in the closed arms of the EPM and increased time spent immobile in the FST. Fluoxetine exposed offspring (both SD and WKY rats) demonstrated an increase in time spent in the closed arms of the EPM (~40%; $p<0.001$) and decreased time spent in the open arms of the EPM (~90%; $p<0.001$), reaching significance in males only. In addition, fluoxetine exposed offspring, spent increased time immobile in the FST (28%; $p<0.05$), an effect that was independent of strain or sex.
Conclusions: Maternal Fluoxetine treatment resulted in significant increases in anxiety-like and depressive-like behaviours in exposed offspring, largely independent of the rat model used. Current studies are exploring the molecular mechanisms underlying this phenotype. Further studies in various models of maternal depression are required to confirm these preliminary findings and establish the effects of Fluoxetine exposure on the developing brain.

2. WHITE MATTER CONNECTIVITY DISRUPTIONS IN EARLY AND CHRONIC SCHIZOPHRENIA

Authors: Maria A. Di Biase, Vanessa L. Cropley, Bernhard T. Baune, James Olver, Paul Amminger, Christina Phassouliotis, Patrick McGorry, Ian Everall, Christos Pantelis, Andrew Zalesky

Affiliations: Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne; Discipline of Psychiatry, The University of Adelaide Department of Psychiatry, The University of Melbourne, Parkville; Orygen, The National Centre of Excellence in Youth Mental Health

Background: White matter disruptions in schizophrenia have been widely reported, but it remains unclear whether these abnormalities differ between illness stages. We mapped the connectome in patients with recently diagnosed and chronic schizophrenia and investigated the extent and overlap of white matter connectivity disruptions between these illness stages.
Method: Diffusion-weighted magnetic resonance images were acquired in recent-onset (n=19) and chronic patients (n=45) with schizophrenia, as well as age-matched controls (n=87). Whole-brain fiber tracking was performed to quantify the strength of white matter connections. Connections were tested for significant strength reductions in recent-onset and chronic groups, relative to separate age-matched controls. Permutation tests were used to assess whether disrupted connections identified in the patient groups significantly overlapped. Linear regression was performed to test whether connectivity was strongest in controls, weakest in chronic patients and midway between these extremities in recent-onset patients (controls>recent- onset>chronic).
Results: Compared to controls, chronic patients displayed a widespread network of connectivity disruptions ($p<.01$). In contrast, connectivity reductions were circumscribed to frontal and parietal regions in recent-onset patients ($p<.01$). A significant proportion of disrupted connections in recent-onset patients (86%) coincided with disrupted connections in chronic patients ($p<.01$). Linear regression revealed that chronic patients displayed reduced connectivity relative to controls, while recent-onset patients showed an intermediate reduction compared to chronic patients ($p<.01$).
Conclusions: Connectome pathology in recent-onset patients with schizophrenia is selectively confined to a more extensive network of white matter connectivity disruptions found in chronic illness. These findings may suggest a trajectory of progressive deterioration of connectivity in schizophrenia.

3. ALLOSTATIC LOAD IS ASSOCIATED WITH POSITIVE SYMPTOMS IN SCHIZOPHRENIA AND FIRST-EPISODE PSYCHOSIS

Authors: Maximus Berger, Robert-Paul Juster, Johann Steiner, Zoltan Sarnyai

Affiliations:
Laboratory of Psychiatric Neuroscience, Australian Institute of Tropical Health and Medicine (AITHM); Columbia University Medical Center, Columbia University Department of Psychiatry; University of Magdeburg Laboratory of Psychiatric Neuroscience

Background: Current pathophysiological models of psychotic disorders suggest that stress contributes to the aetiology and trajectory of the disorder. We investigated if cumulative exposure to stress, quantified by allostatic load (AL), an integrative index of immune, metabolic and neuroendocrine dysregulation, is elevated in patients with schizophrenia (SCZ) and first-episode psychosis (FEP) and related to psychotic symptoms and social and occupational functioning.
Method: We assessed AL in patients with SCZ (n=28), FEP (n=28) and healthy controls matched for age and gender (n=53). Biomarkers for the AL index were selected based on (1) representation of several physiological systems including the cardiovascular, neuroendocrine, immune, and metabolic systems, (2) use in previous AL research, and (3) associations with disease risk. We adopted a scaled AL algorithm whereby each marker proportionally contributes to the overall AL index. Unadjusted and adjusted differences between patients with SCZ, FEP and controls in AL were tested with ANCOVA and partial correlations were used to test associations of AL with psychometric variables.
Results: AL was higher in patients with SCZ compared to controls (4.91 ± 1.89 vs. 2.87 ± 1.62, $p<0.001$), patients with FEP compared to controls (3.80 ± 1.66 vs. 2.87 ± 1.62, $p=0.020$), but not different between patients with SCZ and patients with FEP ($p=0.302$). Adjusting for age and smoking, we found that positive symptoms were positively correlated with AL across all patients with a psychotic disorder (adjusted $R = 0.520$, $p<0.001$) and Global Assessment of Functioning (GAF) scores were negatively correlated with AL at trend level (adjusted $R = -0.251$, $p=0.070$). No significant associations were found for negative symptoms ($p=0.582$).
Conclusions: Our data provide evidence for cumulative physiological dysregulation in patients with SCZ and FEP that is linked to the experience of current positive psychotic symptoms. AL could be a useful model that takes exogenous (e.g., stress) and endogenous factors into account to further understand the pathophysiology of schizophrenia.

Data Blitz 1

Thursday, 13 Oct, 10:00 am – 10:30 am

1. ALTERED STEROIDOGENESIS IN FETAL BRAIN AND PLACENTA IN A DEVELOPMENTAL VITAMIN D DEFICIENT FETAL RAT

Authors: Asad Ali, Xiaoying Cui, Gregory Medley, Darryl Eyles

Affiliations: Queensland Brain Institute

Background: Emerging evidence suggests that prenatal vitamin D deficiency is a risk factor for ASD. A meta-analysis of 12 studies clearly shows the incidence of autism is higher in children of vitamin D deficient pregnant women. A recent study showed that important neurosteroids including progesterone, 17 hydroxyprogesterone, testosterone, androstenedione and cortisol were significantly increased in the amniotic fluid of children who developed ASD. Consistent with these findings, aromatase is reduced in autistic post-mortem brains. Vitamin D has been shown to regulate expression and activity of many of these enzymes in vitro. Here, we investigated the effects of DVD-deficiency on fetal steroidogenesis.
Method: Fetal steroidogenesis was examined in the brain, placentas and amniotic fluids of male fetuses. To generate DVD-deficient fetal brains and placentas, female Sprague-Dawley(SD) rats were offered vitamin D depleted diet for the period of 6 weeks before mating until embryonic day (E)18, which represents peak period of steroidogenesis in SD rats. Male fetuses positioned between two neighbouring males (2M-males) or downstream to a male fetus (1M-males) were selected. Gene expression of steroidogenic enzymes and ASD-related genes were examined by Real-Time PCR in whole fetal brains and matching placentas. We also intend to profile steroids in the amniotic fluid by LC-MS.
Results: In fetal brains DVD-deficiency produced 16% reduction in aromatase, which is a major testosterone catabolic enzyme. When we analysed 2M-males only, aromatase was further reduced to 26% compared to similar positioned controls. The catabolic enzyme for progesterone cyp21a1 and foxp2 which is involved in language disorder and the enzyme involved in the formation of the active vitamin D hormone cyp27b1 were significantly reduced in 2M-DVD-deficient brains. In placenta catechol-O- methyltransferase (COMT), which converts estradiol to 2-methoxyestradiol and the cholesterol side-chain cleavage enzymes were down-regulated in DVD-deficient males compared to controls. In contrast to fetal brains, cyp27b1 was up-regulated in DVD- deficient placentas.
Conclusions: The alterations in the aromatase and cyp21a1 expressions are likely to enhance testosterone, progesterone and 17 hydroxyprogesterone productions in DVD-deficient male brains. Hypermasculinization due to exposure of high levels of prenatal steroids leads to extreme manifestation of cognitive and emotional behaviour observed in ASD individuals. Interestingly, these are the hormones which were elevated in the amniotic fluid of children who developed ASD. Reduction in 2-methoxyestradiol due to down regulation of COMT leads to placental insufficiency and preeclampsia which is a well-known risk factor for ASD. Profiling these steroids in the amniotic fluid of DVD-deficient and control males is ongoing.

2. THE EFFECTS OF ACUTE CBD IN A MOUSE MODEL FOR FRAGILE X SYNDROME

Authors: Jerzy Zieba, Duncan Sinclair, Steven Siegel, Donna Gutterman, Terri Sebree, Tim Karl

Affiliations: Neuroscience Research Australia; Schizophrenia Research Institute; Perelman School of Medicine, University of Pennsylvania; Zynerba Pharmaceuticals; Western Sydney University

Background: Fragile X Syndrome (FXS) is an X-linked neurodevelopmental disorder and arises due to mutation of the Fragile X mental retardation 1 (FMR1) gene. Mutant FMR1 is the primary known genetic cause of intellectual disability and autism and leads to cognitive deficits, altered sensory sensitivity, social communication deficits, and increased anxiety. Recently, the endocannabinoid system has become a target of preclinical research into FXS. For example, pharmacological blockade of the cannabinoid receptor 2 (CB2) normalizes anxiety behaviours of Fmr1 KO mice. The non-psychoactive phytocannabiniod cannabidiol (CBD) has anti-anxiety effects and has been shown potential to ameliorate social deficits.
Method: Thus, in a first step, we tested whether different doses of CBD can rescue some of the established behavioural deficits of germline Fmr1 knockout (KO) mice (C57BL/6J background). Adult, male mice treated acutely with vehicle or CBD (5 mg or 20 mg/kg body weight) were assessed 30 min post injection in the open field test, the elevated plus maze, the three-chamber social preference test, and the continuous Y maze task using a within subject design and an interest interval of at least 72 h.
Results: Fmr1 KO mice exhibited a hyperlocomotive and hyperexplorative phenotype in the open field and a pronounced anxiolytic-like response across anxiety tests. Acute CBD treatment had an anxiolytic-like effect across genotypes in the plus maze. In the social test, only Fmr1 KO mice treated with 5 mg/kg CBD showed a significant aversion of the novel mouse [t(11) = -9.8, p .05). No genotype or CBD effects were detected for the cognitive domains investigated.
Conclusions: Our study detected established behavioural characteristics of Fmr1 KO mice including hyperlocomotion and reduced anxiety. The effects of acute CBD treatment on anxiety domains are in line with earlier reports on the acute characteristics of this phytocannabinoids. CBD had no impact on either cognitive domain. Other studies found that modulating the endocannabinoid system ameliorated cognitive impairments. Thus, future work should follow up on these findings and determine the effects of chronic CBD treatment on the phenotype of Fmr1 KO mice.

3. THE INTEGRATION OF RNA SEQUENCING AND EXOME SEQUENCING HIGHLIGHTS THE FUNCTIONAL SIGNIFICANCE OF RARE MUTATIONS IN PATIENTS WITH SCHIZOPHRENIA

Authors: Chantel Fitzsimmons, Josh R Atkins, Rodney Scott, Paul A Tooney, Vaughan Carr, Melissa J Green, Murray J Cairns

Affiliations: School of Biomedical Sciences and Pharmacy, University of Newcastle; School of Psychiatry, University of New South Wales; Schizophrenia Research Institute, Sydney

Background: Schizophrenia (SZ) is a complex neuropsychiatric disorder with substantial genetic and epigenetic contributions to etiology. While the heritability is predicted to be very high, a mixture of common, rare and epigenetic variants gives an individual the predisposition to the disorder. Understanding this predisposition is an important challenge and will involve integration of all the complexities of this genomic information. Gene expression can provide insight into the disruption of the genome at both the genetic and epigenetic levels. In this study we investigate the functional significance of rare variants identified by exome sequencing though personalised integration of gene expression.
Method: DNA and RNA samples (41 cases and 15 controls) were sourced from the Australian Schizophrenia Research Bank (ASRB). RNA-sequencing was performed at a depth of 40 million reads per sample and mapped to hg19 using Hisat2. Transcript assembly, abundance and differential expression analysis was performed by the Tuxdeo/Cufflinks packages. Exome-Seq reads were mapped using bowtie2, post-alignment/recalibration was performed using the GATK gold standard pipeline. Functional annotation was conducted using SNPeff and their allelle frequency/rarity estimated with respect to dbSNP and ExAC databases. Individual expression analysis was conducted on a per case basis and cross-matched with their high-risk variants called by SNPeff.
Results: After case/control group comparison, 34 genes were identified as having differential expression after correction for multiple testing, with 97% upregulated. Five novel transcripts were identified including a differential expressed novel long-non coding RNA on chromosome 11. After integration with exome sequence data, individual gene expression identified 41 genes that had high-risk genomic variants. Five of these had very low minor allele frequencies and 28 variants had no previous record and were assumed to have very low frequencies. Kremen 1 was differentially expressed in both the SZ group analysis and an individual with a rare deletion that affected that individual's expression of the gene.
Conclusions: Integration of genomic data highlighted the impact of rare variants on the function of genes in individual patients with SZ. We identified 28 mutations not previously seen in databases of genomic variation that influenced gene expression. Interestingly, Kremen1 was discovered in both group and individual analyses, suggesting it is sensitive to disruption in the disorder. Alteration of this gene in SZ is also supported by previous observations reported in the literature. Another gene, CD177 was also differentially expressed in the group analysis, and has been reported before in relation to anti-psychotics. In summary, analysing expression over genotype provides insight into the functional significance of genomic alterations that affect individuals with SZ, and could ultimately be informative in establishing more precise treatment options.

4. CHARACTERISATION OF 500 WHOLE-GENOMES; A PILOT STUDY INTO PERSONALISED APPROACHES INTO SCHIZOPHRENIA

Authors: Joshua R Atkins, Chantel Fitzsimmons, Vaughan Carr, Melissa J. Green, Murray J Cairns

Affiliations: School of Biomedical Sciences and Pharmacy, University of Newcastle; School of Psychiatry, University of New South Wales

Background: The affordable of whole genome sequencing will eventually allow population based screen and personalised approaches in identifying and treating disorders. While monogenic disorders will be the first to benefit from this technology, more complex disorders such as schizophrenia (SZ) will remain challenging till we find the best analytical solutions to unravel this information. This is due to the many components that contribute to the genetic risk, such as types of variation and their position in the genome. In this study we focused on common variation with known effect and rare mutations that have predictable functional significance. This pilot study aims to develop approaches to identify the high-risk variation and its functional significance in people with schizophrenia.
Method: 500 DNA samples (168 controls, 165 patients define as cognitive impaired and 167 cognitve spared) were source from the ASRB, and sequenced at the Garvin Instuite Sydney. PRS analysis was conducted using –log(odd_ratio) with a p value cut off of 10-8 . Variant calling was based on a BED file contain coordinates using the GATK Unified Genotyper. VCFTools and Plink 1.9 was utilised to generate PGS for each individual, group analysis was performed in R studio. Functional annotation was performed by SNPeff, this provided summary stats for each individual and group analysis was performed using R studio.
Results: Using common variant profiling, there were differences between SZ and control. (t-test 1.47-5 , logistic regression 2.57-5) just using the 108 loci. Preliminary analysis of summary statistics for the functional annotation reveals a overall increase in variants compared to controls(p 0.0126) with the majority of these variants occurring in intergenic (p 0.007) and intronic (p0.027) regions. On average, SNPeff called 980 high risk variants, these include frame shift, codon deletions or splicing mutations. These mutations appear to be mostly rare and many of them only occur in that individual
Conclusions: In this study we are testing a variety of approaches to comprehensively understand the genome of individuals with the disorder. When we examine PRS of common variant we found that many individuals with SZ had a high burden of risk as expected. A large proportion of cases, however, had relatively low burden of common risk variants. We predict that these individuals will have a higher risk burden from rare variants. Even in the groups as a whole we observed elevated levels of high-risk variants in the patients compared to the non-psychiatric controls. We are now working towards understand how these variants are functionally distributed and if they cluster with severity of illness or anatomical and cognitive phenotypes. While the work already conducted in the field has paved the way for common variant profiling using PRS, we are now focussing on the integration of common and rare variants revealed by WGS into models that will provide greater insight for precision medicine. This will ultimately lead to a personalised genomics approach to the diagnosis and treatment of people with SZ.

5. EVIDENCE FOR IMPAIRED GLUCOSE METABOLISM IN THE STRIATUM FROM SUBJECTS WITH SCHIZOPHRENIA

Authors: Brian Dean, Natalie Thomas, Elizabeth Scarr, Madhara Udawela

Affiliations: FINMH

Background: Evidence from postmortem CNS studies suggests pathways involved in energy and metabolism contribute to the pathophysiology of schizophrenia with neuroimaging studies suggesting glucose metabolism is particularly affected in the striatum. It is possible to gain information on the status of pathways involved in glucose metabolism by measuring key substrates within those pathways as well as levels of rate-limiting enzymes.
Method: we measured levels of glucose, pyruvate, acetyl-CoA and lactate as well as the ? sub-unit of pyruvate dehydrogenase (PDHB), a rate limiting enzyme, in the striatum from subjects with schizophrenia and age / sex matched controls. The subjects with schizophrenia were made up of two sub-groups, which could be divided because they have (MRDS), or do not have (non- MRDS), a marked deficit in cortical muscarinic receptors.
Results: Compared to controls, levels of PDHB were lower and levels of pyruvate, lactate and acetyl-CoA were higher in the striatum from subjects with schizophrenia. Notably, in subjects with non-MRDS, striatal levels of PDHB were lower whilst levels of pyruvate, acetyl-CoA and glucose were higher whereas levels of lactate were higher in MRDS. Finally, discriminate analyses using levels of levels of PDHB and glucose, or better still, PDHB and glucose in combination with pyruvate, lactate or acetyl- CoA could separate subjects with non-MRDS from controls with high levels of specificity (93%) and selectivity (91%).
Conclusions: Our data shows the benefit of being able to study defined sub-groups within the syndrome of schizophrenia and suggest that changes in glucose metabolism are significant contributors to the pathophysiology of schizophrenia, particularly non-MRDS.

Data Blitz 2

Thursday, 13 Oct, 12:30 – 1:15 pm

1. ADULT VITAMIN D DEFICIENCY AND ATTENTIONAL PROCESSING

Authors: Karly M Turner, Kyna Conn, Thomas H J Burne

Affiliations: Queensland Brain Institute, The University of Queensland

Background: Adult vitamin D deficiency (AVD) has been associated with altered cognitive function and may worsen symptoms in neuropsychiatric disorders. AVD in BALB/c mice has been found to alter excitatory/inhibitory neurotransmitter balance and leads to attentional impairments on the 5-choice serial reaction time task (5C-SRT). The aim of this study was to further characterise these attentional impairments using a signal detection task.
Method: Male BALB/c mice (n=12/group) were placed on either a control or vitamin D deplete diet from 10 weeks of age to induce adult vitamin D deficiency (AVD). After 10 weeks on these diets, the mice were food restricted and trained on an operant signal detection task. Briefly, mice were required to attend to a visual stimulus and respond appropriately to signal and non-signal trials to receive a strawberry milk reward. Various task manipulations were used to probe cognitive performance in control and AVD-deficient mice.
Results: Mice performed well on the task with >95% accuracy. When the signal durations were reduced, AVD-deficient mice were less accurate at detecting signals compared to control mice, particularly at the shortest durations. However on non-signal trials AVD- deficient mice had greater accuracy than controls. These results demonstrate altered decision-making in AVD-deficient mice. After a set-shift from visual to spatial cues, AVD-deficient mice acquired the new rule in fewer trials than control mice. AVD- deficient mice did not differ from controls on the majority of measures after reward devaluation or extended session manipulations, indicating differences were not due to motivational factors.
Conclusions: Vitamin D deficiency has been associated with reduced cognitive performance, although the mechanism is unclear. Here we demonstrate that altered cognitive performance occurs in a mouse model of AVD deficiency. AVD-deficient mice were more conservative in their decision-making when brief stimuli were presented, but more easily shifted responding when the cue modality was changed. These results demonstrate that vitamin D deficiency in adulthood impact cognitive functions in a mouse model.

2. THE IMPACT OF VITAMIN D ON L-TYPE CALCIUM CHANNEL TRAFFICKING AND FUNCTION IN DEVELOPING CORTEX

Authors: Xiaoying Cui, Men Chee Tan, Helen Gooch, Darryl Eyles, Thomas Burne, Victor Anggono, John McGrath

Affiliations: Queensland Brain Institute, The University of Queensland

Background: Developmental vitamin D deficiency is an established risk factor for schizophrenia. The impact of 1,25(OH)2D3 (the active form of vitamin D) on the brain development via its classical genomic pathways are well documented, however, less is known about the non-genomic function of this vitamin. One non-genomic function of 1, 25(OH)2D3 is to rapidly enhance calcium influx via L- type voltage-gated calcium channels (L-VGCC) and this has been demonstrated in bone and muscle. Considering developmental vitamin D deficiency and common genetic variants in L-VGCC are both independent risk factors for schizophrenia, we investigated the impact of 1, 25(OH)2D3 on L-VGCC activity in the mouse prefrontal cortex and the molecular mechanisms of action.
Method: The effects of 1, 25(OH)2D3 on the activity of L-VGCC were examined using single cell nucleated patch recordings and wide- field calcium imaging in the prefrontal cortex (PFC) of postnatal day 10 (P10) mice. We further examined the modulation of surface expression of L-VGCC by 1, 25(OH)2D3 using biotinylation in primary cortical neurons from embryonic day 18 rat foetuses. The protein kinases mediating L-VGCC surface expression were examined using kinase inhibitors (KN62 for calmodulin kinase II, H89 for PKA, Go6983 for PKC, LY294002 for PI-3K, Uo1206 for MEK1/2). The endocytosis was blocked with Dyngo4a and nifedipine was used as L-VGCC blocker.
Results: Application of 1, 25(OH)2D3 rapidly increased the somatic calcium fluorescence levels in a subset of layer 2 and 3 cells in the PFC slice, which was blocked by nifedipine. Nucleated patch recording further confirmed this finding. In cultured cortical neurons, 10 minutes of 1, 25(OH)2D3 treatment significantly reduced surface levels of the pore-forming unit of L-VGCC (subunit A1C). Inhibition of the activation of CaMKII, PKA, PKC and PI3K blocked L-VGCC-A1C internalisation. Blocking endocytosis or the use of nifedipine also diminished the internalization of L-VGCC-A1C induced by 1, 25(OH)2D3
Conclusions: Our results clearly demonstrated that 1, 25(OH)2D3 induced a fast calcium influx via L-VGCC. This subsequently activated the protein kinases and induced a surface L-VGCC internalisation. The modulation of calcium entry into neurons by 1, 25(OH)2D3 may have effects on neuronal differentiation and maturation, axonal growth and synapse formation. Experiments are ongoing to verify this.

3. SMALL RNA REGULATION OF NEURAL GENE EXPRESSION IN RESPONSE TO ENVIRONMENTAL EXPOSURE

Authors: Sharon Hollins, Murray Cairns, Frederick Walker, Katerina Zavitsanou

Affiliations: School of Biomedical Sciences and Pharmacy, University of Newcastle; School of Psychiatry, University of New South Wales, Sydney, NSW 2052

Background: The developmental processes that establish the synaptic architecture of the brain while retaining capacity for activity-dependent remodelling, are complex and involve a combination of genetic and epigenetic influences. Postmortem molecular analysis of the human brain during development and aging suggests there are epigenetic changes reflecting early life experiences. This includes changes in the expression of small non-coding RNAs. These dynamic and influential molecules play an important function in both brain development and its adaption to stress via the regulation of gene expression. Dysregulation of these processes, and interaction with underlying genetic risk factors, can lead to problems with neural circuitry which can manifest in humans as a range of neurodevelopmental syndromes, such as schizophrenia, bipolar disorder and autism spectrum disorders.
Method: We investigate the role of miRNA in the brains' response to maternal immune activation and adolescent cannabinoid exposure, alone and in combination, as both have been identified as environmental risk factors for in psychiatric disorders.
Results: Interestingly, combined exposure to significantly altered miRNA expression in the left hemisphere of the entorhinal cortex as compared to the right. These changes were dominated by a large subgroup of miRNA transcribed from a single imprinted locus on chromosome 6q32, which we previously observed to be dysregulated in schizophrenia. These changes correlated with altered gene expression in the combined treatment group, with miRNA-gene interactions predicted to regulate neuronal growth and differentiation; development of specific cortical layers; synaptic plasticity and transmission; axonogenesis; and learning and memory formation. In addition, we examined radiolabelled coronal sections from each group for changes in morphology and 5HT1AR binding intensity and observed neuropathological changes in the entorhinal cortex.
Conclusions: These findings suggested that the interaction of both an early and late environmental insult enhances changes in offspring miRNA expression in the brain with possible outcomes related to psychiatric disorders in adulthood.

4. PATHWAY ANALYSIS IN SCHIZOPHRENIA IDENTIFIED MULTIPLE CONVERGING PATHWAYS ACROSS THREE POPULATIONS.

Authors: Chenxing Liu, Weihua Yue, Christos Pantelis, Ian Everall, Chad Bousman

Affiliations: Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne; Institute of Mental Health, The Sixth Hospital, Peking University

Background: Schizophrenia is a severe psychiatric disorder. Genome wide association studies (GWAS) have identified numerous single nucleotide polymorphisms (SNPs) associated with schizophrenia, yet common SNPs with small effect lack the potential for utility in clinical practice. Simultaneously considering the effect of multiple SNPs in a pathway is an effective way to explore GWAS datasets and to investigate the pathological significance of biological pathway in schizophrenia. In this study, a machine learning algorithm has been applied to GWAS datasets to test pathway-based SNP sets in three ethnic populations.
Method: GWAS data from individuals with schizophrenia (n = 5,033) and healthy controls (n = 5,332) across European-American, African- American and Han Chinese ethnic populations were collected both from the dbGap database and the Chinese Schizophrenia Coordination Group. Genes were mapped to biological pathways curated by the Kyoto Encyclopedia of Genes and Genomes (KEGG) category. A feature selection algorithm named Minimum Redundancy Maximum Relevance (MRMR) was introduced to filter redundancy information in each of 300 KEGG pathways. The MRMR-selected SNP sets were then used to build diagnosis classifiers via the random forest algorithm. Performance metrics (accuracy, area under the receiver operating curve, and odds ratio) were calculated for each of the pathways.
Results: A total of 85, 61 and 71 pathways significantly differentiated individuals with schizophrenia from healthy controls in three cohorts, respectively. Examination of the overlap between the three cohorts showed four pathways (ubiquitin mediated proteolysis, hedgehog signaling, adipocytokine signaling, renin secretion) were common but the relative contribution of the SNPs and genes within these four pathways differed considerably. Nagelkerke R2 test demonstrated no pathway explained more than 1% of the variance in the liability to schizophrenia.
Conclusions: Four KEGG pathways were identified as common risk factors across three ancestral distinct populations. However, each pathway on its own could explain only a small proportion of disorder variance.

5. FMRI INVESTIGATION OF FEAR EXTINCTION IN ADOLESCENT AND ADULT HUMANS

Authors: Despina E Ganella, Eleni Ganella, Sarah Whittle, Jee Hyun Kim

Affiliations: The Florey Institute of Neuroscience and Mental Health; University of Melbourne

Background: The increased prevalence of anxiety disorders during adolescence is thought to be due to fear extinction deficits involving immature ventromedial prefrontal cortical (vmPFC) function, which has been demonstrated in preclinical rodent studies. We investigated the neural correlates of fear extinction learning and recall using functional magnetic resonance imaging (fMRI) in adolescent and adult humans.
Method: Healthy adults (n=15, aged 25-35) and adolescents (n=9, aged 14-16) underwent fMRI using a fear-learning paradigm involving the pairing of a neutral face (CS) with a fear face plus scream (US). The paradigm involved four phases: (A) Conditioning, where one of two CSs was paired with the US on 100% of trials. The other neutral face was a control stimulus (CS-) that was never paired with the US, (B) Extinction, CSs were presented without the US, (C) Reinstatement, two CS US pairings, and (D) Extinction recall, where both CSs were again presented without the US.
Results: fMRI analysis using SPM12 revealed that adults demonstrated the expected vmPFC activation during extinction (CS+>CS-); however adolescents showed decreased vmPFC and increased rostral/dorsal anterior cingulate cortex activity during both extinction learning and recall, compared to adults. Male adolescents showed reduced vmPFC activity during extinction learning compared with females. When tested for fear recall, however, female adolescents showed reduced vmPFC activity compared to males.
Conclusions: It appears that extinction deficits relate to vmPFC functional immaturity in adolescents. Additionally, vmPFC deficits may not appear until after the initial extinction learning phase in female adolescents. These findings have implications for understanding risk factors and developing novel treatment strategies for adolescent anxiety. (249 words)

6. INCREASED EXPRESSION OF ATP13A4 IN THE PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA

Authors: Laura Bell, Andrew Gibbons, Madhara Udawela, Brian Dean

Affiliations: Florey Institute of Neuroscience and Mental Health

Background: Our recent microarray study highlighted ATPase type 13A4 (ATP13A4) as one of the top 5 genes with the most significantly increased levels of mRNA in the dorsolateral prefrontal cortex from subjects with schizophrenia compared with non-psychiatric controls. Human ATP13A4 was recently characterised as a protein-coding gene that produces a P5-type ATPase with an as yet unknown function. In the present study, we aimed to determine whether ATP13A4 mRNA levels are altered in other regions of the prefrontal cortex in subjects with schizophrenia and whether the expression of ATP13A4 is affected by antipsychotic medication.
Method: Real-time quantitative PCR (qPCR) was used to measure ATP13A4 mRNA levels in Brodmann's area (BA) 8 and BA44 from subjects with schizophrenia (n = 30) and non-psychiatric controls (n = 30). Similarly, Atp13a4 mRNA levels were also measured in cortical tissue from male Sprague Dawley rats treated for 1 month with 1mg/kg/day haloperidol, 10mg/kg/day chlorpromazine, 10 mg/kg/day thioridazine or vehicle (n = 5 per treatment arm). All qPCR data was normalised against the expression of 3 stably expressed reference genes.
Results: A significant increase in ATP13A4 mRNA was observed in both BA8 and BA44 in schizophrenia compared to controls when the data was normalised. In BA44, statistically significant differences were seen in the raw Ct values ($p = 0.0006$) and the relative expression normalised to reference genes (p 0.05). There were no significant differences in Atp13a4 mRNA levels in any of the antipsychotic treatments in rats compared to controls ($p > 0.05$).
Conclusions: In schizophrenia, compared to controls, ATP13A4 mRNA levels were higher in both cortical regions studied. However, we argue that the altered expression was more prominent in BA44, as the analyses demonstrated a significant difference between groups in both raw and normalised expression data. This study adds to our previous microarray findings in BA 9, and suggests there are widespread increases in ATP13A4 gene expression in the prefrontal cortex. The absence of significant differences in Atp13a4 levels in the rat cortex following antipsychotic treatment suggests that these differences are not due to an antipsychotic drug effect and may, therefore, play a role in the underlying pathophysiology of schizophrenia.

7. ELEVATED DOPAMINE RELEASE IN THE DORSAL STRIATUM: A NEW ANIMAL MODEL OF SCHIZOPHRENIA

Authors: Alice Petty, Xiaoying Cui (PhD), Assoc. Prof. Darryl Eyles

Affiliations: The Queensland Brain Institute; Queensland Centre for Mental Health Research

Background: Increased dopamine synthesis and release in the dorsal striatum is one of the strongest pathophysiological findings in patients with schizophrenia. This finding has been reliably documented through PET studies. Intriguingly, this abnormality is also found prior to schizophrenia proper, in the 'prodromal' period but to a lesser extent. Thus the progressive nature of this abnormality suggests there may be a window of opportunity for therapeutic intervention. In order to develop interventions however, greater understanding of the transition from the prodrome to schizophrenia is necessary. We are therefore developing an animal model of this prodromal abnormality.
Method: We used an adeno-associated viral vector to stereotactically insert genetic material into the substantia nigra pars compacta (SNpc) of rats. This vector expresses tyrosine hydroxylase (TH) and GTP cyclohydrolase 1 (GCH1); rate limiting factors in the synthesis of dopamine. The SNpc innervates the dorsal striatum. Six weeks after the injection (in adolescence - PND35), animals were tested for spontaneous and amphetamine-induced locomotion (0.6mg/kg AMPH). Brain tissue was collected, and immunohistochemistry was performed to examine TH protein expression in the site of injection. Neurochemistry of the dorsal striatum, nucleus accumbens (NAc) and pre-frontal cortex (PFC) was analysed with HPLC and rtPCR.
Results: Six weeks after the active virus was injected bilaterally, we saw a clear increase of TH+ staining in the SNpc, and an increase in human TH mRNA in the dorsal striatum, but not in the NAc or PFC. Although there was no change in dopamine levels in the dorsal striatum, a significant increase in the metabolite HVA was seen in this region. There were also indications of decreased COMT mRNA levels in the PFC and dorsal striatum. While no change in spontaneous locomotion was observed, the animals injected with the active virus showed significantly increased hyperlocomotion following the amphetamine treatment.
Conclusions: The viral injection appears to be selective to the region of interest, since the increase in TH mRNA levels is confined to the intended projection area - the dorsal striatum. We have also found changes in dopamine metabolites, which might suggest increased DA turnover in the treated animals. Crucially, treated animals demonstrated a hypersensitive locomotion response following a moderate dose of amphetamine. Since this is a classic test for the positive symptoms of schizophrenia, this strongly suggests that this novel animal model is indeed replicating some schizophrenia-like phenotypes. Further behavioural and neurochemical studies are now planned

Data Blitz 3

Friday, 14 Oct, 10:00 – 10:15 am

1. ATHEROGENICITY INDEX AND OXIDATIVE STRESS BIOMARKERS IN BIPOLAR DISORDER AND TOBACCO USE DISORDER

Authors: Kamila Landucci Bonifacio, Chiara Cristina Bortolasci, Carine Coneglian de Farias, Luciana Higachi, Francis Fregonesi Brinholi, Ana Paula Michelin, Laura Oliveira Semeao, Srisaiyini Kidnapillai, Ken Walder, Estefania Gastaldello Moreira, Decio Sabbatini Barbosa

Affiliations: Universidade Estadual de Londrina; Deakin University

Background: High rates of tobacco smoking have been described in bipolar disorder (BD) and are associated with worse outcomes. Tobacco smoking is approximately 2-3 times more prevalent in BD than in the general population, with estimates ranging from 60-70% in bipolar patients compared to 25-30% in the general population. Tobacco use disorder (TUD) associated with depressive disorder and atherogenicity are related to oxidative stress (OS) and comorbidities, such as cardiovascular diseases (CVD). The aim of this study was to investigate the alterations in the oxidant/antioxidant status and Castelli indexes in subjects with BD, BD smokers and healthy controls.
Method: Blood samples were collected from men and women, aged 18–65, with BD (n = 30) who were recruited from outpatients at the Psychiatric ambulatory and BD smokers (n=36) from the Center of Cigarette Smoking Cessation Service, State University of Londrina (UEL). We also included control group (without mood disorder and TUD) (n=29) recruited from staff at the UEL, Londrina, Parana, Brazil. We measured lipid hydroperoxides (LOOH) and advanced oxidation protein products (AOPP) as oxidative biomarkers, Paraoxonase 1 activity (PON1) as antioxidant biomarker, and atherogenicity using the Castelli 1(Cast1) and 2 (Cast2) index.
Results: BD smokers patients have significantly higher levels of LOOH, AOPP and Cast1 and lowered levels of PON1 and HDL compared to controls. AOPP was also increased in BD patients compared to control group. There were no significant differences in total cholesterol or Cast2 between groups. Multiple Regression results with AOPP or Cast1 as dependent variables showed that AOPP was positively associated with LOOH, PON1 and Cast1, and that Cast1 was positively associated with AOPP and negatively with LOOH and PON1. Using Spearman's correlation, the AOPP was associated with LOOH and Cast1 respectively ($r=0.42$, $p=0.01$; $r=0.73$, $p<0.001$).
Conclusions: Our results are consistent with OS theory of BD and suggest that OS could be exacerbated by TUD. It's likely that BD smokers have higher levels of AOPP and Cast1, due to (authors please check edit) a significant decrease in PON1 and disturbances in lipid profile. AOPP also have been considered as a novel marker of oxidant-mediated protein damage. The mechanisms that lead to and regulate OS and how it contributes to neuropsychiatric diseases are likely to be complex. However, some OS pathways are sensitive to pharmacological manipulation, which makes this pathway a putative target for pharmacological therapies for BD and TUD.

2. INFLAMMATORY CYTOKINES ARE ELEVATED IN THE SUBSTANTIA NIGRA OF A SUBSET OF PEOPLE WITH SCHIZOPHRENIA

Authors: Tertia Purves-Tyson, Debora A Rothmond, Kate Naude, Cynthia Shannon Weickert

Affiliations: Schizophrenia Research Laboratory, NeuRA; School of Psychiatry, UNSW; Schizophrenia Research Institute, NeuRA

Background: Neuroinflammation is gaining traction as a candidate mechanism contributing to the neuropathology of schizophrenia in some people. In ~40% of people with schizophrenia, pro-inflammatory cytokines are elevated in both post mortem prefrontal cortex and in peripheral blood of living patients. Inflammation may contribute to the dysregulation of midbrain dopamine neurons responsible for symptoms and cognitive deficits; however, cytokine gene expression in the post-mortem midbrain has not been examined in schizophrenia. We hypothesised that inflammatory markers will be elevated in the midbrain of a subset of people with schizophrenia.

Method: Gene expression of pro-inflammatory cytokines, interleukin (IL) 1β, IL6, IL6 signal transducer (IL6ST), IL8, 1L18, tumor necrosis factor (TNF) α, the acute phase inflammatory marker, SERPINA3, and the microglia marker, allograft inflammatory factor 1 (AIF1), was examined by qRT-PCR in the midbrain from 28 schizophrenia cases and 29 healthy controls. Students's t-tests or analysis of variance, with demographic variables as covariates when appropriate, were used to detect diagnostic differences. Inflammatory subgroups were defined using two-step cluster analysis of pro-inflammatory cytokine gene expression on the entire case/control post mortem cohort and chi-squared analysis revealed inflammatory groups based on diagnosis.

Results: SERPINA3, IL1β, IL6 and IL6ST mRNAs increased 200%, 154%, 173%, and 17%, respectively, in the midbrain of schizophrenia patients compared to controls ($F>4.0, p0.05$). TNFα mRNA showed a trend to be increased. Cluster analysis revealed 13 individuals in a high inflammatory group and 44 in a low-inflammatory group. Chi-squared analysis revealed all 13 individuals in the high inflammatory group had schizophrenia and the remaining 15 individuals with schizophrenia and all individuals in the control group exhibited low inflammatory markers ($\chi 2=57.0, P<0.0001$). The high inflammation group received higher daily chlorpromazine-equivalent doses ($t(20)=-2.91, P<0.01$).

Conclusions: Inflammatory markers were elevated in the midbrain in ~50% of schizophrenia cases, whilst no healthy controls were classified as having high inflammation in the midbrain. As PET studies have related increased microglia activity to at-risk symptom severity in medication naïve people at ultra-high risk for schizophrenia, we suggest that the higher dose of antipsychotics in the high inflammation group indicates that these patients were more symptomatic and thus more highly medicated, rather than antipsychotics increasing inflammatory markers. This data suggests that increases in pro-inflammatory cytokines extend to midbrain regions and may contribute to the underlying neuropathophysiology of the disorder.

3. STRESS-ENVIRONMENTAL ENRICHMENT PARADIGMS IN A TWO-HIT MODEL: SEX-SPECIFIC HIPPOCAMPAL CHANGES

Authors: Adrienne Grech, Udani Ratnayake, Rachel Hill, Maarten Van den Buuse

Affiliations: Monash University; The Florey Institute of Neuorscience & Mental Health; La Trobe University

Background: This study investigated the impact of environmental enrichment (EE) upon learning and memory in a two-hit model of developmental stress. The two-hit hypothesis suggests that a combination of genetic and environmental insults during critical periods of development lead to schizophrenia in adulthood. Epidemiological studies demonstrate clear sex differences in the severity and onset of schizophrenia but this knowledge is not reflected in standard treatment practises. Ignorance of the molecular mechanisms underlying these gender disparities contributes to incomplete treatments. This study therefore investigated potential molecular sex-dimorphic responses to positive and negative environmental factors.
Method: To model this we used both wildtype (WT) and Brain-Derived Neurotrophic Factor (BDNF) heterozygous (HET) mice (first 'hit'). BDNF is a vital neurotrophin implicated in schizophrenia. Adolescent mice received the experimental treatment of the major stress hormone corticosterone (CORT) and/or EE. Protein expressions of phosphorylated and full length TrkB receptors and downstream molecules involved in BDNF-TrkB signalling were measured by Western blot analysis in the dorsal hippocampus of male and female mice, an area highly implicated in learning and memory.
Results: Across all markers there were striking sex-specific results. In males chronic CORT significantly increased phosphorylated TrkB (pTrkB) expression at the Y515 and Y816 residues, but no effect of genotype nor genotype x CORT interaction was observed. Increased pTrkB following CORT was accompanied by a downstream increase in phosphorylated ERK1 (pERK1) expression. For females, changes in pTrkB were genotype dependent; a significant genotype x CORT interaction showed increased pTrkB Y816 protein expression in BDNF-HET CORT mice. In both males and females there was a significant EE effect upon pERK1 but no CORT X EE nor genotype x EE interactions were observed.
Conclusions: This study provides a detailed profile of the BDNF pathway, highlighting fundamental differences between the sexes in response to BDNF deficiency and negative and positive environmental factors. This lends further support for the development of sex- focused treatments for schizophrenia. Further work is needed to elucidate the regional specificity of the results and future studies will need to investigate more upstream markers of the BDNF-TrkB signalling pathways.

Poster Session 1

Thursday, 13 Oct, 1:30 – 2:30 pm

P1. OPTOGENETIC INVESTIGATION OF MEDIAL AMYGDALA CONNECTIVITY TO THE PARAVENTRICULAR HYPOTHALAMUS

Authors: Cameron D. Adams, Jiann Wei Yeoh, Erin J. Campbell, Jaideep S. Bains, Brett A. Graham, Christopher V. Dayas

Affiliations: School of Biomedical Sciences and Pharmacy and the Centre for Translational Neuroscience and Mental Health Research, University of Newcastle and the Hunter Medical Research Institute, Newcastle, NSW, Australia

Background: The amygdala plays a central role in the generation of adaptive responses to stressful stimuli including modulation of the hypothalamic-pituitary-adrenal (HPA) axis. Previous work suggests the medial amygdala (MeA) is necessary for HPA axis responses to psychological stressors (noise, restraint). These actions are thought to be mediated via relays through the bed nucleus of the stria terminalis (BNST). However, we have previously identified, using tract tracing, the existence of a direct, psychological stressor-sensitive projection from the MeA to the medial parvocellular paraventricular nucleus (mpPVN). Here, using a targeted optogenetic approach, we investigated direct MeA mediated control of mpPVN neuroendocrine cells.
Method: Adult male Sprague-Dawley rats (n= 25) were prepared with bilateral, MeA targeted injections of the light-sensitive cation channel, channelrhodopsin-2 (AAV5-CaMKIIa-EYFP or AAV5-hSyn-EYFP) with a subset receiving bilateral MeA fiber optic probes (n= 16). In experiment 1, AAV-ChR2 and AAV-EYFP animals implanted with probes received blue light stimulation and after 1.5 hours were overdosed with anaesthetic and brains processed for Fos-protein immunohistochemistry. In experiment 2, AAV-ChR2 animals were sacrificed and slices of the hypothalamus incorporating the PVN were prepared for whole cell patch clamp electrophysiology and an examination of blue light evoked post-synaptic currents.
Results: Blue light delivered to the MeA in behaving animals significantly increased numbers of Fos-positive cells in the MeA ($p < 0.05$) and mpPVN ($p < 0.05$). In hypothalamic slices, blue light stimulation evoked post-synaptic currents in 41% of recorded neurons (n= 24/58). 22% of these neurons received excitatory inputs (CNQX-sensitive), 12% received inhibitory inputs (picrotoxin-sensitive) while 7% received both excitatory and inhibitory inputs.
Conclusions: Together our findings demonstrate a functionally relevant direct projection from the MeA to PVN capable of eliciting direct control over of the apex of the HPA axis.

P2. IMPAIRED SPATIAL LEARNING IN ADULT VITAMIN D DEFICIENT BALB/C MICE IS ASSOCIATED WITH A REDUCTION IN NITRIC OXIDE LEVELS AND SPINE DENSITY IN THE HIPPOCAMPUS

Authors: M. M. Al-Amin, R. Sullivan, S. Alexander, D. Blackmore, T.H.J. Burne

Affiliations: Queensland Brain Institute, The University of Queensland, Brisbane QLD 4072

Background: The effect of adult vitamin D (AVD) deficiency on hippocampal-dependent spatial learning and memory has been tested in rodents. However, the results are inconsistent. Nevertheless, AVD deficiency resulted in an imbalance between excitatory and inhibitory neurotransmitters and reduced expression of glutamic acid decarboxylase 67 in brain (Groves et al., 2013). In addition, AVD deficiency altered the expression of proteins involve in neurotransmission and synaptic function in hippocampus. Here we examined the effect of AVD deficiency on hippocampal-dependent spatial learning, glutathione, nitric oxide (NO) and spine density in the hippocampus in mice.
Method: Adult male BALB/c mice (10 weeks old) were fed a control diet (1500 IU vitamin D3/kg, n=16) or deficient diet (0 IU vitamin D3/kg, n=16) for a minimum duration of 10 weeks and during the behavioural testing period. We used active place avoidance test to measure spatial learning and memory formation. We performed Golgi-cox staining to measure the spine density of hippocampal dentate gyrus neurons. We also determined the levels of glutathione and NO in hippocampal tissue.
Results: We found a significant main effect ($p<0.05$) of Diet on the latency to enter the shock zone in the active place avoidance test, with AVD-deficient mice having a shorter latency than control mice. We also found a significantly lower spine density in the proximal branches of the dentate gyrus neurons in AVD-deficient mice. Hippocampal glutathione levels were not affected by diet but NO levels were significantly depleted in AVD-deficient mice.
Conclusions: Consistent with several previous studies, we found that AVD deficiency impairs spatial learning and memory formation. These results suggest that lower spine density and NO levels may affect synapse formation, and this could be associated with the spatial learning deficits (i.e. longer latency to enter the shock zone) we observed in AVD-deficient mice. We suggest that pharmacological and genetic approaches be used to investigate the role of nitric oxide in hippocampal-dependent spatial learning deficits in future studies.

P3. THE EFFECT OF DEVELOPMENTAL VITAMIN D DEFICIENCY IN MOUSE MODELS RELATED TO AUTISM SPECTRUM DISORDER

Authors: M.L. Langguth, S.A. Alexander, K.M. Turner, M.C. Sanchez-Vega, T.H.J. Burne

Affiliations: University of Queensland, Queensland Brain Institute Queensland Centre for Mental Health Research

Background: Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders commonly characterised by verbal and non- verbal communication deficits, impaired social interaction and repetitive, stereotypic behaviours. Increasingly, the aetiology of ASD is thought to be a combination of genetic and environmental factors. Amongst other risk factors, epidemiological evidence suggests a role for gestational and early developmental vitamin D (DVD) deficiency in the incidence of ASD. Vitamin D is thought to regulate neuronal proliferation, apoptosis and neurotrophic factors during development. The aim of the present study was to analyse the effect of DVD deficiency in different mouse models related to autism.
Method: To implement DVD deficiency, dams were placed on vitamin D deplete diets for ten weeks, including mating and gestation, until littering. Three different inbred strains; C57BL/6J, BALB/c and BTBR T+tf/J (BTBR) were used to address the three key behavioural symptoms related to ASD; isolation-induced ultrasonic vocalisations to measure communication, the three- chambered social interaction task to observe social novelty and preference and finally the open field test to examine repetitive movements and hyperlocomotion. BTBR, a commonly used mouse model for autism, has previously been shown to have social interaction and communication deficits and display excessive self-grooming and hyperlocomotion.
Results: Preliminary results suggest that BTBR mice display significantly increased ultrasonic vocalisations and narrowed vocal repertoire in comparison to C57BL/6J and BALB/c mice as well as impairments in social interaction, increased repetitive movements and significant hyperlocomotion. Using active place avoidance, we show for the first time cognitive impairments in spatial memory and reversal learning in BTBR mice. BTBR mice were found to perform better during spatial learning and memory tasks but performed poorly compared to C57BL/6J and BALB/c mice in reversal learning tasks. Data will be presented on the extent to which DVD deficiency has on behaviours related to autism.
Conclusions: In conclusion, we have explored different behavioural characteristics related to ASD and found that BTBR do have behavioural deficits in communication, social interaction and repetitive, stereotypic movements in comparison to C57BL/6J and BALB/c. Experiments are currently ongoing and the effect of diet will be analysed once experiments are completed prior to the meeting. Using different mouse models, we can explore the autism behavioural phenotype and better understand gene and environmental risk factors and how they interact to contribute to the aetiology of ASD.

P4. THE EFFECT OF ADULT VITAMIN D DEFICIENCY IN C57BL6/J AND APP23 MICE.

Authors: K. Jaeschke, S.A. Alexander, J. Götz, T.H.J. Burne

Affiliations: Queensland Brain Institute, Queensland Centre for Mental Health Research, Clem Jones Centre for Ageing Dementia Research, Queensland Centre for Mental Health Research

Background: It is well established that vitamin D is essential in calcium homeostasis and bone metabolism. However recent evidence has exposed further roles of vitamin D in adult brain function, specifically that low vitamin D levels during adulthood may be related to adverse brain-related outcomes. Furthermore, a recent meta-analysis has revealed a large association between low serum vitamin D concentrations and Alzheimer's disease (AD). The primary aim of this research was to investigate the role of varying vitamin D levels on the healthy adult brain and to highlight the impact vitamin D has on brain function in AD.
Method: Male adult C57BL/6J mice were exposed to varying levels of vitamin D (0, 150, 1,500 and 15,000 IU/vitamin D/kg referred to as deficient, insufficient, replete and excess, respectively) for 10 weeks and then assessed on a test for spatial learning and memory, active place avoidance (APA). Male and female APP23 mice, a transgenic mouse model of AD, and littermate controls exposed to replete or excess levels of vitamin D were also assessed on APA. Both C57BL/6J and APP23 mice were also assessed for locomotion, using activity monitors, and response to noxious stimuli, using a hot plate test.
Results: There was no significant effect of diet on performance in APA in C57BL/6J mice or male APP23 mice. However female APP23 mice on excess vitamin D diets showed significantly impaired performance on APA compared to APP23 mice on replete diets and littermate controls. There was no significant effect of diet or genotype on performance on the hot plate test in C57BL/6J and APP23 mice. There was no significant effect of diet on locomotor performance in C57BL/6J mice. However, APP23 mice on replete vitamin D diets showed significantly decreased locomotor activity compared to APP23 mice on excess diets and littermate controls.
Conclusions: Overall, these results indicate that varying vitamin D levels in healthy adult C57BL/6J mice do not underlay differences in spatial learning and memory. However, excess vitamin D levels in female APP23 mice, who show a greater disease burden than males at a younger age, is linked to an impairment in spatial learning and memory. Excess vitamin D could also correct the hypolocomotive phenotype in APP23 mice. As the APP23 are a model of AD, these results show that excess vitamin D could potentially exacerbate the progression of AD in females and these findings warrant further scrutiny.

P5. NORADRENERGIC B-RECEPTOR ANTAGONISM IN THE BASOLATERAL AMYGDALA IMPAIRS RECONSOLIDATION, BUT NOT EXTINCTION, OF ALCOHOL SELF-ADMINISTRATION.

Authors: Rose Chesworth, Laura Corbit

Affiliations: School of Medicine, Western Sydney University, Australia; School of Psychology, University of Sydney, Australia

Background: Alcohol addiction is a chronic, relapsing disorder, and a critical barrier to recovery is the propensity for relapse. Cues associated with alcohol use can trigger relapse, and pharmacologically facilitating processes such as extinction, which decreases the associative strength of these cues, may help prevent relapse. Previous research in our laboratory demonstrates the noradrenergic system mediates extinction learning for alcohol in rats; however, the neural locus of this effect is unknown. The present study was designed to determine whether the basolateral amygdala (BLA), a region critical for the extinction of fear and appetitive learning, also mediates extinction of alcohol seeking.
Method: Hooded Wistar rats were trained to lever press for 10% ethanol in the presence of auditory and visual cues. Following task acquisition, rats were implanted with bilateral cannula targeting the BLA and retrained. Infusions of the β-receptor antagonist propranolol (PROP; 2µg/µl) were administered prior to extinction (Experiment 1), and rats assessed for relapse-like behaviour at spontaneous recovery 2 weeks later. In a subsequent experiment, rats were infused with PROP after extinction (targeting consolidation, Experiment 2A), and assessed for spontaneous recovery. Rats were retrained and infused after self-administration (targeting reconsolidation, Experiment 2B) and prior to self-administration (targeting reinforced responding, Experiment 2C).
Results: We expected intra-BLA PROP to impair extinction learning; however, rats infused with PROP prior to extinction exhibited reduced responding at spontaneous recovery (Experiment 1), suggesting enhanced extinction learning. We sought to explain this unexpected result by determining if PROP treatment was affecting processes other than extinction, and assessed consolidation of extinction as well as reconsolidation of self-administration. PROP treatment had no effect on consolidation of extinction learning (Experiment 2A), but impaired reconsolidation of self-administration (Experiment 2B). In addition, PROP treatment had no effect on reinforced responding (Experiment 2C).
Conclusions: Extinction and reconsolidation are opposing processes which can be triggered by specific test conditions. We suggest our test conditions led to modulation of reconsolidation of self-administration memory by PROP, rather than modulation of extinction memory. Thus, our data implicates noradrenergic β-receptors in the BLA in reconsolidation of alcohol self-administration memory. Furthermore, we highlight how specific test conditions can bias the targeting of one memory process over another.

P6. EFFECT OF MATERNAL IMMUNE ACTIVATION AND SEX ON ELECTROPHYSIOLOGY RELATED TO SCHIZOPHRENIA

Authors: Ariel Dunn, Lauren Harms, Juanita Todd, Ross Fulham, Aaron Wong, Deborah M. Hodgson, Ulrich Schall, Patricia T. Michie

Affiliations: Priority Research Centre for Brain and Mental Health Research, University of Newcastle

Background: Maternal immune activation (MIA) in response to gestational infection is a risk factor for developing schizophrenia in the offspring. Several studies have determined that MIA in rats or mice, induced by the non-infectious viral mimic Poly (I:C) produces a wide-range of schizophrenia-like behavioural and neurobiological alterations in the offspring. Two electrophysiological features that are consistently altered are gamma activity and mismatch negativity (MMN). Preliminary data suggests that MIA alters gamma oscillations similarly to in schizophrenia, while MMN has not been previously investigated in MIA animals. Furthermore, such features have not been explored sufficiently in female rodents.
Method: Pregnant Wistar rats were exposed to either a viral mimetic polyriboinosinic-polyribocytidilic acid (PolyI:C; MIA group) or an equivolume of saline (Control group) during late gestation (gestational day 19). Both male and female offspring underwent surgery in adulthood to implant skull electrodes in the frontal and auditory cortices. These electrodes were used to assess the schizophrenia-like neurophysiological phenotypes of MMN and gamma activity after the animals' recovery. Gamma activity was measured via an auditory steady-state response (ASSR) task, while MMN was measured using an oddball and many-standards control paradigm.
Results: Reliable ASSRs (where the largest frequency response was to the corresponding stimulation frequency) were found from 40 to 80 Hz, with the largest ASSRs occurring at 60 Hz. However, MIA had no significant effect on ASSRs at any frequency, nor were any sex differences found. MMN responses were found for three waveform components. Females were found to have higher overall MMN responses than males in the early components of the waveform, a novel finding. In contrast, no significant MIA effects were found for any MMN component.
Conclusions: MIA was not sufficient to produce schizophrenia-like electrophysiology alterations in gamma activity and mismatch negativity in adult offspring. Previous studies have found changes in behaviour and schizophrenia-like neurobiology, therefore it is possible that a multiple or 'two-hit' model of MIA is needed for electrophysiological changes to be observed. In schizophrenia, sexual dimorphisms are important to understanding the aetiology and development of the disorder across the lifespan of diagnosed individuals. Our novel findings of sex differences suggest that animal research should consistently include both sexes to improve the validity of current models.

P7. PERINATAL PROGRAMMING OF FEMALE SUBFERTILITY AND STRESS VULNERABILITY BY EARLY-LIFE IMMUNE STRESS

Authors: Erin A Fuller, Marissa C.J. Carey, Luba Sominsky, Lauren Harms, Deborah Hodgson

Affiliations: Laboratory of Neuroimmunology, School of Psychology, University of Newcastle, Newcastle, Australia; School of Health and Biomedical Sciences, RMIT University. Laboratory of Neuroimmunology

Background: Regardless of technological advances, female subfertility remains prevalent and leads to psychological distress. Increasingly, younger women are presenting with inflammatory reproductive disorders. Accumulating evidence suggests developmental factors, such as immune status, are involved in the aetiology of subfertility. Interestingly, pro-inflammation and early-life stress are associated with both reproductive dysfunction and depressive symptomology. Reproductive fundamentals are established in early life via immunoendocrine mediators. Immune disturbances, such as infection, may dysregulate these processes. Importantly, these effects may be compounded with later-life stress. The current study aimed to determine central and peripheral inflammation following early-life stress and a 'second-hit' of stress.
Method: Using a rat model of neonatal immune activation (NIA), Female rats were exposed to bacterial lipopolysaccharide (LPS) (0.05mg/kg) or equivolume saline on postnatal days 3 and 5 via intraperitoneal injection. In adulthood, animals underwent either a 'Stress' (3 day restraint stress) or 'No Stress' condition to mimic a psychological stressor. Inflammatory mediators were assessed in blood, as well as ovarian and brain tissue in early-life (PND 5) and adulthood (~PND 85). To evaluate the effects of NIA only, adult females underwent behavioural testing to assess depressive-like behaviours and mating behaviours.
Results: Neonatal immune activation (NIA) upregulated circulating proinflammatory mediators and lead to advanced puberty onset ($p<0.05$). NIA paired with an adulthood psychological stressor lead to upregulated circulating interleukin (IL)-6 and altered gene expression of pro-inflammatory mediators and pathways. NIA altered female mating behaviours; however depressive-like behaviours were not significantly expressed in the model.
Conclusions: Neonatal immune stress may alter early life immune-regulated female reproductive development, contributing to female subfertility. Immune stress exposure during critical periods of reproductive development may lead to permanent ovarian alterations such as a reduction in the quality and quantity of ovarian follicles, and this could contribute to early onset puberty and premature exhaustion of the ovarian reserve. Disruptions to the early-life immune milieu has the potential to perinatally program long-term ovarian inflammatory functioning which may detrimentally affect female fertility, fecundity and affect behaviours.

P8. THE EFFECTS OF MID-LATE GESTATIONAL MATERNAL IMMUNE ACTIVATION IN RATS ON DOPAMINE-RELATED BEHAVIOUR

Authors: Anita Gray, Lauren Harms, Patricia Michie, Deborah Hodgson

Affiliations: University of Newcastle

Background: Prenatal infection is a risk factor for schizophrenia in offspring and is believed to be mediated by maternal immune activation (MIA) in response to an infection. Previous studies in our lab investigating the effect of MIA during mid or late gestation found PPI deficits, but only in males, and did not observe changes in drug sensitivity, indicating that these periods of gestation are not the most sensitive with regard to the effects of MIA on the developing dopamine system. Therefore, the current study aims to determine whether MIA at another gestational time-point leads to significant changes to dopamine-related behaviour.
Method: Polyinosinic:polycytidylic acid (PolyI:C) was injected to induce MIA in pregnant Wistar rats at gestational day (GD) 14. Control dams were given saline injections. Control and MIA dams gave birth, and their offspring were raised to adulthood, at which time they underwent testing for a range of dopamine-related behaviours. Prepulse inhibition of acoustic startle was assessed, as was locomotor sensitivity amphetamine and MK-801.
Results: Blood taken from the saphenous vein of pregnant dams 2 hours after exposure to PolyI:C was tested using an ELISA Kit for interleukin-6 (IL-6). Analysis found significant elevation of IL-6 in MIA dams compared to controls. Collection of behavioural data is still ongoing.
Conclusions: The current project is in the preliminary stages. We have successfully induced MIA at GD14 using the viral mimic PolyI:C on pregnant rats, confirming the effectiveness of the viral mimic in the MIA model. It still remains to be seen if the PolyI:C exposed rats will exhibit a schizophrenia-like phenotype. Our findings hope to strengthen the MIA model and characterize behaviours related to dopaminergic functioning to produce a valid animal model of schizophrenia.

P9. EFFECTS OF MID VS. LATE MATERNAL IMMUNE ACTIVATION ON SCHIZOPHRENIA-LIKE BEHAVIOUR IN ADULT RATS

Authors: Lauren Harms, Crystal Meehan, Jade Frost, Juanita Todd, Ulrich Schall, Patricia Michie, Deborah Hodgson

Affiliations: Priority Research Centre for Brain and Mental Health Research, University of Newcastle

Background: Exposure to prenatal infection is associated with increased risk for schizophrenia in offspring, an effect that is believed to be primarily mediated by the maternal immune response. Many previous studies have established that maternal immune activation (MIA) via the viral mimic Poly(I:C) in pregnant rodents leads to a variety of schizophrenia-related behavioural consequences in rat or mouse offspring. In the current study, we aimed to establish a similar model in our laboratory, by exposing rats to Poly(I:C) at either mid or late gestation and investigating schizophrenia-related behaviours in the adult offspring.
Method: Pregnant rats were exposed to 4 mg/kg Poly(I:C) (MIA) or saline (Control) at gestational day 10 (GD10) or GD19. Blood samples were collected 2h after injection and were subsequently assessed for interleukin 6 (IL-6) levels to confirm successful immune activation. Their offspring were raised to adulthood, and examined on a range of schizophrenia-related behavioural tests including prepulse inhibition of acoustic startle response (sensiorimotor gating), an operant version of the delayed non-match to position (DNMTP, working memory), and locomotor sensitivity to MK-801 and amphetamine.
Results: Poly(I:C) injection at GD10 and 19 increased circulating IL-6 relative to dams treated with saline, indicating successful immune activation. As adults, the offspring of dams exposed to Poly(I:C) exhibited several behavioural alterations. Males, but not females, exposed to MIA (regardless of GD) exhibited reductions in sensorimotor gating. In addition, transient working memory impairments were observed in GD19, but not GD10 rats exposed to MIA (regardless of sex). MIA did not affect the sensitivity of rats to the locomotor-enhancing effects of MK-801 or amphetamine.
Conclusions: This study demonstrates that MIA at mid and late gestation in Wistar rats does indeed alter the trajectory of brain development to such a degree that behavioural alterations can be observed in adult animals. However, these alterations are subtle, compared to previous reports using other species, or GDs of MIA. It is therefore likely that GDs 10 and 19 are not the most sensitive with regard to the developmental impact of MIA.

P10. IN UTERO ELECTROPORATION OF DEVELOPING DOPAMINE NEURONS IN MICE

Authors: James P. Kesby, Rodrigo Suarez, Darryl W. Eyles

Affiliations: Queensland Brain Institute, The University of Queensland, Queensland Centre for Mental Health Research, The Park Centre for Mental Health

Background: Schizophrenia is a chronic psychiatric disorder with a poorly understood aetiology. Altered brain function and psychotic symptoms present prior to disease onset suggesting a developmental origin. Dopamine is a neurotransmitter that has been implicated in the cause and treatment of schizophrenia and thus represents a core developmental drug target in schizophrenia. Understanding the consequences of altered dopamine neuron development requires the use of techniques that allow for the specific manipulation of dopamine progenitors in the embryonic brain. We are developing a protocol using in utero electroporation that will allow for the transfection of siRNA to dopamine neurons in mice.
Method: Pregnant mice were anaesthetised and the uterine horns exposed. A reporter plasmid encoding a yellow fluorescent protein (pCAG-eYFP, 1.5 ug) was injected into the mesencephalic ventricle of e11 mice embryos. Electroporation was accomplished using a triple electrode configuration. The head of each embryo was held between two positive circular electrodes, positioned laterally but ventral to the floor plate, with a third negative electrode held dorsal to the embryo head to generate a ventral electrical field vector that transfects the plasmid into the ventral mesencephalon. Five electrical pulses (amplitude, 30 V; duration, 50 ms; intervals, 950 ms) were administered.
Results: We will present preliminary evidence using immunofluorescence microscopy (triple labelling for DAPI, eYFP and tyrosine hydroxylase) that demonstrates the specific transfection of dopamine progenitors in the ventral mesencephalon.
Conclusions: Although preliminary, these data suggest the targeting of developing dopamine neurons early in development is possible with the use of in utero electroporation. Furthermore, this work demonstrates the feasibility of future experiments transfecting siRNA against dopamine differentiation factors in the embryonic mouse brain. When combined, the use of targeted in utero electroporation and siRNA will allow us to gain a better insight into the role of specific differentiation factors in dopamine neuron development.

P11. CHRONIC ADOLESCENT CDPPB TREATMENT CAUSES ALTERATIONS TO MGLUR5 AND HOMER1B/C

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Background: Metabotropic glutamate receptor 5 (mGluR5) positive allosteric modulators (PAMs), such as CDPPB, are currently being investigated for the treatment of schizophrenia, autism and addiction. Recent investigations have shown that CDPPB treatment at adolescence prevents behavioural and molecular phenotypes associated with these disorders. Considering the chronic nature of these disorders a chronic treatment paradigm is required. However, due to the "pharmacological nature" of CDPPB chronic treatment may cause mGluR5 desensitisation, which may not deem it ideal for chronic use.
Method: Male adolescent (postnatal day 28) Sprague-Dawley rats were treated for 7 days with the mGluR5 PAM, CDPPB (30mg/kg, i.p; daily) or vehicle. Ex-vivo [3H]MPEP receptor binding autoradiography was employed to investigate the effect of chronic CDPPB treatment on mGluR5 binding in PFC and hippocampal sections. Furthermore, to investigate protein level changes between treatment groups, western blot analysis was performed to measure protein levels of mGluR5 and two key regulators of mGluR5 activity and cell surface expression, Norbin and Homer1b/c, in the PFC and hippocampus.
Results: Chronic adolescent CDPPB treatment had no effect on [3H]MPEP binding in the PFC ($p=0.147$), yet in the hippocampus, CDPPB treatment significantly reduced [3H]MPEP binding (-30%; $p=0.006$). Immunoblot analysies showed that adolescent CDPPB treatment caused no change in dimeric mGluR5 protein levels in the PFC ($p=0.189$) or hippocampus ($p=859$). However, chronic CDPPB treatment caused a significant reduction in levels of Homer1b/c, the major regulator of mGluR5 cell surface expression, in the hippocampus (-19%; $p=0.019$), yet was unchanged in the PFC ($p=0.868$). In addition, Norbin was unaltered by chronic CDPPB treatment in the PFC ($p=0.533$) or hippocampus ($p=0.439$).
Conclusions: Collectively, our results support that chronic adolescent CDPPB treatment may cause receptor desensitisation and subsequent internalisation in the hippocampus, mediated through its major endogenous regulator, Homer1b/c. CDPPB was recently shown to have agonist-like properties, under certain conditions, which may be responsible for the desensitisation effects observed. Whilst preclinical results hold promise for the therapeutic potential of CDPPB, "pure" mGluR5 PAMs should be considered, which are expected to be void of agonist-like activity and receptor desensitisation.

P12. DISSECTING COGNITION IN MICE WITH MUTATIONS IN POSTSYNAPTIC GENES RELEVANT TO PSYCHIATRIC DISORDERS

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Background: Sensory information from the environment is ultimately processed at the level of synapses, the connection between neurons that form the most fundamental information-processing units in the nervous system. In recent years, human genetic studies have increasingly highlighted that many of the mutations implicated in cognitive disorders converge upon genes associated with the synapse. However, very little is known about the genetic basis of distinct aspects of higher cognitive functions such as complex forms of learning and memory, attention and executive functions that are commonly impaired in disorders.
Method: Bridging the gap between mouse and human cognitive testing, the recently developed touchscreen methodology provides an innovative tool for assessing higher cognitive functions in rodents.
Results: Employing this technology, our recent studies have begun to dissect the functional role of postsynaptic genes in complex cognition in both mice and humans.
Conclusions: Our approach aims to aid our understanding of the genetic basis of these different aspects of cognition, and has significant implications for how we address translation from animal models to the clinic.

P13. WORKING MEMORY CAPACITY FOLLOWING NMDA RECEPTOR ABLATION IN PARVALBUMIN CELLS

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Background: The hypofunction of NMDA receptors (NMDARs) is a strong candidate mechanism underlying the pathophysiology of schizophrenia. In addition, deficits in the function of parvalbumin (PV+) inhibitory interneurons may also be particularly relevant. The aim of the current study is to investigate whether deletion of the NMDAR from PV+ interneurons impairs working memory processes, a core feature of schizophrenia. It is hypothesised that selectively ablating the NR1 subunit of the NMDAR from PV+ interneurons will induce working memory impairments in mice, and that the memory-impairing effects of NMDAR antagonists will not be apparent in these transgenic animals.
Method: PV-Cre; NR1f/f mice (n = 10) and their wild-type (wt) litter mates (n = 10) were trained to perform the trial-unique, delayed nonmatching-to-location (TUNL) task in automated touchscreen chambers. Once the task was acquired, working memory capacity was probed by increasing the delay between the sample and choice phases of the task. Task performance was assessed in terms of accuracy and the perseveration index (no. of correction trials: no. of incorrect responses). Working memory was also challenged by administering MK-801, an NMDAR antagonist.
Results: Somewhat surprisingly, PV-Cre; NR1f/f mice required significantly fewer sessions to reach criterion for the first stage of task- specific training compared to their wt litter mate controls ($p = 0.01$). In line with this, the NR1 flox animals made significantly less incorrect responses during this stage of training ($p = 0.001$). However, there was no difference in the perseveration index or the number of correction trials per session between the two genotypes. This demonstrates that the transgenic mice do not exhibit impairments performing the TUNL task, allowing us to accurately investigate challenging working memory paradigms.
Conclusions: This study hypothesised that disturbing NMDAR signalling exclusively on PV+ interneurons will generate a deficit in working memory. Specifically, it was predicted that increasing the delay between stimuli in the TUNL task will decrease accuracy, and that this effect will be more pronounced in the transgenic animals. Our initial studies demonstrate that the mice can perform the task successfully. Subsequent investigations will establish whether a reduction in NMDAR signalling specifically on PV+ interneurons is sufficient to disturb working memory performance, suggesting that this deficit may represent one pathological entity underlying working memory impairment in schizophrenia.

P14. MODELLING AN AUTISM RISK FACTOR IN RATS. THE IMPACT OF PERINATAL IMMUNE CHALLENGES ON GASTROINTESTINAL INTEGRITY

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Background: There is a growing subset of Autism Spectrum Disorder (ASD) sufferers presenting with co-morbid gastrointestinal (GI) abnormalities. With emerging knowledge of bi-directional gut-brain axis communication, there is a critical need to identify the aetiological factors contributing to these GI deficits. Animal models of maternal immune activation (MIA) have provided researchers a translational tool to investigate the risk factors that contribute to biological abnormalities underlying neurodevelopmental disorders such as ASD. The current study aimed to determine the effect MIA has on GI barrier integrity and inflammation.
Methods: Pregnant dams were exposed to their allocated treatment (5mg/kg polyIC or 0.1M phosphate buffered saline) on gestational day 10 (GD10) and GD19. Offspring were allocated to a tissue collection age across postnatal day 7 (P7), P21 and P84. Tissue collection involved extracting a 2cm segment of the distal large intestine which was PBS-flushed and homogenized. GI RNA was extracted and converted to cDNA. Quantitative real time polymerase chain reactions (qPCR) were used to assess the abundance of tight junction protein mRNA (zonula occludens-1, zonula occludens-2, claudin-2 and occludin) and pro-inflammatory cytokine mRNA (interleukin-6 (IL-6), tumour necrosis factor- α (TNF-α) and Interleukin-1β (IL-1β)).
Results: Results indicate that polyIC treatment is associated with increased levels of peripheral IL-6 in pregnant dams. Furthermore, in comparison to controls, MIA offspring exhibited decreased gene expression of TNF-α and IL-1β at P7 and an increased gene expression of IL-6 at P84. P21 cytokines were not altered by MIA exposure. The tight junction protein zonula occludens-1 was decreased in MIA P84 intestinal tissue while other tight junction proteins were not altered by MIA exposure at any other time points.
Conclusions: Our results show that the MIA induces changes in the gene expression of pro-inflammatory cytokines and tight junction genes in the large intestine of MIA offspring. Moreover, these changes are only observed at specific time-points. Although it is hard to speculate the biological meaning of these changes in relation to ASD at this stage, our results show that perinatal immune challenge is a risk factor that induces life-long changes on the GI tract of offspring in relation to integrity and inflammation. This seems to corroborate with the notion of a role for the gut-brain axis in the development of ASD.

P15. EFFECTS OF MATERNAL IMMUNE ACTIVATION ON NEGATIVE AND COGNITIVE SYMPTOM-RELATED BEHAVIOUR IN ADULT RATS

Authors: Rebecca Tattoli, Lauren Harms, Patricia Michie, Deborah Hodgson

Affiliations: University of Newcastle

Background: Maternal immune activation (MIA) related to prenatal infection increases the risk of schizophrenia in offspring. Previous studies in our lab have demonstrated that rats exposed to MIA during mid- or late-gestation exhibit some schizophrenia-like behavioural changes, but do not exhibit marked changes in behaviours related to the negative symptoms or cognitive deficits of schizophrenia. Other studies suggest that gestational day 14 (GD14) may be the most sensitive gestational time-point with regard to MIA exposure. Therefore, we aim to replicate these findings and characterise the effects of GD14 MIA on rodent behaviour related to the negative and cognitive symptoms of schizophrenia.
Method: On GD14, pregnant rats were injected with either a viral mimic, PolyI:C (MIA) or saline (Control). Two hours post injection, blood was collected from the saphenous vein to assess maternal levels of the cytokine interleukin 6 (IL-6). Control and MIA dams gave birth to their pups and when the offspring reached adulthood, they were tested on a battery of examinations aimed at assessing behaviour analogous to the negative symptoms and cognitive deficits of schizophrenia. Behaviour was assessed on the novel object recognition test, the social interaction test, the sucrose preference test and the elevated plus maze.
Results: Maternal IL-6 cytokine levels on GD14 were increased in MIA dams relative to controls. Behavioural examinations from the adult offspring are currently underway.
Conclusions: This project is in its preliminary stages. The increase in IL-6 in MIA-exposed rats demonstrates that MIA did occur and that behavioural assessments on the MIA and control offspring will produce valid results.

P16. NOVEL THERAPEUTIC APPROACHES TO TREATING COGNITIVE DEFICITS OF SCHIZOPHRENIA

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Background: Cognitive impairment is experienced by 85% of people with schizophrenia. The MATRICs initiative identified deficits in recognition, working memory, learning and executive functioning as key cognitive domains impaired in schizophrenia. Antipsychotic drugs (APDs) do little to improve cognition in schizophrenia and can cause obesity and diabetes side-effects. Therefore, novel therapeutic approaches are required. Liraglutide is an anti-diabetic and anti-obesity drug that may also improve cognition via glucagon-like peptide 1 receptor agonism. In addition, cannabidiol improves cognition in numerous pathological states. Aim: examine the effects of liraglutide and cannabidiol on cognition during APD treatment and an inflammatory model of schizophrenia, respectively.
Method: Study 1: Liraglutide was administered to female Sprague Dawley rats in combination with olanzapine or clozapine (6 treatment groups, n=12/group) for 6 weeks. Body weight was measured weekly. Study 2: Pregnant rats were administered Poly:IC and male and female adolescent offs pring were treated with cannabidiol for 3-weeks (8 treatment groups, n=12/group). Cognitive behaviours were examined using novel object recognition and T-Maze tests in both studies. Social behaviour was examined using the social interaction test in Study 2.
Results: Study 1: Olanzapine and clozapine impaired recognition memory ($p<0.001$ vs controls). Liraglutide co-treatment prevented this deficit ($p<0.001$ vs APD). Olanzapine (not clozapine) caused minor impairment to working memory ($p<0.05$ vs controls), but the co-treatment group did not differ to controls. Olanzapine induced weight gain ($p<0.05$ vs controls), that was prevented by liraglutide ($p<0.001$ vs olanzapine). Study 2: Poly:IC offspring exhibited impaired social interaction ($p<0.01$ vs controls) that was treated by cannabidiol ($p<0.01$ poly:CBD vs poly:control). Male (not female) poly:IC offspring exhibited impaired working ($p<0.05$) and recognition memory ($p<0.05$) that was restored by cannabidiol ($p<0.05$ poly:CBD vs poly:control).
Conclusions: These results demonstrate potential benefits of liraglutide and cannabidiol as novel therapeutic candidates to improve cognition in schizophrenia. Mechanistic studies are currently underway. Further studies are required to examine whether liraglutide co- treatment with APDs can provide benefits in a schizophrenia model, and to determine whether the animal model results of these studies can translate to clinical benefits.

P17. LONGITUDINAL ASSOCIATION OF OMEGA-6/3 POLYUNSATURATED FATTY ACID RATIO AND MOOD DISORDERS

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Background: Cross-sectional studies suggest a higher ratio of omega-6 to omega-3 polyunsaturated fatty acids (PUFAs) and lower levels of long-chain omega-3 PUFAs in patients with mood disorders. Omega-3 PUFAs are essential for neural development and brain function and may consequently play a causal role in depression pathophysiology. However, it remains unclear if high omega-6 to omega-3 PUFA ratio and low levels of omega-3 PUFAs are risk biomarkers for mood disorders. Here, we test the hypothesis that higher omega-6 to omega-3 PUFA ratio increases the risk for mood disorders in young individuals at ultra-high risk (UHR) for psychosis.
Method: We conducted a secondary analysis of the Vienna Omega-3 study, a randomized-controlled trial (RCT) of omega-3 PUFA supplementation for individuals at UHR for psychosis, using long-term (median 7 years) follow-up data from 69 individuals at UHR for psychosis. Mood disorder diagnosis was ascertained with the Structured Clinical Interview for DSM Disorders (SCID I/P) and confirmed by review of medical records and interviews with of caregivers. Levels of long chain omega-6 and omega-3 PUFAs were measured in the phosphatidylethanolamine fraction of erythrocyte membranes at intake into the RCT.
Results: Twenty-six (37.7%) individuals received a mood disorder diagnosis during follow-up. Adjusting for age, gender, severity of depressive symptoms at initial presentation, smoking and omega-3 supplementation, higher ratio of omega-6 to omega-3 PUFA (adjusted odds ratio [OR]=1.82, 95%CI=1.024 – 3.289, p=0.04) predicted mood disorders in UHR individuals over a 7-year (median) follow-up. The predictive capacity of higher omega-6 and lower omega-3 PUFA was specific for mood disorders as no associations were found for psychotic disorders, anxiety disorders, substance use disorder, or other psychiatric disorders.
Conclusions: Our data provide evidence that low levels of omega-3 PUFAs and high levels of omega-6 PUFAs are associated with an increased risk for mood disorders in young people exhibiting an ultra-high risk phenotype. In the context of current early intervention strategies, these findings may have important implications for risk stratification beyond clinical characteristics.

P18. BRAIN STRUCTURE AND COGNITION IN FIRST EPISODE SCHIZOPHRENIA ARE ASSOCIATED WITH SERUM NCAM LEVELS

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Background: Abundant evidence indicates that schizophrenia is characterized by cognitive impairments and abnormalities of brain structure. Neural cell adhesion molecule (NCAM) is a glycoprotein which plays important roles in cell–cell adhesion, neural migration, neurite outgrowth, synaptic plasticity and brain development that are related to cognition impairments. Moreover, schizophrenia patients show alterations of central or peripheral NCAM levels. So in this study, we investigated whether serum NCAM levels correlate with the cognitive impairments and changes of brain structure in first episode drug naïves schizophrenia patients compared to healthy controls.
Method: Thirty first episode drug naïves schizophrenia patients and thirty healthy controls were recruited. Psychiatric symptoms were assessed by the positive and negative syndrome scale (PANSS). Cognitive functions were assessed by measurement and treatment research to improve cognition in schizophrenia (MATRICS) and consensus cognitive battery (MCCB). Serum levels of NCAM were determined by ELISA. Structural neuroimaging were obtained using 3T Scanner and T1 images were processed in order to obtain a grey matter volumetric map.
Results: Schizophrenia patients revealed significantly decreased serum NCAM levels ($p<0.001$), which positively correlated with lower MCCB total score($r=0.438$, $p=0.003$). Also, left occipital middle grey matter volumes were decreased in schizophrenia patients compared to healthy controls ($p<0.001$). These reduced left occipital middle grey matter volumes were positively correlated with the serum NCAM levels($r=0.331$, $p=0.027$). Moreover, left occipital middle grey matter volumes showed a significant positive association with the MCCB total score ($r=0.429$, $p=0.001$).
Conclusions: In this study we found decreased serum NCAM levels and reduced occipital middle grey matter volumes in first episode drug naïve schizophrenia patients. Furthermore, these alterations both displayed a close relationship with impaired cognitive function. Since NCAN has an important role in neurodevelopmental processes, these results provide further evidence to support the neurodevelopmental dysfunction hypothesis of schizophrenia and suggest altered NCAM may be the potential biomarker of schizophrenia especially for cognitive impairments.

P19. IDENTIFICATION OF RARE CIRCULAR RNAS IN DEPOLARISED SH-SY5Y CELL LINES

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Background: In the last 20 years the transcriptome as we know has been enriched by the discovery of several new forms of transacting non- coding RNAs. A large number of these molecules are enriched in the brain and are thought to participate in multiple important neurological functions. Circular RNA (circRNA), one of the latest to emerge, is an RNA molecule whose 3 and 5 ends covalently link in a circle and has been discovered in a wide range of species including humans. This new class of ubiquitous RNAs species is relatively abundant and evolutionary conserved. They have also been implicated in several important biological functions, including, microRNA repressors (sponges) and transcriptional regulators. In this study, we investigate circRNAs expression in differentiated human SH-SY5Y neuroblasts.
Method: High throughput RNA-Seq was employed analyse total RNA depleted of both linear RNA and ribosomal RNA. Subsequently, cDNA libraries were generated according to the Illumina TruSeq total RNA-Seq protocol and sequenced (150 cycles) using an Illumina NexSeq500.
Results: After bioinformatic analysis we were able to reveal over 20,000 circRNAs, which were stably expressed in this cell line, of which, a large proportion were either rare or novel. Our analysis showed many of these circRNAs are derived from the genes involved in important neurological activities, such as synaptic function. Validation experiments by qPCR were performed using outward primers sets designed to uniquely amplify circular transcripts. The results confirmed existence, stability and circularity of the molecules observed by sequencing.
Conclusions: Taken together, our findings provide more evidence of abundance of this type RNA in the brain, suggesting a role for circRNA in the neuron function.

P20. SINGLE MARKER AND HAPLOTYPE META-ANALYSIS OF NEUREGULIN-1 (NRG1) GENETIC VARIATION AND SCHIZOPHRENIA

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Background: Genetic, post-mortem and neuroimaging studies repeatedly implicate neuregulin-1 (NRG1) as a critical component in the pathophysiology of schizophrenia. Although a 290 kilobase risk haplotype along with several genetic markers in both 5' and 3' region of the NRG1 gene have been linked with schizophrenia, results have been mixed. To reconcile these conflicting findings we conducted a meta-analysis of all NRG1 case-control and family based studies in schizophrenia.
Method: We conducted systematic searches using Medline (Ovid), PubMed & PsychInfo databases to find case-control and family based studies published between 2002 and 2016. Search terms included combinations of the following key words: "schizophrenia", "NRG1", "neuregulin 1", and "association". Data from each case-control and family study were used to create 2X2 and 1X2 tables respectively. For each NRG1 haplotype and single marker with three or more independent published studies a pooled odds ratio and 95% confidence interval was calculated using random-effects models. Heterogeneity in effect sizes across studies was tested using the Q-statistic and its magnitude was quantified by the I-squared statistic.
Results: Two markers (SNP8NRG241930 and 478B14-848) located in the 5' Icelandic schizophrenia risk haplotype (HapICE) region and one marker (rs2954041) in the 3' region of NRG1 were found to be significantly associated with schizophrenia. Carriers of the G allele at SNP8NRG241930 had 1.10 (95%CI=1.01-1.20, P=0.018) greater odds of having a schizophrenia diagnosis. Furthermore, 478B14-848(0) and T allele carriers at rs2954041 had 1.11 (95%CI=1.02-1.20, P=0.008) and 1.21 (95%CI=0.97-1.52, P=0.038) greater odds of having a schizophrenia diagnosis, respectively. There was evidence of heterogeneity for all three significant markers (rs62510682, I2=55.8; 478B14-848, I2=18.5; rs2954041, I2=43.3) but no publication bias was identified. No significant haplotype associations were found.
Conclusions: Our results suggest single markers in the 5' region and for the first time the 3' region of NRG1 are associated with schizophrenia. Although we did not find support for any haplotype, the number of studies and samples sizes available as well as allelic and clinical heterogeneity provides reasonable explanations for this observation. Nevertheless, our results provide renewed evidence that genetic variation in NRG1 is associated with schizophrenia.

P21. ABERRANT ACTION SELECTION AND CORTICOSTRIATAL ACTIVITY IN OBSESSIVE-COMPULSIVE DISORDER

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Background: Aberrant action selection may be the sine qua non of obsessive-compulsive disorder (OCD). The frontostriatal circuits hyperactive in OCD also control instrumental actions. Dissociable circuits mediate action-selection driven by instrumental outcome to actions prevoked by stimuli predicting the outcome, in the medial and lateral orbitofrontal cortex (OFC) respectively. This study explored the capacity of adolescents with OCD to integrate Pavlovian and instrumental learning to select actions and update their preferences.
Method: Participants were adolescents with OCD (n = 20) and matched healthy adolescents (n = 21). Participants instrumentally learned to earn food. They also learnt the Pavlovian relationship between conditioned stimuli and food availability. Flexible action selection was tested using Pavlovian-to-Instrumental Transfer and outcome devaluation tests during fMRI respectively. Blood oxygen level dependent (BOLD) data were analysed using whole brain and region of interest analysis within limbic-OFC and caudate- pre-frontal circuitries.
Results: Both groups showed Pavlovian and instrumental associations. The group with OCD were unable to use sensory information of conditioned stimuli to direct action selection; they exhibited hyperactive BOLD responses in the bilateral lateral and rostral OFC during this task. After devaluation, there was no significant between-group difference in action selection, however the OCD group displayed medial prefrontal cortex hypoactivity. Symptoms were unrelated to action selection, however medial OFC BOLD during stimuli-guided action selection increased with compulsions.
Conclusions: The novel finding of a failure to use sensory information from stimuli to guide action selection in adolescents with OCD was associated rostral and lateral OFC to hyperactivity. This deficit may represent an early risk factor for inflexible behavior with disease onset and progression. The normal response to devaluation may represent an area to intervene to maintain behavioural flexibility.

P22. ANTI-INFLAMMATORY EFFECTS OF BIPOLAR DISORDER DRUGS IN C8-B4 (GLIAL) CELLS

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Background: Bipolar (BD) disorder is a neuroprogressive, chronic mental health condition with progressive social and cognitive function disturbances. Most patients' treatments are based on 2 or more drugs to control their disease, but little is known of their interactions, and there is no biological basis for selection of drug combinations to improve efficacy. Additionally, the effect of these drugs on cytokine profiles are unclear. Understanding the actions of BD medications, especially the interactions between two or more of these drugs together, may increase our understanding of the pathophysiology of BD, and also allow new, more effective, targeted treatments to be identified.
Method: C8-B4 cells (ATCC®CRL-2540™) were seeded onto 24-well plates at 1.32x105cells/well in DMEM with 10% FBS. Cells were stimulated with 1ng lipopolysaccharide (LPS) and treated with Lithium (5mM), Valproate (0.5mM), Quetiapine (0.05mM) and Lamotrigine (0.05mM) individually and in all possible combinations. Vehicle control cells were treated with MilliQ water, 0.2% DMSO or both. A panel of 20 cytokines (IL-1a, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13,IL-17a, Eotaxin, GCSF, GMCSF, IFNg, KC, RANTES, TNF-α) was measured in media from the cells with a Bioplex Pro™ mouse cytokine 23-plex kit according to the manufacturer's protocol.
Results: Individual drugs had minor effects on the release of cytokines by LPS-stimulated C8-B4 cells after treatment. Quetiapine however robustly increased the release of all cytokines measured except for GCSF, suggesting a pro-inflammatory effect. Various drug combinations containing lithium and valproate also tended to have pro-inflammatory effects on the cells. On the other hand, the combination of lamotrigine and quetiapine robustly reduced cytokine release, suggesting a strong anti- inflammatory effect. Multiple three drug combinations that include both lamotrigine and quetiapine were also shown to have strong anti-inflammatory effects on the glial cells. All drugs used in combination also robustly lowered cytokine release.
Conclusions: To our knowledge, this is the first study investigating differences in cytokine production between commonly prescribed bipolar disorder drugs alone and in combination in glial cells. These data suggest that these drugs when acting alone have little overall effect on cytokine release from activated glial cells. However, the results suggest an additive anti-inflammatory effects of psychopharmacological combinations on cytokine production, which would have a protective effect in neurons.

P23. THE HETERODIMERIZATION OF HISTAMINE RECEPTOR-1 AND GHSR1A IS DECREASED BY OLANZAPINE: IMPLICATIONS FOR ANTIPSYCHOTIC-RELATED OVERWEIGHT

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Background: The atypical antipsychotics (APD), including olanzapine and clozapine possess potent histamine H1 receptors (H1R) antagonist properties, which plays an important role in APD-induced obesity. We previously have demonstrated that olanzapine consistently increases hypothalamic ghrelin receptor type 1a (GHSR1a) expression and causes obesity. GHSR1a exhibits an unusually high level of constitutive activity and dimerization with other receptor. Both H1R and GHSR1a acts through a common downstream signaling AMPK to regulate food intake, suggesting an interaction between these two receptors. This study aimed to identify the endogenous heterodimerization of H1R and GHSR1a in hypothalamic neurons and effects of olanzapine and H1 agonist.
Method: Primary hypothalamic neurons and mHypoA-NPY/GFP cells were treated with olanzapine (50μM) and H1 agonist (FMPH, 1μM). After 1.5 hour, the neurons were detected by H1R and GHSR1a primary antibodies and the secondary antibodies labelled with AlexaFlu 647 (acceptor) and Alexa Flu 568 (donor) dyes. Fluorescence Energy Transfer efficiencies (FRETeff) for the sensitized emission (SE) were calculated and analysed by FRET-SE Wizard Software. The NPY expression levels were quantified following olanzapine or FMPH treatments by the FlexStation.
Results: H1R and GHSR1a are colocalized in primary hypothalamic neurons and hypothalamic NPY-GFP neurons. H1R formed heteromers with GHSR1a in primary hypothalamic neurons and hypothalamic NPY-GFP neurons. Olanzapine significantly decreased the FRETeff in the NPY-GFP neurons ($P<0.001$), while FMPH increased the FRETeff ($P<0.001$). Furthermore, olanzapine increased NPY level by 75% ($P=0.006$) in the NPY-GFP neurons, while FMPH decreased NPY level by 37% ($P=0.008$).
Conclusions: The colocalized expression of endogenous H1R and GHSR1a are identified in the hypothalamic NPY neurons. The heterodimerization of H1R and GHSR1a has been demonstrated in the hypothalamic NPY neurons. The opposite effects olanzapine and H1 agonist on FRETeff suggest a physiological relevance of the interaction of H1R and GHSR1a. Furthermore, H1 agonist decreasing NPY level and olanzapine increasing NPY level suggest H1R may be involved in APD in stimulation of orexigenic NPY in the hypothalamus. Overall, these results reveal that the decreased H1R-GHSR1a dimerization may contribute to olanzapine-induced obesity, which warrants further investigation to clarify the detailed mechanism.

P24. REGULATION OF NEURONAL IMMUNE PATHWAY GENES BY DICER1

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Background: MicroRNAs (miRNA) are ~22 nucleotide non-coding RNAs that silence gene expression by guiding the RNA-Induced Silencing Complex (RISC) toward target mRNAs. Their biogenesis involves processing by numerous proteins, including the nuclear microprocessor components DGCR8 and DROSHA, and the cytoplasmic RNase III DICER. Their short target recognition sequence, or "seed region", allows a single miRNA to potentially target hundreds of genes, and miRNAs are thought to be involved in polygenic disorders such as schizophrenia (SZ). Previously we demonstrated that SZ is associated with an elevation of DGCR8, DROSHA, and DICER1 within the dorsolateral prefrontal cortex and superior temporal gyrus.
Method: The aim of the current study was to model the elevated expression of these genes in a neuronal cell culture and identify the consequence of this for neuronal miRNA and gene expression. We achieved this by overexpressing either DGCR8 or DICER1 in differentiated, neuron-like SH-SY5Y cells in vitro. Total RNA was then extracted from these cells by TRIzol extraction. We then utilised RNA sequencing technology to assess the differential expression of miRNA and mRNA. We further analysed this data by pathway analysis to identify common themes amongst dysregulated genes.
Results: This predicted that neuronal pathways such as axonal guidance signalling, glutamate signalling, and wnt/β-catenin signalling – all of which have relevance to SZ – would be significantly perturbed. Additionally, following overexpression of DICER1, several of the most highly dysregulated genes were involved in inflammatory and immune pathways, including MX dynamin-like GTPase 1 (MX1), ubiquitin-specific peptidase 18 (USP18), and interferon-induced proteins 44 (IFI44) and 6 (IFI6). This result is of particular significance, since the immune system is thought to play a significant role in the pathophysiology of SZ.
Conclusions: Overall, the results of this study suggest that the overexpression of neuronal miRNA biogenesis genes is able to influence neuronal function, including synapse formation and synaptic signalling, and in particular, increased levels of DICER1 may influence neuronal immune gene pathways. As such this study adds support to the hypothesis that dysregulation of miRNA biogenesis is part of the pathophysiology of SZ.

P25. NEURAL DIFFERENTIATION OF HUMAN NEUROBLASTS IS ASSOCIATED WITH TRANSCRIPTOME POLY(A) TAIL MODULATION

Authors: Dylan J. Kiltschewskij, Traude Beilharz, Murray J. Cairns

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Background: Differentiation of neural progenitors requires multiple levels of posttranscriptional regulation to dictate the location and timing of intracellular protein synthesis. A critical component of this system is the cis-acting mRNA 3′ poly(A) tail which acts as an enhancer of mRNA stability and translation. Shortening of this structure decreases transcript cytoplasmic half-life and translatability and is thus believed to represent a key stage in microRNA-mediated gene repression. At present, Poly(A) tail modulation is known to contribute to long term memory formation, circadian rhythms, Fragile X Syndrome and Alzheimer Disease, underscoring the importance of this RNA feature in neural function.
Method: In the current study, we sought to profile transcriptome-wide changes in poly(A) tail length in differentiated SH-SY5Y cells and investigate its association with steady-state mRNA levels. SH-SY5Y neuroblastoma cells were differentiated to near-pure cultures of neuron-like cells via sequential all-trans retinoic acid and brain-derived neurotrophic factor treatment over 14 days. mRNA expression and poly(A) tail length changes were characterized and compared via high-throughput deep sequencing using conventional RNA-Seq and a novel high-throughput library preparation for transcriptome-wide analysis of mRNA poly(A) tail length, designated PAT-Seq.
Results: Following genome alignment, read-count, poly(A) tail length attribution, we observed a differentiation-associated change of poly(A) tail length for over 1,700 mRNAs whereby a robust preference for lengthening was observed (93%). Interestingly, this was not coupled with increased expression of polyadenylation machinery nor an overall decrease in regulators of deadenylation. In addition, a striking correlation between mRNA expression and poly(A) tail length ($R2 = 0.3064$, $p < 0.0001$) was discovered, lending support to existing research suggesting increased poly(A) tail length confers mRNA stability. mRNAs subject to differentiation-associated changes in poly(A) tail length also exhibited significant enrichment in neurogenesis and cell cycle gene ontologies.
Conclusions: These results provide compelling support for the role of poly(A) tail length in the posttranscriptional regulation of mRNA during neural development. We suspect widespread tail lengthening represents a stabilization of the intracellular translational environment to facilitate the protein synthetic requirements for the transition from a pluripotent to neuronal phenotype. We believe that factors involved in the modulation of mRNA stability and translation may also have a significant role in the dysregulation of neuronal function and the pathogenesis of psychiatric disorders.

P26. VITAMIN D, VDR AND THE DIFFERENTIATION OF DOPAMINERGIC CELLS

Authors: Renata A. N. Pertile, Xiaoying Cui, Darryl W. Eyles

Affiliations: Queensland Brain Institute

Background: Schizophrenia is a polygenetic group of diseases of neurodevelopment influenced by a variety of pre and post-natal environmental factors. Abnormalities in dopamine (DA) regulation remain central to the neurobiology of schizophrenia. Vitamin D regulates multiple factors involved in the ontogeny of dopaminergic systems. Our group showed that in neonatal rats maternally deprived of vitamin D, DA turnover is decreased and the levels of the enzyme catechol-o-methyl transferase (COMT) is reduced. Although alterations in the COMT gene are implicated in schizophrenia, the mechanism for how 1,25(OH)2D3 may regulate COMT expression remains unknown.
Method: We have over-expressed the vitamin D receptor (VDR) in human neuroblastoma SH-SY5Y cells. We examine the effects of the active vitamin D hormone, 1,25(OH)2D3, on the production of DA by HPLC and the expression of dopaminergic-associated genes using QPCR and Western Blot. For those enzymes altered by vitamin D signalling we further examined gene regulation by Chromatin immunoprecipitation (ChIP-QPCR assay).
Results: Our results show that VDR overexpression increases DA neuron differentiation by increasing tyrosine hydroxylase expression and DA production. In the VDR-overexpressing cells, 1,25(OH)2D3 further increased the levels of the DA-metabolites 3-MT and HVA and elevated COMT gene expression. Chromatin immunoprecipitation revealed that 1,25(OH)2D3 increased VDR binding in 3 regions of the COMT promoter, suggesting direct regulation. The expression of other genes involved in the maturation of DA neurons such as VMAT2, MAOA and DRD2 were also altered by VDR-overexpression and 1,25(OH)2D3 treatment. Furthermore, the expression of NEUROG2, a marker of immature DA neurons, was decreased by VDR-overexpression.
Conclusions: These results show VDR and 1,25(OH)2D3 are directly involved in the regulation of dopaminergic-associated gene expression in neuronal cells and this model is a useful tool for identifying the role of 1,25(OH)2D3 in DA neuronal development and maturation.

P27. OPTIMISING WHOLE TISSUE CLEARING TECHNIQUES TO VISUALISE NEURONS IN THREE DIMENSIONS

Authors: Jessie Rowan, Jamie Flynn, Paul Tooney, Phillip Jobling

Affiliations: School of Biomedical Science and Pharmacy, University of Newcastle; PRC for Brain and Mental Health

Background: Investigation of neuroanatomy is essential for studying neuropsychiatric disorders. Historically, this was achieved via sectioning of samples. However, new 3D histology techniques enable examination of nervous tissue with intact, transparent samples. There are a variety of clearing protocols, each with their distinct advantages and disadvantages. Preservation of endogenous fluorophores and ability to keep tissue integrity is a priority for successful imaging and examination of neuronal morphology. In this study, we aim to compare two established tissue clearing techniques, CLARITY and CUBIC and introduce a new technique, CUBITY.
Method: CUBIC uses aminoalcohols and urea to facilitate lipid removal, while CLARITY embeds a hydrogel matrix to stablise tissue before removal of lipids via an anionic surfactant. Tissue harvested from transgenic mice expressing green fluorescent protein (GFP) under the parvalbumin, calretinin or choline acetyl transferase promoter was processed using CLARITY and CUBIC. We also combined aspects of both procedures in a new technique, CUBITY. All protocols were performed in accordance with the University of Newcastle animal care and ethics committee guidelines.
Results: Both protocols quenched fluorescence, though CUBIC preserved endogenous signals better than CLARITY. Tissue integrity was sustained throughout the CLARITY protocol, with the samples remaining intact throughout the process. Significant tissue degradation was observed with CUBIC, and breakdown of the sample was problematic. CUBITY provided better durability of tissue as well as significant visual clarity.
Conclusions: 3D clearing techniques such as CLARITY and CUBIC has great applicability to imaging and visualizing nervous tissue without the need for tissue sectioning. CUBITY introduces a way to preserve tissue integrity without compromising tissue transparency. Combined with transgenic mouse models and confocal/light microscopy, these techniques allow for in-depth investigation of morphology of nervous tissue. This technique could be developed to study in 3D the abnormal connections between neurons in animal models of psychiatric disorders.

P28. OLANZAPINE DECREASES THE 5-HT2CR AND GHSR1A DIMERIZATION IN THE HYPOTHALAMIC NPY NEURONS

Authors: Yinghua Yu, Tiantian Jin, Xiaoqi Chen, Zehra Boz, Xu-Feng Huang

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Background: Treatment with second generation antipsychotics (SGAs), notably olanzapine and clozapine, can cause severe obesity. These obesogenic SGAs possess potent 5-HT2cR antagonist properties. Antagonism of the serotonin 5-HT2c receptor (5-HT2cR) has been identified as a main cause of SGA-induced obesity. New evidence demonstrates a pivotal regulatory role for the 5-HT2cR in ghrelin-mediated appetite signalling. The 5-HT2cR dimerizes with the ghrelin receptor type 1a (GHSR1a) to inhibit orexigenic activity, while 5-HT2cR antagonism increases GHSR1a-induced food intake. In this study, we investigated the effects of olanzapine, 5-HT2cR antagonist and agonist on the 5-HT2cR - GHSR1a dimerization, GHSR1a expression and orexigenic NPY level.
Method: Primary hypothalamic neurons and mHypoA-NPY/GFP cells were treated with olanzapine (25 and 50µM), 5HT2cR antagonist (SB242084, 10, 50 and 100µM) and olanzapine (50µM) + 5HT2cR agonist (lorcaserin, 10 and 50µM). After 1.5 hour, the neurons were detected by 5-HT2cR and GHSR1a primary antibodies followed by the secondary antibodies labelled with AlexaFlu 594 (acceptor) and Alexa Flu 488 (donor) dyes. Fluorescence Energy Transfer efficiencies (FRETeff) for the sensitized emission (SE) were calculated and analysed by FRET-SE Wizard Software. The NPY expression levels were quantified following olanzapine and/or lorcaserin treatments by the FlexStation.
Results: Olanzapine (50µM) significantly decreased the FRETeff in both primary hypothalamic neurons ($p<0.05$) and NPY-GFP neurons ($p<0.05$) after 1.5 hour treatment. SB242084 (10, 50 and 100µM) also decreased FRETeff in the primary hypothalamic neurons. Furthermore, olanzapine treatment for 24 hours increased (+44.1%) the level of GHSR1a immunofluorescence in NPY-GFP neurons. Lorcaserin (10 and 50µM) counteracted the olanzapine's effect on decreasing FRETeff in the primary hypothalamic neurons. In the NPY-GFP neurons, the NPY level is significantly lower in co-treatment of olanzapine and lorcaserin compared with olanzapine group.
Conclusions: Olanzapine decreases 5-HT2cR dimerization to the GHSR1a in hypothalamic NPY neurons via its 5-HT2cR antagonistic property. Given the well-documented role for GHSR1a-NPY in hyperphagia and weight gain, this study provides critical evidence of the causal mechanisms of olanzapine-induced weight gain. Furthermore, lorcaserin prevented the reduction of 5-HT2cR and GHSR1a dimerization and increased NPY in the hypothalamic neurons induced by olanzapine. It suggests that lorcaserin may attenuate SGAs induced obesity and metabolic disorders, which needs further investigation in preclinical and clinical study.

P29. DOPAMINE HYPERACTIVITY INDUCED BY QUINPIROLE AND PCP INHIBITED NEURITE GROWTH VIA D2R-DISC1 COMPLEX

Authors: PENG ZHENG, YINGHUA YU, HONGQIN WANG, XUFENG HUANG,

Affiliations: School of Medicine, University of Wollongong, and IHMRI, NSW 2522, Australia

Background: Disturbances in neuronal connectivity underlie schizophrenia and associated disorders. The dopamine hyperactivity has been involved in the pathogenesis of schizophrenia. Previously, quinpirole, the agonist of dopamine 2 receptor (D2R) and phencyclidine (PCP) exhibit schizophrenia-like hyperactivity of dopamine. The present work was designed to elucidate effects of quinpirole on neurite outgrowth and synaptic plasticity in primary cortical neurons. Disrupted-In-Schizophrenia 1 (DISC1) plays a role in the etiology of schizophrenia. Recently, D2R-DISC1 complex levels are increased in postmortem brain tissue from schizophrenia patients. In this study, D2R-DISC1 complex levels in striatal neurons were examined in response to PCP-induced dopamine hyperactivity.
Method: Primary prefrontal cortical and striatal neurons were cultured from postnatal day 0 mice. At 7 days in vitro, the cell viability was measured 48 hours after quinpirole (1-700 µM) administration. Furthermore, after administration of quinpirole (300 µM) and PCP (10 µM) for 48 hours respectively, neurons were labeled with microtubule associated protein 2 (MAP2) and postsynaptic density 95 (PSD 95) antibodies for immunocytochemistry. In addition, the neurons were detected by D2R, DISC1 primary antibodies followed by the secondary antibodies labelled with Alexa Flu 594 (acceptor) and Alexa Flu 488 (donor) dyes. Fluorescence resonance energy transfer (FRET) were calculated and analyzed.
Results: Quinpirole reduced cell viability in a dose-dependent manner. Quinpirole at 300 µM significantly decreased cell viability (-40%) compared with control. The neurite length and the number of neurite branches were significantly decreased by quinpirole in primary cortical neurons stained with MAP2. The PSD95 immunoreactivity was significantly decreased 62.67% by quinpirole administration ($P < 0.05$). FRET exhibited endogenous co-localization and interaction of DISC1 and D2 receptor in the primary striatal neurons. This interaction was significantly increased by the PCP administration after 48 hours.
Conclusions: Our results suggest that the hyperactivity of dopamine induce by D2R agonist impairs neurite outgrowth and post- synaptogenesis in prefrontal cortical neurons. Furthermore, we found that the DISC1-D2R complex increased in the striatal neurons in response to PCP, which is well known to stimulate D2R and induce schizophrenia-like behavior. Therefore, it implies that alteration of D2R-Disc1 complex mediated by dopamine hyperactivity may be a critical mechanism underlying schizophrenia neuropathology.

P30. CHOLINERGIC M1 RECEPTOR POSITIVE NEURONS IN THE HUMAN HIPPOCAMPUS: A STEREOLOGICAL INVESTIGATION.

Authors: Shaun Hopper, Myoung Suk Seo, Brian Dean, Elizabeth Scarr

Affiliations: The Florey Institute of Neuroscience and Mental Health

Background: The hippocampal formation and cholinergic muscarinic receptors (CHRMs) play roles in diverse higher-order brain functions and their dysfunction have been implicated in multiple neurological and neuropsychiatric diseases including schizophrenia and Alzheimer's disease. However until now, the distribution and density of specific CHRMs has remained to be elucidated due to difficulty in targeting the receptors individually. Recently, we validated an antibody specific to CHRM1 for use in immunohistochemistry. Thus, we present here a stereological investigation of the density of CHRM1 positive neurons in multiple fields within the human hippocampus.
Method: We used immunoperoxidase to determine the distribution and density of neurons positive and negative for CHRM1 in post mortem samples from the right hippocampus of five individuals with no history of neurological or neuropsychiatric disease. We counterstained the sections with haematoxylin in order to distinguish neurons from glia. To aid with identification of the cornu ammonis (CA) fields and polymorphic layer of the dentate gyrus one section from each case was stained with cresyl violet. Images were captured with a Leica DMLB microscope and analysed using StereoInvestigator software.
Results: The density of neurons showed little individual variation with CA2>CA3=CA1>Polymorphic layer (CA2: 48200±6200; CA3: 37000±4900; CA1: 35100±3900; and the polymorphic layer: 21500±4000; all results are MEAN±S.D. cells/mm3). Conversely, the immunogenic reactions showed highly variable distributions of positive cells within regions, with neurons expressing CHRM1 in CA3 (25100±9900), CA2 (22000±16900), polymorphic layer (16100±6800), and CA1(5300±3400). The density of glia was approximately equal in the polymorphic layer, CA3, and CA2 (Polymorphic layer: 160000±28000; CA3: 151000±21000; CA2: 163000±17000) and lower in CA1 (98000±17000).
Conclusions: This study characterises the distribution and density of neurons specifically positive for the CHRM1 in the human hippocampus. The differences between the distribution of our results and previous [3H]pirenzepine binding studies, particularly in CA1, suggests that [3H]pirenzepine is also binding to CHRM4 in this region. Recently, specific drugs targeting CHRM1 have been synthesised and knowing where the targets are located will help elucidate the mechanism of action of these compounds.

P31. TYROSINE HYDROXYLASE AND DOPAMINE TRANSPORTER PROTEINS IN THE NIGROSTRIATAL PATHWAY IN SCHIZOPHRENIA

Authors: Samantha J Owens, Tertia D Purves-Tyson, Danny Boerrigter, Debora A Rothmond, Glenda M Halliday, Kay L Double, Cynthia Shannon Weickert

Affiliations: Schizophrenia Research Laboratory, Neuroscience Research Australia; School of Psychiatry, UNSW; Ageing and Neurodegeneration, Neuroscience Research Australia and School of Medical Sciences, UNSW; Discipline of Biomedical Science and Brain and Mind Centre, University of Sydney

Background: Increased subcortical dopamine neurotransmission is thought to underlie psychosis in schizophrenia. In vivo imaging studies indicate a presynaptic dysfunction within axon terminals and/or cell bodies of dopamine neurons; however, consistent changes in molecules regulating presynaptic dopamine have not been identified in schizophrenia. Tyrosine hydroxylase (TH), the rate- limiting enzyme in dopamine synthesis, and dopamine transporter (DAT), responsible for dopamine reuptake from the synaptic cleft, are proteins key to dopamine neurotransmission. We hypothesised that subcortical hyperdopaminergia in schizophrenia could result from decreased DAT protein, and/or increased protein levels of TH and/or a more active phosphorylated TH state (TH-Phos) in the nigrostriatal pathway.
Method: Western blotting was used to measure TH, TH-Phos and DAT proteins in post-mortem midbrain (26 schizophrenia/28 controls), and TH and DAT proteins in post-mortem caudate (19 schizophrenia/22 controls). For all protein measures, correlations were conducted with demographic variables (age, pH, post-mortem interval) and with antipsychotic drug measurements and duration of illness in the schizophrenia group. TH and TH-Phos in the midbrain correlated with brain pH and analysis of covariance was used to test for diagnostic differences. No correlations were detected for all other proteins of interest and independent sample 2-tailed t-tests were used to detect diagnostic differences.
Results: No changes in the protein levels of TH ($F=0.30$; $df=53$; $p=0.58$), TH-Phos ($F=1.94$; $df=53$; $p=0.17$) or DAT ($t=-1.36$; $df=52$; $p=0.18$) were found in the midbrain in schizophrenia patients compared to controls. TH ($t=-1.30$; $df=25.04$; $p=0.21$) and DAT ($t=-1.21$; $df=39$; $p=0.24$) protein levels in the caudate were also unchanged between schizophrenia patients and controls.
Conclusions: Our results suggest that presynaptic dopaminergic dysfunction in schizophrenia is not mediated by changes in steady-state protein levels of TH or DAT in the nigrostriatal pathway or TH-Phos in the midbrain. However, alterations in the activity of these proteins or dysregulation of other dopamine-related molecules may contribute to increases in presynaptic dopamine neurotransmission in schizophrenia. Future investigations examining DOPA decarboxylase, also involved in dopamine synthesis, and DAT activity are ongoing aspects of this work.

P32. MATERNAL IMMUNE ACTIVATION INDUCES GESTATIONAL DAY SPECIFIC AND SEX SPECIFIC ALTERATIONS IN GAD67 GENE EXPRESSION IN THE RAT MEDIAL PREFRONTAL CORTEX.

Authors: Ryan Duchatel, Crystal Meehan, Mark Bigland, Douglas Smith, Philip Jobling, Katerina Zavitsanou, Lauren Harms, Deborah Hodgson, Patricia Michie and Paul Tooney

Affiliations: Priority Centre for Brain and Mental Health Research, University of Newcastle; Schizophrenia Research Institute, Neuroscience Research Australia

Background: Prenatal immune challenge is an environmental risk factor for the development of psychiatric illnesses including schizophrenia. Modelling this epidemiological link in animals shows that maternal immune activation (MIA) is capable of inducing long-lasting deficits in brain structure, function and behaviour in the offspring. Alterations in the GABAergic system are among the most robust findings within schizophrenia. Our aim was to determine if MIA induced in pregnant rats by administration of the viral mimetic Poly(I:C) (polyriboinosinic-polyribocytidilic acid) resulted in alterations to prefrontal cortical GABAergic gene expression in the offspring.
Methods: MIA was induced in pregnant Wistar rats using an injection of PolyI:C at either gestational day (GD) 10 or GD19. Brains from offspring of MIA affected dams (n = 12 GD10 PolyI:C, n = 12 GD19 PolyI:C) and control animals (n = 12 GD10 Saline, n = 12 GD19 Saline) were collected at postnatal day 84. Gene expression of Glutamic Acid Decarboxylase-65 *(GAD65)*, *GAD67*, neuronal nitric oxide synthase *(nNOS),* parvalbumin *(PV),* somatostatin *(SST)*, calretinin *(CR)*, calbindin *(CB)*, neuropeptide Y *(NPY)* was then examined using real-time quantitative PCR in the medial prefrontal cortex (mPFC). Statistical significance was determined by student's t-test.
Results: The expression of GAD65, nNOS, PV, SST, CR, CB or NPY was not altered in either GD10 or GD19 MIA offspring, however a significant increase in GAD67 was identified in female offspring at GD19, but not GD10. This demonstrates a potential GD-specific and sex-specific effect of MIA on GAD67 gene expression in the mPFC.
Discussion: We identified that prenatal exposure to immune challenge leads to increases in GAD67 gene expression, in females, which is inconsistent with postmortem brain tissue studies from people with schizophrenia. Intriguingly, the timing of the prenatal insult, and sex is crucial in our model, suggesting that MIA in late but not early gestation affects GAD67 gene expression and only in females. This data emphasises the impact of prenatal immune-related insults on long-term changes in the cortex, but how these changes relate to those seen in people with schizophrenia requires further investigation.

Poster Session 2

Friday, 14 Oct, 1:30 – 2:30 pm

P1. ESTABLISHING THE RODENT SIGNAL DETECTION TASK IN MICE

Authors: K.A. Conn, K.M. Turner, T.H.J. Burne

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Background: Schizophrenia is a complex disorder characterised by positive, negative and cognitive symptoms, including deficits in executive functioning, attentional processing and decision-making. A current lack of effective treatments has resulted in the foundation of major initiatives to guide research on cognitive dysfunction and the development of better preclinical models. This study presents the establishment of the rodent Signal Detection Task (SDT) to assess attention and decision-making in the mouse, which is based on an existing task initially developed for the rat.
Method: Two inbred strains of mice were chosen, BALB/c (n=48) and C57BL/6J (n=24), as they are commonly used background strains for modelling disease. After acquisition, as mice reached baseline performance, a series of manipulations were used to assess the validity of this task to model attentional processes. These techniques included varying the procedural parameters, devaluing the reward, and a pharmacological manipulation using amphetamine due to its known effects on cognition. By examining cognitive performance and motivational bias on the SDT, we were also able to apply signal detection theory, a well-established mathematical method used to study attention in humans.
Results: Both strains of mice were able to perform the task with high accuracy and at a fast pace with differences due to differing genetic backgrounds being observable, such as BALB/c mice acquiring the task in a shorter amount of time than C57BL/6J ($p<.001$). By increasing the attentional load on the task we were able to decrease performance in accordance with increased load ($p<.001$). In turn, by devaluing the reward, we found that motivation was altered but accuracy was not affected, indicating the task's ability to separate motivational bias from cognitive performance. Finally, the administration of systemic amphetamine in the mouse resulted in both altered performance and response parameters.
Conclusions: This study supports the SDT's ability to probe attention and decision-making processes validly in mice. Additionally, the application of signal detection theory is valuable not only due to its use in human studies of attention, but as it provides variables which can eventually be correlated with activity on a neuronal level. Consequently, the establishment of the rodent SDT is an improvement for valid cross-species translational studies of mouse to human behaviour.

P2. BDNF MAINTAINS NORMAL ESTROUS CYCLING – RELEVANCE TO SCHIZOPHRENIA

Authors: Xin Du, Cushla McCarthny, Anna Schroeder, Michael Notaras, Adrienne Grech, Rachel Hill

Affiliations: Monash University; The Florey Institute of Neuroscience and Mental Health

Background: Recent clinical evidence suggests that a large proportion of women with schizophrenia exhibit abnormal menstrual cycles and reduced circulating estradiol levels, which may contribute to symptoms such as cognitive decline. While this phenomenon is partially accounted for by antipsychotic-induced hyperprolactinaemia, it is likely induced by innate pathophysiological changes related to the disease. We propose that a reduction of brain-derived neurotrophic factor (BDNF), as observed in schizophrenia, may be sufficient in inducing pathological changes in the hypothalamic-pituitary-gonadal (HPG) axis, thereby leading to alterations of menstrual cycling and reductions in estrogen production.
Method: We used both wild-type and BDNF heterozygous female mice, which express ~50% of normal BDNF levels. To assess estrous cycling, vaginal smears were performed 2-3 times a day for 3 weeks from 7-9 weeks of age. Furthermore, brain tissue was collected in separate cohort of female mice at both low and high estradiol-expressing phases of the estrous cycle to assess expression levels of key regulators of HPG-axis function, including GnRH receptor protein expression in both the hypothalamus and the hippocampus.
Results: Female BDNF heterozygous mice exhibit dysregulation of their estrous cycle in a remarkably similar manner to that reported in female schizophrenia patients. Where the patients showed elongation of menstrual cycle, the BDNF heterozygous mice show increased cycle lengths compared to wild-type mice. Particularly, in the BDNF heterozygous mice there is elongation of the pro- estrus and estrus phases, a sign of transitioning to acyclicity. This alteration is accompanied by a significant reduction in GnRH receptor protein expression in both the dorsal and ventral hippocampus, which is associated with reduced ability to locally produce estrogen, reduced neuronal plasticity and ultimately cognitive deficits.
Conclusions: Our data is the first to show that reduced BDNF is a sufficient pathoaetiological factor to cause a shift in the estrous cycle of female mice; that in the hippocampus, GnRH receptor loss suggest that the local estrogen production is also affected; and that these changes may contribute to symptoms such as cognitive decline observed in patients. These findings provide a new prism through which symptoms like cognitive decline, hitherto untreatable in schizophrenia, could be studied and ameliorated.

P3. CLOZAPINE IMPROVES THE IMPAIRMENT OF NEURITE OUTGROWTH INDUCED BY NR2B ANTAGONIST IN PFC NEURONS

Authors: Fu Q, Wang HQ, Yu YH, Huang XF

Affiliations: School of Traditional Chinese Pharmacy, China Pharmaceutical University; School of Medicine, University of Wollongong; IHMRI

Background: Accumulation evidence has demonstrated that various mental diseases, such as schizophrenia, are closely related with impairment of neurite outgrowth. N-methyl-D-aspartate receptor (NMDAR) plays a critical role in neuron plasticity, neurite outgrowth, dendritic arborisation and synapse formation. The hypofunction of NMDA receptor is related with degeneration of neurite outgrowth. NMDA receptor subunit 2A (NR2A) or NMDA receptor subunit 2B (NR2B) predominate in the forebrain and play the differential role in regulating cortical long-term potentiation. This study aims to identify the role of NR2A and NR2B in neurite outgrowth as well as to examine the protect effects of common used atypical antipsychotics, clozapine.
Method: The primary prefrontal cortical neurons of wild type (WT) and neuregulin 1 (Nrg1) knockout mice were prepared and cultured in 24-well plate with PDL coated coverslips. After 4 hours culture, NR2B antagonist (ifenprodil, 5-50μM), NR2A antagonist (PEAQX, 5-50μM), clozapine (0.001-50μM) and co-treatment of clozapine and ifenprodil were administrated to the neurons for 5 days. The neurite outgrowth was monitored by optical microscope and finally the neurons were stained with MAP2, synaptophysin or PSD95 primary antibodies and labelled with AlexaFlu 594 and Alexa Flu 488 dyes.
Results: NR2B antagonist ifenprodil (5-50μM) significantly inhibited the neurite outgrowth of prefrontal cortical neurons in both WT and Nrg1 knockout mice. However, NR2A antagonist PEAQX (0-50μM) did not affect the neurite outgrowth of prefrontal cortical neurons. Clozapine (0.01-0.1μM) significantly reversed ifenprodil impaired neurite outgrowth of cortical neurons in both WT and Nrg1 knockout mice, although clozapine at low concentration (<0.1μM) did not affect neurite outgrowth. Furthermore, clozapine at 10μM significantly inhibited the neurite outgrowth of primary prefrontal cortical neurons in both WT and Nrg1 knockout mice.
Conclusions: We characterized NR2B, but not NR2A is mainly responsible for NMDA receptor in regulating neurite outgrowth. Clozapine significantly prevent of the impairment of neurite outgrowth induced by NR2B antagonist in the prefrontal cortical neurons independent on Nrg1. The over dose of clozapine may have deleterious effect on neurite out-growth of cortical neurons. Overall, atypical antipsychotic clozapine at optimal dose may improve neurite outgrowth in the status of hypofunction of NMDA, especially NR2B subtype.

P4. EFFECTS OF RALOXIFENE TREATMENT IN THE POLY I:C MODEL OF SCHIZOPHRENIA

Authors: Anna Schroeder, Jay Nakamura, Dr. Xin Du, Michael Notaras, Dr. Rachel Hill

Affiliations: The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Australia; Department of Psychiatry, Monash University, Melbourne, Australia

Background: Schizophrenia is a mental illness with strong sex differences, females showing less severe outcomes thought to be due to the neuroprotective effects of estrogens. While estradiol treatment itself is limited due to its peripheral actions, the selective estrogen receptor modulator, raloxifene, has shown promise in the clinic but lacks mechanistic understanding. Our novel previous data, using an ovariectomized mouse model, demonstrate that both estradiol and raloxifene synchronize hippocampal gamma frequency oscillations, (thought to underlie cognitive dysfunction in schizophrenia) enhancing spatial memory. This project now sought to determine the effects of raloxifene on behavior and electrophysiology in a mouse model with high relevance to schizophrenia; the poly-I:C-induced maternal immune activation model.
Method: Breeder dams were injected with either the viral mimetic - Poly I:C, or saline, in late pregnancy (GD17) to elicit an immune response. Offspring of these dams were used as subjects in this experiment. Slow release raloxifene pellets or matched placebo control pellets were subcutaneously administered at the mouse age equivalent to human late adolescence/early adulthood, a likely period of diagnosis and prescription of medications in schizophrenia. Learning, memory, anxiety and sensorimotor gating was probed using the y-maze, cheeseboard maze, elevated plus maze and pre-pulse inhibition chambers. Electrophysiological recordings were simultaneously taken from the dorsal hippocampus to investigate the effects of raloxifene on gamma frequency oscillations during behavioural testing.
Results: Prenatal Poly I:C treatment resulted in a variety of sex specific behavioural deficits. Briefly, male Poly I:C mice had increased anxiety, disrupted spatial learning, and decreased sensorimotor gating. Female mice demonstrated perseverative behaviour and shared the decreased sensorimotor phenotype with males. Raloxifene treatment reversed the changes to male spatial learning and female preservative behaviour. Interestingly, raloxifene treatment had the added effect of reducing anxious behaviour in both control and Poly I:C treated females. Electrophysiological data again shows sex specific changes, mainly, a disrupted gamma power during decision making in female Poly I:C treated mice which is then reversed with raloxifene treatment.
Conclusions: Behavioural deficits were indeed observed in the Poly I:C mouse model of schizophrenia, and these deficits were highly behaviour and sex dependent. Raloxifene was able to recover some but not all of these deficits, warranting further investigation of its physiological effects and mechanism of action in future studies. Prenatal Poly I:C treatment and raloxifene also displayed the ability to modulate gamma oscillations in the dorsal hippocampus, an area known to regulate spatial memory ability. This hints at possible mechanisms by which raloxifene may alter learning and memory behaviours in schizophrenia.

P5. NMDAR ABLATION FROM PARVALBUMIN INTERNEURONS CAUSES DESENSITISATION TO SCHIZOPHRENIA-RELEVANT DEFICITS INDUCED BY MK-801

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Background: It has been suggested that NMDA receptor hypofunction on parvalbumin (PV) positive interneurons is closely tied to the pathophysiology of schizophrenia. Supportive evidence comes from examination of genetically modified mice where the obligatory NMDA receptor subunit NR1 has been selectively ablated from PV+ interneurons. The aim of the current study was to assess the effects of NMDAr antagonists on behavioural and electrophysiological measures with relevance to schizophrenia in PV-Cre; NR1f/f mice.
Method: Mice in which the obligatory NMDAr subunit NR1 was selectively ablated from PV positive neurons (PV-Cre; NR1f/f mice; n=8) and their wild-type littermates (wt;n=8) were generated by crossing PV-Cre mice with mice carrying 'floxed' NR1 alleles. At 12-16 weeks of age, behavioural tests of locomotor activity and prepulse inhibition (PPI) were performed following injection of MK-801 or saline. Following the behavioural tests, mice underwent stereotaxic surgery to allow for the implantation of electrodes in the prelimbic cortex, dorsal hippocampus and nucleus accumbens. Electrophysiological recordings were then made following administration of MK-801 or saline.
Results: Behaviourally, the NMDAr antagonist MK-801 produced an increase in locomotor activity as well as a reduction in PPI in both PV-Cre; NR1f/f mice and WT littermates. The increase in locomotion was substantially blunted in PV-Cre; NR1f/f mice when compared to wildtype littermates. In the electrophysiology study, MK-801 produced an elevation in ongoing gamma power in all regions, and this effect was substantially blunted in the PV-Cre; NR1f/f mice.
Conclusions: Following administration of the NMDAr antagonist MK-801, PV-Cre; NR1f/f mice showed substantially reduced levels of hyperlocomotor activity and a blunted increase in ongoing gamma power indicating a desensitisation to the effects of NMDAr antagonists on these measures. These findings suggest that the increase in locomotor activity and elevation in ongoing gamma power observed following administration of NMDAr antagonists is mediated at least in part by NMDA receptors on PV+ interneurons.

P6. ACUTE MK801 TREATMENT INDUCES WORKING MEMORY DEFICIT IN AUTOMATED TOUCHSCREEN CHAMBERS

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Background: Working memory (WM) deficits are a consistent cognitive impairment in schizophrenia patients. Current treatments do not improve these deficits, which may be attributable to our limited understanding of the underlying causal mechanisms. Neural oscillations in the gamma frequency range (i.e. 30-80Hz) are associated with higher order cognitive function, including WM, and regulation of gamma oscillations is impaired in schizophrenia patients. NMDA receptor (NMDAr) hypofunction is a strong candidate mechanism involved in the pathophysiology of schizophrenia. Administration of NMDAr antagonists can induce aberrant gamma oscillations. Therefore, we investigate whether gamma oscillatory disruptions induced by NMDAr antagonism is related to WM deficits.
Method: Long Evans rats (N=6) were surgically implanted with depth recording electrodes into the prefrontal cortex and hippocampus. Animals were trained to perform the Trial Unique Non-Matching to Location (TUNL) task of working memory in automated touchscreen chambers. When steady state performance was achieved, they were injected with either saline or MK801 (0.06mg/kg), and electrophysiological recordings were simultaneously performed during working memory behavioural trials of varying levels of difficulty.
Results: Rats performed significantly poorer in the more taxing working memory task (i.e. 10 second delay compared to 1 second delay; $p<0.01$). In addition, treatment with MK801 significantly impaired working memory regardless of task difficulty ($p<0.01$). No differences in performance were observed in trials when the electrophysiological recordings were not conducted. Preliminary analysis suggests that the degree of aberrant gamma oscillations is associated with performance.
Conclusions: These results demonstrate that NMDAr hypofunction causes impairment in an advanced working memory paradigm, and suggest that these deficits may be driven by dysregulation of cortical gamma oscillations.

P7. KETOGENIC DIET NORMALISES SENSORIMOTOR GATING DEFICIT IN ACUTE AND CHRONIC NMDA RECEPTOR MICE MODEL

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Background: Recent findings suggest that abnormal glucose/energy metabolism, leading to impaired neural communication and altered production of excitatory and inhibitory neurotransmitters, may play a role in the pathophysiology of SZ. A high fat, low carbohydrate ketogenic diet (KD) improves brain energy metabolism and normalises excitatory/inhibitory neurotransmitter balance by facilitating GABA production in the brain. We have recently demonstrated that KD reverses behavioural abnormalities in an acute NMDA receptor hypofunction model of schizophrenia (Kraeuter et al., 2015). Here we aimed to investigate the effects of KD on sensorimotor gating deficit, a translatable schizophrenia endophenotypes in acute and chronic NMDA receptor hypofunction models.
Method: Young male C57BL/6 mice were randomly assigned to consume standard diet (SD) or KD ad libitum for 3 weeks. Acute NMDA receptor hypofunction was induced by MK-801 (0.25 mg/kg, i.p., n=9 per group). Chronic NMDA receptor hypofunction was induced by repeated daily injection of 1 mg/kg MK-801 for 28 days. KD was initiated after 1 week after the start of chronic MK-801 administration and maintained for the following 3 weeks. Sensorimotor gating was studies by measuring prepulse inhibition of startle (PPI) 30 minutes or 24 hours after the acute or the last of the chronic MK-801m injection, respectively.
Results: KD normalised the impaired pre-pulse inhibition of startle (%PPI) both in acute and chronic NMDA receptor hypofunction models of schizophrenia.
Conclusions: Our findings show, for the first time, that the KD normalizes sensorimotor gating deficits, a key translatable endophenotype of schizophrenia, in acute and chronic NMDA receptor hypofunction models. This raises the possibility that a metabolic therapy approach can be used effectively in the treatment of schizophrenia.

P8. THE EMERGENCE OF BEHAVIOURAL AND NEUROCHEMICAL CHANGES AT ADOLESCENCE IN A RODENT MODEL OF DEPRESSION

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Background: Depression is a severe neuropsychiatric disorder, whereby symptoms typically emerge at adolescence. Alterations to the development of the neurotransmitter system, glutamate, are thought to be implicated in the pathophysiology of depression. Furthermore, the glutamatergic receptors N-methyl-D-aspartate receptor (NMDAR) and metabotropic glutamate 5 receptor (mGluR5) have recently been identified as therapeutic targets. The Wistar-Kyoto rat strain has been extensively employed to model depression pathology. However, examining their behaviour and neurochemistry at critical neurodevelopmental periods will help gain a deeper understanding of the pathophysiology of depression.
Method: Adolescent (~5 weeks old) female WKY (depressive strain) rats were assessed for anxiety- and depressive-like behaviours using open field (OFT) and forced swim test (FST), compared to Sprague-Dawley (SD; healthy control) rats. To investigate neurochemical alterations, we examined the hippocampus of WKY and SD rats at juvenile (2 week old) and adolescent (5 week old) time-points, in a separate cohort. Immunoblots were performed on hippocampal tissue to access glutamatergic alterations, including the NMDA and mGlu5 receptors. Furthermore, we quantified Norbin, a protein which regulates mGluR5 cell surface expression and activity and has recently been implicated in the pathophysiology of depression.
Results: In the OFT, WKY rats exhibited a reduction in the total distance travelled and spent an increased amount of time spent in the corners of the arena compared to the SD strain. Furthermore, in the FST, adolescent WKY rats spend significantly increased time immobile, indicative of depressive-like behaviour. Immunoblot analysis of the hippocampus of 2 week old rats, revealed were no significant differences between WKY and SD rats, of any of the proteins examined. However at 5 weeks of age, we observed decreased expression of NMDAR, mGluR5 and Norbin protein levels in the WKY strain.
Conclusions: These findings show that the WKY rat strain exhibit anxiety- and depressive-like behaviours at adolescence. Furthermore, they exhibit glutamatergic deficits which at adolescence, which provide support for the involvement of the glutamatergic system in the pathophysiology of depression.

P9. OPTOGENETIC STIMULATION OF AN AMYGDALA-TO-ACCUMBENS PATHWAY CAN CONTROL ETHANOL MOTIVATED BEHAVIOUR

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Background: Understanding the neural systems that control reward-motivated behaviour has important implications for pathologies such as drug addiction. The nucleus accumbens is an important locus for this control and plays a role in extinguishing drug seeking in animal models. The basolateral amygdala is a major input to the nucleus accumbens. Here we used an optogenetic approach to stimulate amygdala axonal terminals targeting the nucleus accumbens and sought to disrupt cued ethanol seeking and drinking in a rodent model via this neural pathway.
Method: We trained ethanol-drinking rats to anticipate ethanol following the onset of a discrete conditioned stimulus (ie, ethanol cue). On tests of cued ethanol seeking, we used light-gated channelrhodopsin to stimulate the amygdala-to-nucleus accumbens pathway when the ethanol cue was presented. We also stimulated this pathway during ethanol drinking. Finally, in a control experiment, we used light-gated archeorhodopsin to inhibit amygdala projections to the nucleus accumbens during cued ethanol seeking.
Results: We found that under multiple cue test conditions, optogenetic stimulation of an amygdala-to-nucleus accumbens pathway disrupted cued ethanol seeking. The effect was specific to the moment of stimulation. Stimulation also interrupted ethanol drinking. Finally, inhibition of this pathway had no significant effect on cued ethanol seeking.
Conclusions: These findings suggest that photostimulation of amygdala neurons that target the nucleus accumbens can override the conditioned motivational properties of alcohol-predictive cues and reduce cued alcohol seeking. It can also control in-the- moment alcohol drinking. The findings provide evidence for limbic-striatal control over conditioned alcohol-motivated behaviour and have broader implications for the function of the brain reward circuitry, and its role in alcohol and drug addiction.

P10. MIR-137 OVEREXPRESSION IN THE PRELIMBIC CORTEX IMPROVES PREPULSE INHIBITION IN RATS

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Background: Schizophrenia is complex, heterogenous disorders, affecting 1% of the Australian population. Sufferers of schizophrenia may present with an array of positive, negative and cognitive symptoms. Commonly present are deficits in prepulse inhibition (PPI) of startle, an operational measure of sensorimotor gating. Understanding of the mechanisms contributing to schizophrenia and symptoms such as PPI deficits are incomplete. Schizophrenia has a strong genetic component, and recent studies have shown that polymorphisms in the MIR137 gene are associated with schizophrenia. The current study assessed the role of miR-137 overexpression in regulation of behavioural phenotypes relevant to schizophrenia and psychiatric disorder.
Method: We first assessed baseline levels of sensorimotor gating using PPI tests, anxiety-like behaviour using the elevated plus maze, locomotor activity using the open field test and memory using the novel object recognition task. Rats were then injected with either a miR-137 overexpression lentiviral vector, an 'empty' lentiviral vector or PBS into the prelimbic (PL) cortex. To determine the effect of miR-137 overexpression, we retested behaviour after 3 weeks of viral incubation.
Results: Overexpression of miR-137 in the PL significantly improved PPI compared to controls. Further, miR-137 overexpression decreased both the number of entries to the open arm and the time spent in the open using the elevated plus maze. Importantly, however, no change was seen in locomotor activity in the open field test.
Conclusions: Our results show that miR-137 overexpression in the PL improves sensorimotor gating. Given that deficits in sensorimotor gating is a common symptom of schizophrenia, and that SNPs in the MIR137 gene are commonly associated with schizophrenia, our results suggest that deficits in miR-137 expression may contribute to these symptomatic deficits, and that increasing mIR-137 expression specifically in the PL can help improve these deficits. Understanding the downstream targets of miR-137 will aid our understanding of the nature of these deficits, and may provide potential therapeutic targets to aid in treatment of schizophrenia.

P12. EFFECTS OF MATERNAL OBESITY ON SCHIZOPHRENIA-RELEVANT BEHAVIOURAL DOMAINS IN MICE

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Background: Schizophrenia patients with a diet high in sugar and polyunsaturated fatty acids experience negative consequences for disease symptoms and outcomes.However there is also limited evidence that particular diets can have beneficial, therapeutic-like properties for human brain disorders. For example, a high fat diet (HFD) can ameliorate the behavioural impact of early life trauma in rodents and reduce anxiety levels, depressive-like behaviours and improve stress responses. Interestingly, dietary choices of mothers have been found to affect cognitive domains and anxiety behaviour. Whether behavioural effects of HFD on schizophrenia-related domains are passed on from mothers to offspring is investigated in this study.
Method: We determined the effects of maternal HFD on C57BL/6J mice. Females were fed HFD or chow starting 6 weeks prior to mating, and continued during gestation and lactation. The male offspring of these mothers were weaned onto chow on PND21 and underwent testing for a range of schizophrenia-related behavioural outcomes including prepulse inhibition, anxiety, cognition, and locomotion at 30 weeks of age.
Results: Offspring of HFD mothers had a significantly higher body weight compared to offspring of chow control mothers and this difference was maintained for the duration of the experiment. Interestingly, offspring of HFD mothers had significantly improved sensorimotor gating compared to control mice. However, there was also a trend ($p < 0.06$ and $p < 0.07$) for higher scores on anxiety-related behavioural tests, such as elevated plus maze. No other behavioural domains were affected by maternal HFD.
Conclusions: These results suggest for the first time some positive effects of maternal HFD consumption on schizophrenia-relevant behaviours in adult offspring. Maternal HFD consumption also tended to increase anxiety levels of offspring. Whether the HFD response is related to maternal characteristics, e.g. altered nursing behaviour or metabolic changes, warrants further investigation.

P13. USING CYTOKINE MEASURES FROM BLOOD TO DEFINE HIGH INFLAMMATORY BIOTYPE OF SCHIZOPHRENIA

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Background: Cytokine mRNA transcripts derived from peripheral blood may be used to identify a subset of schizophrenia patients with increased inflammation, however how these mRNA measures correspond to changes in peripheral cytokine proteins has not been tested in people with schizophrenia. In this study, we identified a subset of schizophrenia patients with increased inflammation based on cytokine mRNA transcripts derived from peripheral blood and examined if the inflammatory subgroups showed similar patterns of change in the steady-state peripheral protein level in serum and plasma for a range of pro- and anti–inflammatory cytokines as well as CRP (C-reactive protein) levels.
Method: Peripheral cytokines (IL-1β, IL-2, IL-6, IL8 and IL-18) mRNA levels were measured by RT-qPCR in chronic patients with schizophrenia or schizoaffective disorder (n=90) with mild-to-moderate symptom severity and in healthy controls (n=75). Inflammatory subgroups were identified by a two-step cluster analysis. Peripheral protein concentrations of a range of cytokines (IFNγ, TNFα, IL-1β, IL-2, IL-6, IL-8, IL-10 and IL-12) were measured with the MAGPIX system in both the serum and the plasma. CRP concentration was measured in the plasma by ELISA. Patterns of change were identified between the inflammatory subtypes (for mRNA, serum protein, and plasma protein).
Results: A greater percentage of people with schizophrenia fell into the high inflammatory group (48%) as compared to the controls (33%) and had an overall decrease in the anti-inflammatory IL-2 mRNA ($p<0.01$). The magnitude of increase in IL-1β, IL-6, IL-8 and IL-18 mRNA was comparable in the high inflammatory group in both controls and schizophrenia. Plasma levels of IL-1β protein were significantly elevated in high inflammatory controls and IL-8 protein was increased in high inflammatory schizophrenics. Three serum cytokine protein levels (IL-6, IL-8 and TNFα) as well as CRP levels were significantly elevated in those with schizophrenia regardless of inflammatory status.
Conclusions: The increase in occurrence of the high inflammatory biotype in schizophrenia compared to controls provides further evidence that peripheral cytokine expression is altered in schizophrenia, but only in a subgroup of patients. Increased levels of IL-6, IL-8 and TNF-α in the serum of schizophrenia patients as well as increased CRP levels in the plasma and a decrease in the expression of the anti-inflammatory cytokine IL-2 indicate a role of moderate chronic inflammation in schizophrenia. Anti- psychotic treatment didn't differ between inflammatory states. However, when looking at peripheral inflammatory changes in schizophrenia results could differ depending on the method of measurement chosen.

P14. C-REACTIVE PROTEIN AS A MARKER OF INFLAMMATION IN ACUTE PSYCHOSIS AND SCHIZOPHRENIA

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Background: There is increasing evidence for the role of the inflammation in schizophrenia. Epidemiological and preclinical evidence implicates prenatal infection and subsequent immune activation in the aetiology of schizophrenia. Clinical trials show that adjunctive anti-inflammatories improve cognition in patients with a dysregulated immune system. Immune alterations have been shown in subgroups of patients with schizophrenia. C-reactive protein (CRP) is an acute phase reactant protein mainly produced by hepatocytes in response to an increase in circulating pro-inflammatory cytokines. Recent meta-analyses have reported a high prevalence of elevated CRP in schizophrenia which has been associated with acute psychosis and impaired cognition in schizophrenia.
Method: Here, we examine the prevalence of CRP as a marker of inflammation in two independent samples of individuals with psychosis: 1) individuals with acute psychosis and 2) chronically ill people with a diagnosis of schizophrenia. Elevated CRP levels were defined as ? 3mg/L.
Results: In the acutely ill sample, CRP levels were significantly elevated (60% having a CRP level above normal). Individuals with acute psychosis also displayed significantly increased neutrophil-to-lymphocyte ratio (NLR) levels and a significantly higher proportion (67%) had positive anti-nuclear antibodies. In acutely ill patients with psychosis CRP and NLR levels remained consistently high at repeated admissions. In the chronically ill patients with schizophrenia, CRP levels were significantly elevated compared to healthy controls, with 44% of chronically ill patients displaying clinically elevated CRP levels. The elevated CRP group of chronically ill patients displayed significantly worse current IQ, working memory, and attention/processing speed.
Conclusions: Taken together, the present findings support previous findings suggesting that inflammatory markers decrease with resolution of acute psychotic symptoms and further support the use of adjunctive anti-inflammatory treatments in schizophrenia.

P16. UNRAVELLING THE GENETIC BASIS OF BIPOLAR DISORDER THROUGH EXOME SEQUENCING IN EXTENDED FAMILIES

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Background: Bipolar disorder (BD) is a heritable illness, likely contributed to by common variants of small effect and rare variants of higher penetrance. Pathogenic rare variants of moderate effect are likely to be present in the exome and be shared amongst individuals with BD in extended families.
Method: We selected 117 subjects from 15 extended families, each containing at least four relatives with BD and two unaffected siblings, to perform whole exome sequencing (WES; Ion Proton platform) and copy number variant (CNV) analyses (CytoScanHD array). Rare variants shared amongst either affected or unaffected relatives were analysed for enrichment in gene sets previously implicated in psychiatric disorders, gene ontology terms and KEGG pathways. Rare CNVs (MAF<0.05 in general population) were validated by quantitative PCR (qPCR) and their inheritance defined within each family. Linkage analysis using WES- derived genotypes defined family-specific linkage intervals likely to include highly-penetrant pathogenic variation.
Results: We validated 19 rare CNVs, although no CNVs segregated in all affected. We will present results of our rare variant functional enrichment analysis. A small number of genes carrying shared SNVs coincided with family-specific linkage peaks, including the X linked IRS4 gene, which carried a truncating mutation in five affected siblings of one family. This gene is highly expressed in the amygdala, and IRS4-/- female mice show compromised maternal behaviours. Our study also implicates the protocadherin- alpha gene cluster, which acts to mediate neuronal connectivity, as we observed CNVs and a loss of function SNP affecting this cluster in several families.
Conclusions: Genetic approaches that combine WES, CNV and linkage analyses in extended families are effective in pinpointing genes and pathways that may contribute to the pathophysiology of the disorder.

P17. DOES SELF-REPORT, EEG AND STARTLE REFLEX MODULATION TO EMOTION IMAGES VARY WITH PORNOGRAPHY USE?

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Background: With modern day technological advances allowing an ever increasing access to a wealth of online pornographic material, we question whether any effects of this exposure can be seen below conscious awareness. Studies in the area of pornography use tend to rely heavily on conscious self-report measures, but many physiological studies suggest that so much of our deepest thoughts and emotions stem from processes below the level of conscious awareness. As such, this exploratory study was conducted to investigate whether frequency of pornography use has an effect, if any, on non-conscious and/or conscious emotion processes.
Method: Fifty-two male students from the University of Newcastle completed an online questionnaire in order to determine how often they viewed pornographic material. Participants then attended a laboratory session where they were fitted with EEG and EMG (for startle reflex modulation) recording equipment. Participants were then asked to view and rate one-hundred and fifty IAPS images based on valence and intensity. All images were shown to all participants and divided equally into five categories, these being: Erotic, Pleasant, Unpleasant, Neutral and Violent.
Results: Self-report data obtained via image rating scales data did not reveal any differences based on frequency of pornography use. However, EEG and startle reflex modulation data showed that increased exposure to pornographic content does alter physiological responses to emotion-related information processing.
Conclusions: Self-report, which is reflective of highly conscious cognitive processing does not appear to be influenced by pornography exposure. Startle reflex modulation showed minor effects which suggests that deeply subcortical affective processing may be influenced by increased pornography use. EEG which is reflective of both subconscious sensory and cognitive processes also showed significant effects between emotion categories. The current findings suggest that increased pornography exposure appears to have an influence on our brains subconscious responses to emotion inducing stimuli.

P18. OBESOGENIC ANTIPSYCHOTICS INDUCE BINOMIAL PATTERN OF NPY PRODUCTION IN MOUSE HYPOTHALAMIC NEURONS

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Background: Olanzapine, clozapine and risperidone are commonly used atypical antipsychotics among schizophrenia patients. However, these medications have been shown to be associated to extreme metabolic disorders; such as excessive body weight gain and diabetes. Yet, the mechanism by which antipsychotics cause weight gain in patients remains unknown. Neuropeptide Y (NPY), an orexigenic neuropeptide, is highly expressed in the hypothalamus. In this study, mHypoA-NPY/GFP cells were treated with olanzapine, clozapine and risperidone to reveal their effect on NPY.
Method: Two methods have been used to investigate NPY levels; Flexstation fluorescence readings and western blot. For the western blot, mHypoA-NPY/GFP cells were treated with olanzapine treatment (100µM) for 24 hours and then collected for protein extraction. The anti-GFP antibody was used to detect GFP-NPY levels. Fluorescence was measured using the FlexStation. Cells were treated with either 5µM-200µM of olanzapine, 1.5µM-100µM clozapine or 5µM-200µM of risperidone for 24 hours. The excitation wavelength was set at 488nm while the emission at 510nm. All experiments were performed at 37°C. The data were exported from SoftmaxPro and analysed using SPSS.
Results: Clozapine increased NPY in a binomial-shaped dose response. This increase of NPY was observed at doses 1.5µM ($p<0.05$), 3µM ($p<0.001$), 10µM ($p<0.001$), 30µM ($p<0.001$), 60µM ($p<0.01$), but no effect was seen at a dose of 100µM clozapine treatment. NPY protein level was significantly increased in cells after 24 hour treatment of olanzapine 100µM ($p<0.05$) using western blot analyses. Correspondingly, fluorescence analyses also revealed a significant increase of NPY in a binomial-shaped dose response after 24 hour treatment of olanzapine at both 25µM and 50µM ($p<0.05$).Finally, risperidone caused a significant increase of NPY at 5µM ($p<0.01$), 50µM ($p<0.001$) and 100µM ($p<0.01$).
Conclusions: This study has shown that NPY levels are significantly increased by obesogenic antipsychotics; olanzapine, clozapine and risperidone. Since NPY increase will result in increased food intake and energy storage our findings provide imperative insight for clinicians prescribing these drugs to high-risk obesity patients. Further study is required to examine the mechanism by which antipsychotics increase orexigenic NPY. The outcome of this study provides a basis for further endeavour into the cause of antipsychotic induced obesity.

P20. ARIPRIZAOLE AND HALOPERIDOL UPREGULATE GABAA AND NMDA RECEPTORS IN THE NUCLEUS ACCUMBENS OF RATS

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Background: Deficits of GABAA and NMDA receptors are involved in the pathophysiology of schizophrenia. One important issue is that current antipsychotics target largely at dopamine D2-like and serotonin 5-HT2 receptors to reach their therapeutic effects, but do not bind with GABAA and NMDA receptors. The present study investigated the effects of aripiprazole and haloperidol in modulating GABAA and NMDA receptors, and related cellular signalling pathways in the rat brain.
Method: Rats were orally treated with aripiprazole (0.75 mg/kg), haloperidol (0.1 mg/kg) or vehicle three times per day for 1 week (short- term) or 10 weeks (chronic). The levels of GABAA, NMDA subunits, PKA, Akt-GSK3?, and Dvl-3 signalling were measured in the nucleus accumbens (NAc) by Western Blots and/or qPCR.
Results: One week treatment of aripiprazole and haloperidol increased activation of PKA, GSK3? and Dvl-3 signalling, and the expression of GABAA (?1) receptors. Moreover, the protein levels of GABAA (?1) were positively correlated with the levels of p- PKA and the ratio of p-PKA/PKA. Chronic treatment of these drugs also increased Akt-GSK3? signalling, and the expression of Dvl-3, GABAA (?1), NMDA NR1 and NR2A subunits.
Conclusions: Aripiprazole and haloperidol may upregulate GABAA (?1) receptors via activating PKA signalling in the NAc. Antipsychotics could also activate GSK3? dependent signalling pathways and upregulate NMDA receptors in the NAc. Upregulation of GABAA, NMDA receptors and related signalling pathways may contribute to the therapeutic efficacy of antipsychotics.

P21. RISPERIDONE AUGMENTS THE EFFECTS OF ACETYLSALICYLIC ACID ON PTGS1 LEVELS IN VITRO

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Background: Acetylsalicylic acid (aspirin) is a nonsteroidal anti-inflammatory drug that inhibits the activity of prostaglandin synthase 1 (PTGS1). Clinical studies have shown that using acetylsalicylic acid as an adjunct to antipsychotic medication can further reduce the symptoms of schizophrenia. Furthermore, we have found that the level of PSTG1 protein is lower in the dorsolateral prefrontal cortex of subjects with schizophrenia compared to non-psychiatric controls. We aimed to determine whether treating cells with a combination of acetylsalicylic acid and an antipsychotic drug can alter their levels of PTSG1 protein.
Method: CCF-STTG1 cells (P9) were subcultured on to 9.6cm^2 wells at a concentration of 5x10^4 cell/well and cultured for 10 days in RPMI 1960 media with reduced levels of foetal bovine serum. The media was then replaced with RPMI 1960 without foetal bovine serum, containing 0mM, 0.1mM 0.5mM or 1mM acetylsalicylic acid in combination with 0µM, 1µM or 10µM risperidone and treated for 24 hours. 5 replicates were used for each drug treatment regime. Protein homogenates were then prepared from each replicate and Western blotting was used to measure the PTGS1 protein levels in the cells.
Results: The level of PTGS1 in CCF-STTG1 cells was significantly decreased when the cells were treated with 0.5mM or 1mM acetylsalicylic acid ($p < 0.05$). Treating the cells with risperidone alone did not alter the level of PSGS1 at either concentration used. When the cells were treated with 1µM or 10µM risperidone in combination with 0.5mM or 1mM acetylsalicylic acid, the level of PTGS1 decreased below the levels produced by acetylsalicylic acid, alone ($p < 0.05$).
Conclusions: Risperidone alone does not appear to alter PTGS1 levels, however, when used in combination with acetylsalicylic acid, risperidone has a synergistic effect on lowering PTGS1 levels. These findings may help explain why of acetylsalicylic acid can augment the effects of antipsychotic drugs in patients with schizophrenia. Furthermore, these data suggest that the lower levels of PTGS1 seen in subjects with schizophrenia may be compensating for a dysfunction in the prostaglandin synthesis pathway rather than contributing to the pathophysiology disorder.

P22. A SYSTEMATIC REVIEW OF THE EFFECT OF CANNABIDIOL ON COGNITIVE FUNCTION: RELEVANCE TO SCHIZOPHRENIA

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Background: Cognitive impairment is a core symptom domain underlying many neurological, neuro-inflammatory, substance abuse and neuropsychiatric disorders, including schizophrenia. Current antipsychotic drugs prescribed for schizophrenia treatment are limited in their efficacy to improve cognitive deficits and new therapeutic agents are required. Evidence demonstrates that cannabidiol (CBD), the non-intoxicating component of cannabis, has antipsychotic-like properties; however, its ability to improve cognitive impairment has not been explored. The aim of this systematic review was to critically evaluate the existing preclinical and clinical literature on the effects of CBD on cognitive domains relevant to schizophrenia.

Method: A systematic literature search was performed across the electronic databases MEDLINE, Scopus and Web of Science for English language articles published from January 1990 to March 2016. Studies were eligible for inclusion if they assessed the effect of CBD on cognitive domains relevant to schizophrenia, as identified by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative. A total of 27 studies (18 preclinical and 9 clinical) were examined in the present review. These studies examined the effects of CBD on cognition in cannabis-induced states, pathological (neuropsychiatric, neurodegenerative, neurological and neuroinflammatory disorders) and non-pathological states.

Results: The limited evidence of schizophrenia reported that CBD did not improve attention in schizophrenia outpatients; however, in a preclinical rodent model of schizophrenia, CBD improved object recognition but not social recognition memory. In preclinical models of Alzheimer's disease, CBD treatment improved object and social recognition memory impairment, with no effect on associative learning. CBD administration improved learning and memory deficits in preclinical models of neurological (stroke and hepatic encephalopathy) and inflammatory disorders (sepsis, cerebral malaria and meningitis). Clinical and preclinical evidence reported that CBD treatment attenuated cannabis-induced cognitive impairment. There was no effect of CBD on cognition in non-pathological states.

Conclusions: There is limited evidence investigating the effects of CBD on cognition in schizophrenia; however, CBD can improve learning and memory in other pathological disorders, with no effects on cognition in 'healthy' states. Studies lack consensus on basic parameters such as dose, frequency and duration of CBD treatment needed to elicit optimal cognitive outcomes. Studies investigating the ability of CBD to limit cannabis-induced cognitive impairment should consider the metabolism and interaction of CBD with other cannabinoids (i.e. Δ9-THC) in co-administration and pre-treatment paradigms. Given the limited evidence, further investigation into the effects of CBD on cognition in schizophrenia is required.

P23. EFFECTS OF BIPOLAR DISORDER DRUGS ON CHOLESTEROL BIOSYNTHESIS AND NEURITE OUTGROWTH

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Background: The specific mechanisms by which bipolar disorder drugs exert their therapeutic effects are not fully understood. Although current medications have some clinical efficacy, they are less than optimal in treating many patients. Consequently, a more comprehensive understanding of how the currently used drugs work at molecular and cellular levels may enable the development of new and improved therapeutics. Therefore, this paper aims to characterise the effects of drugs currently used to treat bipolar disorder on gene expression using unbiased whole genome sequencing and gene expression profiling.
Method: NT2-N cells (human neuronal cells; n=20) and rats (Sprague Dawley; n=8) were treated with a cocktail of four established bipolar disorder drugs (lithium, valproate, lamotrigine and quetiapine) or vehicle (DMSO+H2O). RNA was extracted from the cells and the rat brain frontal cortex for next generation sequencing (HiSeq, Illumina). Data were aligned to a reference genome using Bowtie2/TopHat2 and the transcripts were assembled using Cufflinks. Differential expression analysis for the expressed genes was done using EdgeR in R. Pathway analysis was performed using the BROAD Institute's Gene Set Enrichment Analysis software package.
Results: Pathway analysis showed up-regulation of the cholesterol biosynthesis pathway in NT2-N cells (adjusted p=3.4x10-9), with all 15 genes in the pathway up-regulated (all p<0.001). This pathway was also up-regulated in rat frontal cortex, (p=0.005), where 12 of 14 genes including the rate-limiting step revealed increased expression (HMGCS1, p=0.003). Expression of intracellular cholesterol transport genes were increased (NPC1 p=6.7x10-23, NPC2 p=3.2x10-19, APOE p=1.9x10-13), while the major gene involved in cellular cholesterol efflux was decreased (ABCA1, p=1.2x10-6), suggesting that the newly synthesised cholesterol is being utilised within the cells, possibly for synthesis of new membranes in order to facilitate neurite outgrowth.
Conclusions: One of the modes of action of bipolar disorder drugs is increasing cholesterol biosynthesis in the brain. Increased cholesterol in the neuronal membranes enables the protrusion and elongation of neurites, thereby increasing neurite outgrowth. This mechanism possibly underpins clinical efficacy in bipolar patients.

P24. MODELLING THE EFFECTS OF ELECTRICAL STIMULATION ON HYPOTHALAMIC NEURONS IN PSYCHIATRIC DISORDERS

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Background: The therapeutic effect of electrical stimulation has been investigated in psychiatric disorders such as schizophrenia and major depression (Tortella et al. 2015). However, the mechanisms elicited by electrical stimulation at the cellular and molecular level remains unclear. Given the role of hypothalamus in the pathogenesis and treatment of psychiatric disorders, including the therapeutic effects of deep brain stimulation, we have studied the effect of electrical stimulation on hypothalamic neurons and shown that electrical stimulation mediated by a conductive polymer enhances neurite outgrowth.
Method: Adult mouse hypothalamic neurons were seeded onto a conductive polymer polypyrrole (PPy) film doped with dodecylbenzenesulfonate (DBS) and electrically stimulated for 8 hours/day over three days at 0.25 mA/cm2 current and 250 Hz frequency. The cells were subsequently fixed, immunostained with Tuj1, synaptophysin and brain-derived nerve factor (BDNF), and imaging was performed using confocal and wide-field fluorescence microscopy.
Results: Immunostaining showed that electrical stimulation significantly increased neurite outgrowth and branching of the adult hypothalamic neurons, compared to non-electrically-stimulated controls.
Conclusions: Electrical stimulation via PPy-DBS enhances neurite outgrowth and branching of hypothalamic neurons confirming both film biocompatibility and neuronal cell effects. Future research will include subcellular characterisation of the effects of electrical stimulation and phencyclidine (PCP) treatment of hypothalamic neurons as a schizophrenia model.

P25. BIPOLAR DISORDER DRUGS ACT VIA MICRORNAS TO REGULATE GENES INVOLVED IN NEURONAL PLASTICITY

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Background: Agents currently used to treat bipolar disorder have limited efficacy, and the development of new drugs is hampered by our lack of understanding of the underlying pathophysiology of this disease. The aim of this study was to investigate the transcriptional effects of commonly prescribed bipolar disorder drugs in cultured human neuronal cells, including analysis of effects on both mRNA and miRNA.
Method: Differentiated NT2-N (human neuronal) cells were treated with a combination of different drugs commonly used to treat bipolar disorder including lithium, valproate, lamotrigine and quetiapine or vehicle (negative control). RNA was extracted from the cells for next generation sequencing (Hiseq, Illumina). Data were extracted and annotated using Cufflinks and Tophat (bowtie) software, and analysed for differentially expressed genes and miRNAs using edgeR in R. The differential expression of the selected miRNAs was confirmed by real time RT-PCR using TaqMan MicroRNA assays (Applied Biosystems).
Results: Bipolar disorder drugs increased the expression of miR-128, and reduced the expression of miR-129 (both $p<0.02$). Putative target genes of these miRNAs were obtained from microRNA.org and PubMed searches, and subjected to gene set enrichment analysis using DAVID software (NIH). miR-128 target pathways included “neuron projection development” and “axonogenesis”, while miR-129 target pathways included “cell migration”. A large number of genes that are putative targets of these miRNAs were differentially expressed following treatment with bipolar drugs.
Conclusions: For many years it has been proposed that defective neuronal plasticity is an important component of the pathophysiology of neuropsychiatric disorders such as bipolar disorder and schizophrenia. Our data clearly demonstrate that at a transcriptional level, bipolar disorder drugs affect a number of genes in concert that would increase neuronal plasticity, and that this is mediated (at least in part) by modulation of the expression of two key miRNAs.

P26. ELEVATED ADHESION MOLECULE AND IMMUNE CELL EXPRESSION IN SCHIZOPHRENIA ASSOCIATED WITH INFLAMMATION

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Background: Our laboratory has found pro-inflammatory markers elevated in approximately 40% of individuals with schizophrenia and clustered individuals into high and low inflammatory biotypes. Changes in blood-brain barrier genes were previously found in schizophrenia individuals in a high inflammatory biotype. Intercellular adhesion molecule-1 (ICAM-1) expression is induced by inflammation and is involved in leukocyte transmigration. We wanted to determine expression and localisation of ICAM-1 in the prefrontal cortex and measure the expression of immune cells in the cortex.
Method: ICAM-1 mRNA was measured in the dorsolateral prefrontal cortex (DLPFC) and orbital frontal cortex (OFC) using RT-qPCR in 37 schizophrenia and 37 unaffected controls. Immunofluorescence was used to co-localise ICAM-1 with collagen-IV, a marker of vascular basement membrane in the OFC. Gene expressions of cluster of differentiation (CD)14, CD16 and CD163, were measured in the DLPFC as markers of monocytes, natural killer cells and perivascular macrophages, respectively. This cohort was previously clustered into high inflammation schizophrenia (n=14), low inflammation schizophrenia (n=23) and low inflammation controls (n=33) groups based on inflammatory mRNA expression.
Results: ICAM-1 mRNA was increased in people with schizophrenia and further exaggerated in people with high inflammation. Different staining patterns of ICAM-1 expression were observed including ICAM-1 lining the vessel lumen and also at astrocytic end feet- like processes surrounding the vessels. CD14, CD16 and CD163 gene expressions were not diagnostically different. However, people with schizophrenia and high inflammation had increased CD14 ($F(2,62) = 5.155$, $p = 0.008$), CD16 ($F(2,62) = 8.326$, $p = 0.001$) and CD163 ($F(2,60) = 6.261$, $p = 0.003$) gene expression relative to low inflammation schizophrenia and controls.
Conclusions: People with schizophrenia in an elevated inflammatory state have increased adhesion molecules that may be involved in the movement of leukocytes across blood vessels. ICAM-1 expression was found both lining the vessels and also around vessels as processes, which could facilitate migration of immune cells into the perivascular space and parenchyma. Elevation of immune cell marker gene expression further suggests the presence of peripheral immune cells in the cortex either as a result or cause of inflammation.

P27. INTERSTITIAL WHITE MATTER NEURON DENSITY AND CHEMOKINE RECEPTOR EXPRESSION IN PREFRONTAL CORTEX FROM INDIVIDUALS WITH SCHIZOPHRENIA AND BIPOLAR DISORDER

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Background: Cortical gamma-aminobutyric acid (GABA)ergic interneuron deficiencies and reduced glutamic acid decarboxylase mRNA expressions are found in schizophrenia and bipolar disorder (BPD) dorsolateral prefrontal cortex (DLPFC). There is elevated density of interstitial white matter neurons (IWMN) in schizophrenia, but this has not been measured in BPD. Higher IWMN density might be attributable to failed neuronal migration, a process influenced by inflammation and/or dysfunction of chemokines and their receptors. The chemokine receptor, CXCR7, is expressed by migrating cortical interneurons and previously found to be elevated in individuals with schizophrenia. Here we measured CXCR7 expression and IWMN density in BPD and schizophrenia.
Method: Brain tissue was obtained from the Stanley Medical Research Institute (SMRI) with schizophrenia (n=35), BPD (n=34) and unaffected control (n=35) individuals. These samples have previously been characterised by their expression of inflammatory cytokines (Fillman et al., Translational Psychiatry, 2014). For our study, 14 micron sections (1/case) were cut from human frozen DLPFC and neuronal nuclear antigen (NeuN) positive cells below the grey/matter boundary counted to determine DLPFC IWMN densities. Human DLPFC RNA was isolated using Trizol reagent and cDNA was synthesised using Superscript II. Gene expression for CXCR7 was examined by quantitative real-time PCR using a TaqMan Assay.
Results: DLPFC IWMN density was numerically greater in the schizophrenia (65.9±17.7/mm2) and bipolar disorder (64.5±13.0/mm2) groups than in the control group (59.7±13.9/mm2). There was no diagnostic difference in CXCR7 mRNA levels ($F(2,89)=0.148$, $p=0.862$, co-varied for PMI). Cortical CXCR7 expression was increased by 30% in individuals with elevated expression of inflammatory markers ($F(1,90)=21.680$, $p<0.001$, co-varied for PMI), but no statistically significant effect of inflammatory status on IWMN density ($F(1,92)=0.756$, $p=0.387$, co-varied for age). There was also no significant correlation between expression levels of CXCR7 and IWMN density ($r=0.049$, $p=0.648$).
Conclusions: Elevated expression of CXCR7 with high expression of inflammatory markers, irrespective of diagnostic grouping, suggests that neuroinflammation relates to activation of the chemokine system. Our preliminary investigation of IWMNs indicates CXCR7 expression is independent of IWMN density, suggesting signalling by this chemokine receptor is a minor factor in determining IWMN density or is compromised in other ways. Alternatively, cortical grey matter CXCR7 expression may not accurately reflect IWMN expression of CXCR7. Future work will 1) improve precision of the IWMN density measure with two independent measures/case, 2) confirm expression of CXCR7 on IWMNs and, 3) measure expression of CXCR7 ligand.

P28. [3H]PK11195 BINDING IN THE DLPFC IN SCHIZOPHRENIA: A POST-MORTEM STUDY

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Background: Growing evidence implicates neuro-inflammation in the pathogenesis and pathology of schizophrenia. In addition, recent evidence suggests an association between neuro-inflammation and chronic antipsychotic drug treatment. The inflammatory response is mediated by activated microglia, which express an 18kDa mitochondrial translocator protein (TSPO). Radioligands binding to TSPO are increasingly being used as in vivo markers of neuro-inflammation. In the present study, we used the TSPO radioligand, [3H]PK11195, to examine neuro-inflammation in the dorsolateral prefrontal cortex (DLPFC) of schizophrenia subjects, using a post-mortem brain cohort. Moreover, we explored the association between [3H]PK11195 binding and markers of disease progression and antipsychotic treatment.
Method: The post-mortem cohort consisted of 37 schizophrenia subjects (including 7 schizoaffective) and 37 control subjects matched for age at death, post-mortem interval, brain pH and RNA integrity. The age of subjects ranged from 18-78. The age of schizophrenia onset ranged from 14-40 years, while the duration of illness ranged from 3-52 years. All subjects were medicated and estimated lifetime antipsychotic drug exposure calculated as chlorpromazine equivalents. [3H]PK11195 binding density was measured in grey and white matter of the DLPFC and data analysed to determine any diagnostic effects, or relationships with age, age of schizophrenia onset, duration of illness or estimated.
Results: There was no significant difference in [3H]PK11195 binding density in the DLPFC in schizophrenia subjects compared to controls. There were however positive associations between age at death and [3H]PK11195 binding in grey matter (r=0.427, p=0.008) and white matter (r=0.375, p=0.022) of control subjects. In schizophrenia subjects, there was no association between age at death and [3H]PK1 1195 binding, however there was a significant negative correlation between binding in grey matter and age of schizophrenia onset (r=-0.359, p=0.029). Duration of illness was not associated with [3H]PK11195 binding. Finally we report a positive association between [3H]PK11195 binding and lifetime chlorpromazine equivalents (r=0.356, p=0.026).
Conclusions: These results suggest that TSPO binding may not be altered in the DLPFC in established cases of schizophrenia however patients with an earlier age of schizophrenia onset demonstrate higher TSPO binding. Further, these data suggest a potential contribution of antipsychotic medication to the TSPO marker of brain inflammation. It will be important in future studies to determine whether these findings are influenced by the underlying TSPO genotype (defining high-, medium- and low-affinity TSPO binding profiles).

P29. VALIDATING B-ACTIN AS A REFERENCE GENE FOR WESTERN BLOT

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Background: B-Actin has become the go-to reference gene for protein, and particularly Western Blot, analysis. As an important member of the cell cytoskeleton, B-Actin is believed to be ubiquitously expressed in all tissue and cell types and to be unaffected by diagnosis. While the expression of B-Actin is most often presented visually, it is rarely quantified. Here we quantify the raw values of β-Actin protein levels in the CNS of subjects with Schizophrenia and healthy controls, and assess its validity as a reference gene compared to quantifying total protein via Ponceau.
Method: Postmortem tissue from BA9 of the human prefrontal cortex was obtained from the Victorian Brain Bank. β-Actin expression was measure in 20 µg total protein homogenate on 15% SDS-PAGE gels (MAB1501). Total protein was analysed by probing the post-transfer nitrocellulose membrane with 0.2% Ponceau in 3% TCA, and imaged using white light settings on the UVP Biospectrum.
Results: Levels of β-Actin and total protein were not affected by diagnosis. Linear regression analyses of β-Actin and total protein against homogenate concentration did not intercept zero, and so data was analysed using non-parametric tests. A linear regression analysis of β-Actin against total protein did not show a strong correlation ($r^2 = 0.048$).
Conclusions: B-Actin did not appear to accurately represent total protein levels. We therefore recommend thorough analysis of reference proteins and to apply caution when interpreting results that utilize such proteins.

P30. SOMATOSTATIN, CORTISTATIN, AND SSTR2 MRNA IN FRONTAL CORTEX OF SCHIZOPHRENIA BY INFLAMMATORY STATUS

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Background: Approximately 40% of people with schizophrenia have elevated brain inflammation and exacerbated inhibitory interneuron deficits in dorsolateral prefrontal cortex (dlPFC), including reduced somatostatin mRNA. Somatostatin and cortistatin elicit anti-inflammatory properties through somatostatin receptors (SSTRs). People with schizophrenia have reduced somatostatin mRNA in the orbitofrontal cortex (OFC), and SSTR2 mRNA in the dlPFC. Whether gene expression deficits in cortistatin or SSTR2 in dlPFC, or somatostatin, cortistatin, or SSTR2 in OFC, exist based on neuroinflammatory status is unclear. We hypothesized that somatostatin, cortistatin, and SSTR2 expression would be altered in frontal regions from people with schizophrenia based on their neuroinflammatory status.
Method: Gene expression of somatostatin, cortistatin, and SSTR2 was determined by quantitative real-time RT-PCR in postmortem dlPFC and OFC. Previous two-step clustering of pro-inflammatory cytokine gene expression in dlPFC and OFC yielded high and low inflammatory groups for each cortical region. ANCOVAs of each region compared: controls-low (n=31-33); schizophrenia- high (n=21-27); and schizophrenia-low (n=10-14). Controls-high was excluded due to small sample size (n=4-5). Demographic variables (pH, age of death, RIN) that correlated with gene expression were covariates. In schizophrenia cases only, illness duration correlated with OFC SSTR2 ($r=-0.377$) and cortistatin ($r=-0.364$) mRNA, and age of onset correlated with dlPFC SSTR2 mRNA ($r=-0.380$).
Results: Somatostatin mRNA was reduced in people with schizophrenia (high and low; $p<0.05$) compared to controls-low ($F(2,69)=7.461$) in the dlPFC, but was decreased in schizophrenia-high ($p<0.05$) compared to controls-L ($F(2,66)=3.242$) in the OFC. Cortistatin mRNA was elevated in schizophrenia-low ($F(2,68)=4.632$) in comparison to schizophrenia-high ($p<0.01$) and controls-low ($p<0.05$) in the dlPFC, but was unchanged in the OFC ($F(5,65)=2.105$). SSTR2 was reduced in people with schizophrenia (high and low; $p<0.05$) compared to controls-low in the OFC ($F(2,66)=5.109$), but was unchanged in the dlPFC ($F(5,69)=0.883$).
Conclusions: People with schizophrenia with high or low neuroinflammation exhibited reductions of somatostatin mRNA in the dlPFC, and SSTR2 mRNA in the OFC. People with schizophrenia and high inflammation also had decreased somatostatin mRNA in the OFC. People with schizophrenia and low inflammation had increased cortistatin mRNA in the dlPFC. This supports the hypothesis that people with schizophrenia may have altered frontal SSTR signalling linked to their neuroinflammatory profile. Our findings may indicate that some people with schizophrenia may have mechanisms to respond to neuroinflammation in distinct brain regions. Reducing neuroinflammation, perhaps via SSTRs, may benefit some people with schizophrenia.