Characterisation of Genetic Risk Factors for Mental Illness in Rodent Models: Impact of *Map2k7+/-* and *Fxyd6-/-* Mice Upon Neural Systems and Working Memory

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Abstract

Even in wealthy and seemingly prosperous countries like the United Kingdom, the spectre of mental illness and psychiatric disorders remains highly prevalent. These disorders present a huge economic burden to societies, where in the UK alone, mental disorders cost the economy an estimated €134 billion a year; along with the unmeasurable societal and human costs. This has led to an intense debate over the past few decades just as to what factors contribute to these illnesses. It is now understood that a number of biological and non-biological factors contribute. These include socio-economic pressures, early-life trauma, gestational and peri-natal infections; genetic and familial factors, and molecular and cellular factors. However, while the definitions and diagnostic criteria of mental disorders remain based in the subjective realms of the DSM and ICD, treatment and understanding of psychiatric illness has had little chance to progress over the last fifty years. As a result, neuroscientists are starting to direct psychiatric disorder research from the bottom-up; where genetic, cognitive and neuroconnectivity factors are being investigated to serve as a future basis for diagnosis and treatment.

One of the most complex and debilitating psychiatric disorders, schizophrenia, exhibits a complex array of genetic, cognitive and neuroconnectivity abnormalities. Current challenges in schizophrenia research is to understand how identified genetic abnormalities contribute to neuroconnectivity and cognitive impairments which are prominent in schizophrenia. Recently, genetic association studies have implicated two genes as risk factors for schizophrenia – *FXYD6* and *MAP2K7.* Currently it is unclear exactly how these genes contribute to schizophrenia pathology, particularly cognitive symptoms and neural circuitry. This thesis investigates these two genes by utilising two mouse models, first a heterozygous mouse line of *Map2k7*+/- and second, a gene knock-out line of *Fxyd6-/-.*

***MAP2K7* is a gene that expresses a kinase that is involved in the c-Jun N-terminal kinase (JNK) pathway, which is implicated in neuronal activity, receptor function, and cortical and hippocampal plasticity. Recent studies have found a decreased**

expression of MA2PK7 in the PFC, ACC and hippocampal regions in schizophrenia patients; regions associated with memory and decision making. A component of the cognitive profile of *MAP2K7* was therefore investigated using *Map2k7+/-* mouse lines in a working memory paradigm in the radial arm maze. This test is known as the n-back test or the retention interval test. For the first time this investigation reveals that *Map2k7+/-* mice exhibit a subtle yet significant spatial working memory deficit compared to WT mice; as judged by their average performance over the whole experiment. WT mice exhibited an overall average performance of 70% and MAP2K7+/- mice 66% ($p<0.001$). This indicates that *MAP2K7* may play a subtle role in working memory function in rodents, and may represent a component of the aberrations in the genetic architecture that gives rise to working memory impairments in psychiatric disorders, particularly schizophrenia. This experiment also backs up previous evidence for this radial arm maze paradigm as a robust behavioural test for testing rodent working memory.

FXYD6 belongs to a group of proteins that are known to be involved in modulating NaKATPase activity. Previously, NaKATPase has been associated with bipolar disorder and depression, but has now also been implicated in schizophrenia. Previous studies have found that FXYD6 is also abnormally expressed in the PFC of schizophrenia patients, and therefore may contribute to the cognate symptoms of the disorder. This experiment, therefore, investigated how Fxyd6 contributes to local brain activation, particularly in neural systems relevant to cognition, using gene knockout *Fxyd6-/-* mouse models and semi quantitative 2DG autoradiographic imaging. Three regions showed a significant deviation in activity in *Fxyd6-/-* mice compared to WT mice. The subiculum, medial septum and lateral septum all exhibited significant reductions in activity in *Fxyd6-/-* mice compared to WT mice. Notably the subiculum is heavily implicated with memory functions, particularly working memory and disambiguation of previously learned memory. Indicating a possible role for FXYD6 and NaKATPase in working memory processing and memory disambiguation in the subiculum.

Finally, the role of glutamate in relation to FXYD6 function and brain activity was assessed by administering the NMDA receptor antagonist ketamine and analysing

regional brain activity using semi quantitative 2DG autoradiographic imaging. Generally, regions which were affected by ketamine in WT mice including PFC, thalamic and septal regions, were not affected in *Fxyd6*-/- mice. It is hypothesized that this may be down to a compensatory effect that knocking-out Fxyd6 may have on glutamate reuptake. Because NaKATPase is involved in glutamate reuptake into glia and neurons, the blockage of NMDA receptors may have less effect due to a reduction in glutamate reuptake, and therefore higher than normal postsynaptic glutamate concentrations.

In conclusion, this investigation highlights two genes which may have roles in working memory functioning and neural circuitry that contribute to cognitive processes. While the evidence from this investigation does not explicitly associate these genes with symptoms of schizophrenia and other psychiatric disorders; the evidence does provide indication that they are involved in cognitive processes in rodents, and possibly humans. This investigation provides an interesting path of investigation for the potential roles of these genes regardless of their relationship to psychiatric disorders and will inform future research into the genetic architecture of neural circuits and cognition.

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Declaration of originality

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed: Mark McRobbie Hanlon

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Chapter 1: Introduction

1.1 – Background

In a continent as prosperous and wealthy as Europe the spectre of mental illness still looms large over much of the population. The World Health Organisation (WHO) estimated that every single year around one third of Europe's population will be affected by some form of mental ill health (World Health Organisation 2013) and that mental illness accounts for around one third of disability worldwide (World Health Organisation 2013). Moreover mental disorders - as a whole - have been estimated to cost the United Kingdom around €134 billion a year in productivity losses, direct healthcare costs and non-medical costs (Fineberg *et al.* 2013). This highlights the growing need for more effective treatments and preventative measures for mental illness in the world today. This can only be done through a finer understanding of the biological and environmental factors that spawn these disorders. However, mental illness is a complex issue, with ever changing definitions and parameters that move and shift with the prevailing cultural, political and scientific theories of the age (Zandi *et al.* 2008). Adding to the complexity is the seemingly growing levels of depression, anxiety and psychotic disorders in some of the richest and most 'prosperous' countries in the world (SAMHSA 2010, ECNP 2011). This explains why, over recent decades, biologists, psychologists, sociologists, epidemiologists, economists, physicians, ecologists, philosophers, theologians, and even politicians – have all tried to answer why mental illness is still so prevalent in western countries, and what can be done to curtail this trend. The definitions and diagnosis criteria of psychiatric disorders also remain a significant problem, with methods relying on subjective and often ambiguous criteria. This has led neuroscientists and geneticists to search for objective and empirical variables that can guide definitions of psychiatric pathology. This is especially important because recent evidence has shown that certain circuit, genetic and cognitive dysfunctions span various psychiatric illnesses; indicating the prospect of a common aetiology among psychiatric disorders - existing on a spectrum - rather than as separate entities (Adam 2013). However, the investigation of potential empirical criteria remains challenging, particularly because most studies still utilise the subjective definitions of mental disorders. Encouragingly, new directions into understanding

psychiatric disorders from the 'bottom-up' (as guided by the Research Domain Criteria (RDoC) Project) indicate a shift of focus from traditional definitions to a more biologically-based grouping of disorders – which could spell a paradigm shift in diagnosis and treatment in the coming decades.

1.2 – The biological components of mental disorders

Understanding how biological phenomena can impact the aetiology of psychiatric disorder remains one of the biggest challenges in neuroscience and clinical psychological research. While it could be argued that certain disorders and illnesses are largely influenced by external environmental factors, it is undoubted that disorders like autism and schizophrenia arise from significant abrogation from healthy biological mechanisms. A simple thought experiment can illustrate this. First: two soldiers return from a warzone, both have experienced a traumatising event together, one may suffer extreme and debilitating post-traumatic stress disorder (PTSD) and the other may return relatively psychologically unchanged. Where life experience may provide the conditions for one soldier to be more susceptible to trauma or the other to be more resilient, there is a possibility or a likelihood that genetic and developmental factors play a role in the different outcomes for each individual. Understanding how complex environmental and biological relationships give rise to these disorders will most likely be the most important and valuable lesson we learn in our aims to better treat sufferers of mental illness, refine our abilities to define and recognise mental disorders, and most importantly let individuals flourish in their own cognitive identity, without compromising their abilities to experience the full spectrum of human emotions.

From decades of neuroscientific, developmental and genetic research there is now good evidence that a significant component of psychiatric disorder aetiology comes from developmental, genetic and neuropharmacological factors. For example there is growing evidence to suggest that a major component of schizophrenia risk comes from early developmental problems during gestational and perinatal periods. These risk components arise from perinatal stress, malnutrition, infection, early-social isolations and obstetric complications among others (Cannon et al. 2002; Lewis &

Levitt 2002). Modern genetic studies such as linkage analysis and genome-wide association studies are also beginning to uncover hundreds of genes that may contribute to psychiatric pathology (recent evidence is reviewed in section 1.6.2), some of which are passed down through familial inheritance and recessive expression, and others that arise from rare mutations. These developmental and genetic abnormalities are thought to manifest into the cognitive, emotional and psychological symptoms through discrete malfunctions in neuropharmacological processes and larger-scale malfunctions in brain network circuitry. For example, from the relative effectiveness that dopaminergic receptor antagonists have on treating psychotic symptoms in schizophrenia, there is a wide consensus that a component of schizophrenia pathology arises from abnormal dopaminergic signalling. This is just one example of many pharmacological processes that have been identified as possible risk mechanisms in components of psychiatric disorders, where discrete malfunctions in cell to cell communication can have huge knock-on effects on larger-scale brain network communications. Technologies like positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) are further unveiling how these dysfunctions at the molecular level exhibit themselves at the network level too; where a number of abnormalities in network communications and structural aberrations of the brain have been associated with various psychological, emotional and cognitive symptoms of psychiatric disorders (reviewed in section 1.6.3).

1.3 – Environmental and societal factors in mental illness

1.3.1 – External Effects

In the investigation of mental illness it would be foolish to ignore the cultural, societal and economic aspects that play a huge part in the aggravation and maintenance of these disorders across the modern world. Recently, social theorists and epidemiologists have proposed certain cultural, political and economic factors that are contributing to increased incidents of mental illness in western societies. These include: the psychological pressures of living in a materialist-driven society (Carlisle & Hanlon 2007, Kasser 2002); the social isolation associated with the

cultural shift from collectivism to individualism (Bauman 2001; Eckersley 2006); the increasing demands on individuals, coupled with increasing cultural and economic pressures to succeed (Kasser 2002); and the widening economic inequality in western societies (Wilkinson 2010). All of these theories rely on the psychosocial concepts of psychological illness stemming from external societal pressures. Some of these observations may be supported by recent epidemiological research into psychiatric disorders. For example, research has linked higher rates of schizophrenia with urban living and urban birth (Pedersen & Mortensen 2006; Mortensen *et al.* 1999); being the member of a minority group in a population (Boydell *et al.* 2001), and life-time exposure to negative events (Fearon & Morgan 2006).

Furthermore, meta-analytical epidemiological work has shown a strong association between the incidence of psychotic syndrome and minority groups across a wide range of populations (van Os *et al.* 2010). They found that incidence of psychotic syndrome was higher with first and second generation minority group migrants (Bourque *et al.* 2011; Cantor-Graae & Selten 2005) along with minority groups who hadn't migrated recently (Bresnahan *et al.* 2007). Studies have shown that the incidence of psychotic syndrome is directly affected by the ethnic density of the area an individual from a minority is living in. The lower the proportion of the individual's own ethnic group in the area, the greater the risk for psychotic disorder (Boydell *et al.* 2001; Veling *et al.* 2008). Suggesting that occupying an ethnic group is not what increases chance of psychotic syndrome, but it is the level at which an individual is a minority; how much one stands out in the environment, and the resulting level of chronic social adversity and discrimination experienced (Veling *et al.* 2008; van Os *et al.* 2010). In turn, possibly heightening the chance of an individual to experience chronic levels of social exclusion and feelings of inferiority. Therefore increasing the risk of developing psychotic and affective disorders. This relatively recent psychosocial theory is known as 'out-group intolerance' (Abed & Abbas 2011; Abed & Abbas 2014). Research like this could back-up the claims by certain social theorists that modern society is playing a part in increasing risks of mental illness. In a society where huge pressure is put on each person to succeed financially, socially, physically and mentally – the likelihood of individuals in a population to experience chronic

feelings of social exclusion and inferiority may be high. Could these factors be influencing the epidemic levels of mental illness in western society? Possibly. However, what these theories cannot explain is why only a proportion of individuals in a hypothetical population facing extreme societal pressure and social exclusion will go on to develop a form of mental illness. It is clear that in order to tackle the spectre of mental ill-health, it must be viewed through an integrated understanding of the biological and environmental factors that impact it.

1.3.2 – Social and cultural influences on diagnosis & treatment

The diagnosis and perception of mental illness is also inherently intertwined with the social, political and cultural ideals of a society. For example, in 1952 the diagnostic and statistical manual of mental disorders (DSM) included homosexuality as a sociopathic personality disturbance (American Psychiatric Association 1952). A definition which was subsequently removed in 1973 as a clear result in the changing political and sociological outlooks of the age. Furthermore cultural phenomena known as 'culture-bound syndrome' illustrates how certain mental illnesses can exist only in the perceptions of specific cultures (American Psychiatric Association - DSM-IV 2002) – for example: *Dhat* syndrome in India, defined by anxiety, fatigue and guilt (Sumathipala 2004); *Susto* in Latin America – characterised by chronic fear and anxiety (Bayles & Katerndahl 2009), and even – as has controversially been suggested – pre-menstrual stress (Ussher & Perz 2013). This cultural difference in what constitutes mental illness, and what doesn't, can directly affect whether an individual seeks help or not; who will help and what kind of help these individuals will seek. This gives credence to arguments for more biological means of diagnosing mental disorders.

1.4 – Overview of the major psychiatric disorders

Psychiatric disorders pertain to a large group of pathologies that detrimentally affect an individual's ability to think, perceive, feel and act in a way that is beneficial to their health. These disorders are all thought to exist mainly from some sort of dysfunction in the brain. While there is some debate and confusion as to what exactly is a psychiatric disorder and what is more a neurodegenerative disorder or

neurological disorder, it is easiest to think of a neurodegenerative or neurological disorder as a brain/neurological disease with an obvious biological cause. In contrast, psychiatric disorder is an umbrella term for a large group of disorders and conditions that are harder to define biologically and symptomatically. For example, some conditions we define as psychiatric disorders will have as-yet unidentified biological causes, some may have as-yet unidentified social causes, some will have complex biological/social interdependent causes and it is possible that in the future some may not be seen disorders at all (as discussed in section 1.3.2). This important discussion on the semantics and definitions of mental disorders, highlights how the word 'mental' or 'psychiatric' represents a world that is still largely mysterious, complex and far from our understanding. When we have pushed further into our understanding of how the biological creates the phenomena of what we describe as 'mental', we actually reduce the boundaries and refine our idea of what we describe as 'mental'. And at these points of better understanding we can then allow a 'psychiatric' or 'mental' disorder become a *neurodegenerative*, a *neurological*, a *genetic*, or even a *societal* disorder. Therefore, from this point in the thesis, the term psychiatric disorder refers to the diseases that have not as yet been fully defined by their biological or psychosocial aetiology, and as yet are still within the realm of the less tangible, abstract idea of the 'mental'.

The most prominent and prevalent psychiatric disorders include schizophrenia, bipolar disorder, major depression, generalised anxiety disorder (GAD), attention deficit-hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), post-traumatic stress disorder (PTSD) and autistic spectrum disorder (ASD). While most of these disorders have huge impacts on the well-being on individuals and vast consequences for society as a whole, defining, understanding and treating these disorders is still a huge challenge for the world of clinical neuropsychiatry. Three of these disorders, schizophrenia, bipolar disorder and major depression constitute psychotic and affective disorders with arguably the biggest impact on the human population worldwide.

Schizophrenia is complex and multi-faceted psychiatric disorder that has a prevalence of 1% in the human population (Andreasen 2000). A unique disorder that is usually characterised by disordered thoughts, hallucinations, delusions, inhibited social engagement, and cognitive impairments. The symptoms of schizophrenia fall into three categories of positive, negative and cognitive symptoms. Positive symptoms are described as the symptoms most mentally healthy people do not experience (although this is probably not so clear-cut), which include delusions, hallucinations and paranoia. The positive symptoms mainly describe the psychotic phenotypes of psychiatric disorders. Negative symptoms represent the emotional experiences that healthy people also experience but are much more detrimental and pathological to individuals with schizophrenia. These include flat emotional responses, reduced desire to engage in pleasurable experiences, and poor social engagement. Cognitive symptoms are the least studied and understood symptoms of schizophrenia (which are also prominent in other psychiatric disorders, discussed in section 1.6.1) – these include memory dysfunctions, poor attention and reduced problem solving ability. Currently treatments for schizophrenia rely on antipsychotics that have largely been around for decades and while are effective in treating the positive aspects of the disorder, are ineffective in alleviating negative symptoms and ineffective in reducing the cognitive impairment expressed in schizophrenia.

Bipolar disorder is a disorder in which individuals experience cyclical periods of intense mania, major depressive episodes and then normal moods. Mania periods are characterised by extended periods of impulsive, hyperactive and risky behaviours to the point that it is damaging to the individual and leaves them severely impaired to function in everyday life. These manic periods can intensify into psychotic episodes and delusions, similar to the symptoms exhibited in schizophrenia. Depressive episodes, on the other hand, constitute periods where an individual experiences periods of depression, reduced motivation, apathy, attentional problems, and suicidal thoughts. Cognitive impairments are also present, which include attentional difficulties, impairments in components of memory and hindered problem solving abilities. Between manic and depressive periods, individuals can experience normal healthy mental states known as the euthymic period. The

disorder is thought to affect around 1 - 3% of the human population (Merikangas *et al*. 2011) and is sixth in the list of leading causes of disability worldwide (Kleinman *et al.* 2003). Treatments for bipolar disorder include the widely used mood stabiliser lithium, antipsychotics as used for schizophrenia and anticonvulsants.

Major depressive disorder (MDD) is a far-reaching and complex disorder that has almost incalculable effects on human societies, impacting around 400 million people worldwide (Ferrari *et al.* 2013). With a myriad of social, environmental and biological factors that may contribute to its manifestation. MDD is characterised by sustained and permanent low mood, low confidence, self-esteem, lack of motivation and reduced ability to experience pleasure. Risk of suicide is also high with individuals with MDD. Current treatments include selective-serotonin reuptake inhibitors and fluoxetine along with psychotherapeutic treatments like cognitive behavioural therapy (CBT).

1.4.1 – Cognitive impairments in psychiatric disorders

Among the symptoms of mental disorders, a broad set of cognitive dysfunctions exist which present an important line of investigation for research into psychiatric disorders . From facial expression interpretation dysfunction in autism (Adolphs *et al.* 2001; Pelphrey *et al.* 2002), to working memory impairments in schizophrenia (Goldman-Rakic 1994; Manoach 2003). To identify distinct cognitive dysfunctions among different mental illnesses would be desirable for diagnosis and possible treatment. For example, an individual expressing distinctive cognitive dysfunctions, well-defined psychological symptoms and other biological markers would increase the chances of a correct diagnosis. Furthermore if distinct cognitive dysfunctions are identified to be direct risk factors for specific types of mental illness, early intervention and treatment could provide effective abrogation of further psychological problems developing. Identifying cognitive dysfunction as not only a symptom of mental illness, but a possible cause of mental illness may also provide valuable new understandings. For example cognitive dysfunction may lead to poor performance in early education, social embarrassment, reduced life prospects and poverty – therefore increasing the likelihood of mental health aggravation.

1.5 – Diagnosis and definitions of disorders

Providing accurate diagnosis within robust definitions of a disorder is almost certainly the top priority in the treatment and understanding of mental illness. It is essential in directing effective treatments, determining the prevalence of illness in a population, constructing patient groups for research; and cataloguing public health information such as morbidity, mortality and well-being. In recent decades modern western clinical definitions of mental illness have been underpinned by a subjective set of criteria which have shifted and changed slowly with the culture; and to some extent – the science. With the use of subjective symptom-based diagnoses however, comes a variety of problems. These include: the risk of misdiagnosis, wrongfully diagnosing normal behaviour (as a response to environmental factors) as a mental illness, defining normal aspects of human behaviour as psychiatric disorders (for example, bereavement which was included in an early version of the DSM-5), along with the cultural and political influence on diagnosis. With the mounting costs that mental illnesses are putting on health services around the globe – accurate diagnosis is essential for economical and effective treatment for the general population. Discussed below are the currently used classification parameters and diagnosis methods for mental disorders; the problems with current definitions; and future solutions being developed.

1.5.1 – Diagnostic and Statistical Manual of Mental Disorders (DSM) & the International Statistical Classification of Diseases and Related Health Problems (ICD)

The (DSM) has been the prevailing language and standard criteria for defining and diagnosing psychiatric disorders since the 1950s. It is used by a wide range of professionals and institutions including clinicians, researchers, insurance companies, pharmaceutical companies, national legal systems and government institutions. The tool comprises three major components: the diagnostic classification, the diagnostic criteria sets, and the descriptive text. The diagnostic classification consists of the list of mental disorders that are officially included in the DSM. To make a diagnosis one must select disorders from the classification that reflects the observed symptoms

from an individual. For each disorder in the DSM a set of diagnostic criteria outline what symptoms must be exhibited along with specific criteria that must not be present in order to fall into a certain diagnosis. The third component is the descriptive text that provides each disorder with additional information. The DSM, along with the ICD, relies on a subjective interpretation of self-report symptomatology and behavioural-score based tests in order to assign a diagnosis. Where the DSM and ICD offer reliability and consistency for clinicians to make diagnoses, the underlying principles offer up a range of problems.

1.5.2 – DSM and ICD: criticisms

In recent years the traditional methods for defining and diagnosing mental disorders have come under fire. While most diseases over the past decades have moved from diagnosis by observations of external symptoms to diagnosis rooted in an empirical, biological aspect; methods for mental disorders have failed to catch up. The director of the National Institute of Mental Health (NIMH), Thomas Insel, likened the current DSM & ICD methods of diagnosis, to diagnosing other pathologies by the nature of chest pain or intensity of fever (Cuthbert & Insel 2013). Indeed a DSM based diagnosis is normally made from self-reports from a patient combined with a clinician's own subjective interpretations of the patient's behaviour and understanding of psychiatric terms (Casey *et al.* 2013). This type of subjective diagnosis allows for the potential for misdiagnosis (and therefore mistreatment) across patients and practitioners. It also gives individuals the ability to abuse the use of social institutions like the criminal justice and welfare systems; and gives ground the potential abuse by political and governmental powers. This reliance on subjective interpretation also comes with problems in distinguishing normal 'day-to-day' human problems (e.g. bereavement, work stress, sadness) with more chronic pathologies rooted in genetic, developmental, anatomical and circuit dysfunctions.

Another key criticism of DSM and ICD is their tendency to define disorders in discrete, separate categories (Adam 2013). Meaning that for decades, patients whom present symptoms that span a number of categories have frequently been diagnosed with multiple disorders, experienced changing diagnoses over their life-

times, and consequently have suffered ineffective treatments and inaccurate prognoses.

As a result of recent genetic and neuroscientific evidence, many are now arguing for a paradigm shift in our definitions and approach to psychiatric illness. They argue that different mental illnesses exist on a dimensional spectrum rather than existing as distinct, unrelated entities (Adam 2013). According to the 'dimensionality' theory, mental disorders are seen as a product of common risk factors – separated by variable combinations of risk factors and environmental interactions. For example: a substantial body of evidence has arisen recently supporting the theory that schizophrenia and bipolar disorder exist on a dimensional spectrum – manifesting from common genetic susceptibility and shared pathological mechanisms (Craddock & Owen 2010). This answers for the classic Kraepelian division between schizophrenia and bipolar disorder known as 'schizoaffective disorder' – created in the DSM-IV to account for the large number of patients presenting co-morbidities for schizophrenia and bipolar disorder (Adam 2013).

Many also argue that the DSM and ICD's principle of categorisation and inclusion of contradictory parameters has impaired scientific research in the crusade for diagnosis precision (Cuthbert & Insel 2013). For example: establishing specific circuits or genes involved in a specific disorder (like bipolar disorder for example) prove difficult using subjects within DSM parameters because many involve subjects that present with various symptoms of the disorder. The various symptoms bipolar disorder may actually comprise distinct biological entities which are uninvolved with each other; therefore, reducing the likelihood of establishing a unifying genetic or circuitry factor. This top-down investigation where a specific disorder is chosen and then a proceeding search for a corresponding genetic/circuit based malfunction takes place, has pushed mental illness research into a rut. Many scientists are now calling for a bottom-up method where risk circuits or genes are identified as potential contributors to specific symptoms that may be present in a wide range of disorders. A new project named the Research Domain Criteria (RDoC) aims to 'Develop, for research purposes, new ways of classifying mental disorders based on

dimensions of observable behaviour and neurobiological measures' (Cuthbert & Insel 2013).

1.5.3 – Research Domain Criteria (RDoC) Project

The RDoC Project is a new initiative set up by the NIMH that aims to create a biologically based foundation for the understanding and diagnosis of mental disorders (Cuthbert & Insel 2010; Cuthbert & Insel 2013). The RDoC aims to provide a new kind of framework for mental disorders, enabling researchers to investigate from the 'bottom-up' using modern genetic, neurobiological and behavioural techniques. The development of the RDoC is in direct response to the problems in researching mental illness within the taxonomical constraints of the DCM. RDoC propose that since many genetic and neural circuit pathologies span a number of currently recognised syndromes, the DCM definitions restrain current research from identifying illnesses linked to pathophysiology, and hold back the development of more tailored treatments. Currently RDoC is being developed as a framework to guide grouping methods for patients in research studies and operate under three governing principles:

First RDoC is a dimensional system, spanning the full range of variation, from normal to abnormal. This reflects the current doctrine of viewing diseases as multiple interacting traits that are extremes on a spectrum of normal functioning (e.g. hypertension) (Casey *et al.* 2013). These dimensions can be measured quantitatively or qualitatively.

Secondly, RDoC remains free from the current disorder divisions. The aim of RDoC is to build new classifications based on current understandings of biological – behaviour relationships in relation to pathological behaviours.

Third, RDoC uses seven different units of analysis for study constructs – these are: genes, molecules, cells, circuits, physiology, behaviour, and self-reports. This allows researchers to choose an independent variable from several different units of analysis.

The RDoC framework is a matrix whose columns represent the individual units of analysis (genes, circuits etc) and rows represent 'constructs' – a concept summarising an arbitrary functional dimension of behaviour. Associated constructs are classed into 1 of 5 domains that represent contemporary views on motivation, cognition, and social behaviour. These are: Negative Valence Systems (systems for aversive motivation), Positive Valence Systems, Cognitive Systems, Systems for Social Processes and Arousal/Regulatory Systems. These constructs were picked on three criteria: first, constructs were included by whether a brain circuit or area could be defined that underpins a particular behaviour; second constructs were chosen to span the whole area of human behaviours and cognition while attempting to balance out specificity with conciseness; third, constructs were backed up by current research on neurobehaviour. The domains and constructs are there to serve as a guide and starting point rather than a rigid set of parameters. RDoC expect these constructs to develop and change as research progresses.

Alternatively from DCM-guided 'top-down' research, RDoC offers a research framework to investigate mental disorders from a bottom up approach. RDoC explains this will come from two approaches. First, a study may include all patients who attend a clinic for particular type of treatment. Secondly, a specific criterion for selecting multiple groups could be used e.g. patients whom score under the average on a working memory test – and then compare these patients with the average (control) group. From here researchers could establish biological links to specific variables with a lower chance of excluding patients because of co-morbid conditions.

1.5.4 – RDoC: implications and challenges

In light of this new approach to investigating psychiatric disorders, it is safe to say that the way neuroscientific research is directed and evaluated will have to change dramatically in order to fit this framework. It requires a general paradigm shift in the way that researchers look at mental disorders. For example, a study looking at underlying genetic or circuit based causes to auditory hallucinations should include a whole group of patients reporting these symptoms, regardless of specific disorder as defined by the DSM. Thus, a large component of a researcher's study design must be

changed. Even previous research may have to be re-evaluated and critically analysed with respect to the new RDoC framework. Furthermore, because whole institutions, lab groups, charities, policy makers, legal professionals and clinicians guide much of their work based completely on DSM & ICD definitions – which may turn out to be quite inaccurate or even completely wrong – this could have huge implications that extend way beyond the scientific world. Not only that, but insurance claims, welfare benefits, criminal law decisions, drug treatments and therapeutic programmes would all have to be revised. In light of this, it can be concluded that the way that RDoC may change the definitions, approaches and perceptions of mental illness could be staggering for society.

These challenges and implications, however, pale in comparison to the huge benefits and progress that will arise from such changes. This shift from researching subjectively defined mental disorders into investigating specific symptoms on a spectrum of intensity will not only benefit psychiatric research; but neuroscience as a whole. After all; neuroscience has always exploited what information can be gathered from brain injuries, genetic mutations and other biological aberrations, in order to gain valuable insight into how biological mechanisms (e.g. genetic, neural) give rise to behaviour and cognition. Up until now, neuroscience has failed to make use of mental disorders in a similar way. However, by realigning mental disorder research into this framework there is potential to utilise the spectral distribution of the cognitive and emotional range (from normal to pathological, and everything in between) to unlock the biological processes of these phenomena. For example, a neuroimaging combined genetic study into attention using a spectrum of subjects based on their attentional abilities (e.g. from excellent, hyper focused subjects, to normally attentive subjects, and then all the way to subjects with extreme attentional difficulties) regardless of their formal psychiatric diagnoses (e.g. healthy, schizophrenia, autism, bipolar disorder etc) could provide insight into genetic and circuit architecture of attentional systems; along with a new framework in defining and tracing the aetiology of various disorders. This paradigm shift will be slow, costly and not without its problems but is absolutely essential in furthering our

understanding of the human brain and how biological variations give rise to the vast plethora of our behavioural and cognitive apparatus.

1.6 – Current phenotypes for psychiatric disorder research

1.6.1 – Cognitive impairment

Symptoms of psychiatric disorders tend to be most well-known for the psychotic and emotional aspects of the disorders. For example in schizophrenia the psychotic aspects (or positive symptoms) are characterised by thoughts, feelings or experiences that most individuals do not normally experience. For example auditory hallucinations, paranoid delusions, and disordered thoughts (Kay, Fiszbein, & Opler, 1987). Emotional aspects (or negative symptoms) are characterised by deficits in normal emotional processing including reduced emotional response, reduced motivation for reward and little interest in forming emotional relationships (Kay *et al.*, 1987; Foussias & Remington 2010). These symptoms are generally harder to treat than positive symptoms (Greden & Tandon, 1991). However, while the emotional and positive symptoms of psychiatric disorders remain the main focus for medical practitioners, and are the most well-known aspects of mental illness in popular culture, a third category of symptoms exist - cognitive impairments. Cognitive impairments are just as detrimental to an individuals' well-being; and can have great influence on the positive and emotional aspects of mental disorders like schizophrenia (Pessoa 2008). As with the negative symptoms, cognitive deficits are minimally affected by current drugs for the treatment of schizophrenia and other disorders, and are strongly associated with functional outcome (Hill *et al.* 2010; Mueser & McGurk, 2004; Green *et al.* 2004). Cognitive impairments also exist out-with standard diagnostic definitions, with differing underlying neurobiological pathology from emotional and psychotic symptoms. Despite this, this major primary symptom of the psychiatric disorders is generally overlooked in diagnosis and treatment. Nonetheless, new research is promising to uncover the neurobiological and categorical aspects of cognitive impairments in psychiatric disorders which could serve to sharpen diagnosis and enhance treatment.

1.6.1.1 – Cognitive Parameters

Cognitive processes constitute the 'higher order' functioning that integrates various types of information (e.g. sensory, numerical, memory), in order to execute and learn complex behaviours, motor processes and mental constructs that the pressures of survival constantly demand on organisms. It is especially important in the understanding of our own species, *Homo sapiens*, as they help to describe the multiple facets of our complex behaviours, expressions, and creations that have put us in our unique position on Planet Earth today.

Cognitive processes in humans tend to fall under five distinct, but inter-linking categories which are: attention, memory, emotional and social cognition; symbolic representation; and executive processing. There are sub-types under each category which constitute separate processes and can be identified using various behavioural tasks, where many have been characterised by distinct neural circuits. All these cognitive processes influence each other and work together to create particular mental and behavioural processes. Therefore when one process is disrupted it can cause a cascade of dysfunction in mental processes further down the line, leading to an amalgamation of various behavioural phenotypes and disorders. These disruptions in cognition occur in every psychiatric disorder and in each discrete category of cognition (Millan *et al.* 2012), and one of the major challenges is quantifying the exact changes in cognition that take place among these mental disorders. This is complicated by the close relationship between changes in emotional state, reward motivation, motor performance and fluctuations in cognitive processing speed. Furthermore along with treatment, many other factors can impede on a subject's cognitive ability, these include: education and age; physiological status, disease stage, co-morbidity, motivation and reward value; type of cognitive assessment used, and the quantification method – e.g. self-rate, quantitative (Kalkstein *et al.* 2010; Hauber & Sommer 2009). Despite the complications, various patterns are emerging. Outlined below are the various aspects of cognitive impairment in relation to mental disorders with a summary of cognitive impairments related to mental disorder featured in table 1.

	Attentional	Executive Function	Social Cognition	Symbolic Representation / Language	Working Memory	Semantic	Episodic Memory	Non-Declarative Memory	References
ADHD	**P**	**P**	**P**	**/**	**P**	**/**	**/**	**/**	Arnsten 2006, Martinussen *et al.* 2005; Uekermann *et al.* 2010; Snyder *et al.* 2014
ASD	**P**	**P**	**P**	**P**	**?**	**/**	**P**	**/**	Baron-Cohen & Belmonte 2005; Hill & Frith 2003; Goh & Peterson 2012; Gaigg *et al.* 2014; Lord 2004; Young *et al.* 2005; Kjelgaard 2001; Baron-Cohen 2005; Pelphrey *et al.* 2002; Hill 2004;
Bipolar Disorder	**P**	**P**	**P**	**P**	**P**	**/**	**P**	**/**	Robinson *et al.* 2006; Torres *et al.* 2007; Gallagher *et al.* 2014; Dere *et al.* 2010; Robinson *et al.* 2006; Kurtz 2009; Samamé 2013
GAD	**P**	**/**	**/**	**/**	**/**	**/**	**P**	**/**	Coles *et al.* 2007.
MDD	**P**	**P**	**P**	**/**	**P**	**/**	**P**	**/**	Castaneda *et al.* 2008; Gotlib & Joormann 2010; Airaksinen *et al.* 2007; Christopher & MacDonald 2005; Schreiter *et al.* 2013; Loi *et al.* 2013; Lee *et al.* 2012;
OCD	**P**	**P**	**P**	**/**	**P?**	**/**	**/**	**E**	Burdick *et al.* 2008; Nakao *et al.* 2009; Tolin *et al.* 2001; Roth *et al.* 2004; Sayin *et al.* 2010; Snyder *et al.* 2014
PTSD	**P**	**P**	**/**	**/**	**E?**	**/**	**/**	**/**	Moore 2009; Schönenberg 2013; Williams *et al.* 2007; Aupperle *et al.* 2012; Olff *et al.* 2014;
Schizophrenia	**P**	**P**	**P**	**P**	**P**	**?**	**P**	**/**	Luck & Gold 2008; Dere *et al.* 2010; Elvevåg 2000; Cohen *et al.* 1974; Allen *et al.* 1993; Savla *et al.* 2013; Morrison *et al.* 2013); Kalkstein *et al.* 2010; Ihara *et al.* 2000; Orellana & Slachevsky 2013

Table 1: Cognitive Impairments in the major psychiatric disorders. Key: '**P**' = present; '?' = Disputed; 'E' = enhanced; '/' = no data or not present. ADHD = Attention Deficit Hyperactivity Disorder, ASD = Autism Spectrum Disorder, GAD = Generalized Anxiety Disorder, MDD = Major Depressive Disorder, OCD = Obsessive Compulsive Disorder, PTSD = Post Traumatic Stress Disorder.

1.6.1.2 – Attention

Attention is the cognitive process that allows one to focus on a particular stimuli or mental activity while filtering out other potentially equally weighted stimuli. It is the process that allows one to listen to a piece of music as a whole and then selectively listen to the percussion piece, and then bass and then the brass. It is a fundamental apparatus for humans to maintain goal directed behaviour and focus on social exchanges, and many other important every-day activities. Importantly, this is a cognitive process that seems to be impaired in almost all of the major psychiatric illnesses; highlighting a major line of investigation into the aetiology of these disorders. Attention deficit hyper-activity disorder (ADHD) represents a disorder with a fundamental impairment in attentional systems. In the attentional domain this mainly manifests as an overall loss of focused attention, and severe problems with distractibility (Arnsten 2006). Deficits in attention are common in schizophrenia too. These are specifically in input-selection attention and rule selection (Luck & Gold 2008). Also in inhibitory attentional control and sustained attention (Galaverna *et al.* 2012). Patients with bipolar disorder are found to have marked deficits in sustained attention and speed of processing during euthymic (Robinson *et al.* 2006; Torres *et al.* 2007; Martino *et al.* 2014) and depressed (Gallagher *et al.* 2014; Kurtz & Gerraty 2009) phases of the disorder. Furthermore patients suffering from panic disorder, post-traumatic stress disorder (PTSD), and obsessive compulsive disorder (OCD) all exhibit hypervigilance to threatening stimuli, which serves as a disruption to the stability of attention (Gordeev 2008; Moore 2009; Schönenberg & Abdelrahman 2013; Burdick *et al.* 2008). As a result of this hypervigilance, patients tend to have dysfunctions in selective attention, failing to attend to relevant stimuli while failing to disregard irrelevant stimuli (Clayton *et al.* 1999; Enright & Beech 1990). Attentional bias towards negative thoughts is widely seen in major depressive disorders and anxiety disorders. Along with loss of vigilance in major depressive patients (Castaneda *et al.* 2008; Gotlib & Joormann 2010). Finally, Autistic Spectrum Disorder (ASD) patients exhibit severe deficits in the ability to focus attention to others' emotional state along with a blunting of joint attention. ASD also exhibits

marked hyper-attention to inanimate objects (Baron-Cohen & Belmonte 2005; Hill & Frith 2003).

1.6.1.3 – Memory

Memory systems in mammals are divided under two major categories based on the duration and endurance of the memories – these are long term memory and short term memory. Both categories use similar mechanisms of synaptic plasticity but differ in their cognitive and systems level base. Long-term memory describes the storage and recovery of information over a long period of time (e.g. months, years) and is divided further into two sub-categories – declarative (episodic and semantic memory) and nondeclarative memory (skill learning, priming and conditioning). Short-term memory generally describes the short term memories that are stored for a brief period in order to facilitate short term behavioural goals. A subdivision of short-term memory known as working memory – represents a cognitive apparatus that utilises short-term memories, long-term memories and other kinds of information to rehearse and manipulate information in real time - in order to solve behavioural goals. Working memory can involve visual, verbal and many other types of modalities. Both of the main types of memories are essential in guiding and facilitating other cognitive functions like executive, emotional and social cognition. This means that any deficit in these subtypes of memory has the potential to negatively affect the normal functioning of modes of cognition; and in turn can negatively affect an individual's behaviour and state of well-being.

While memory deficits in brain disorders are most well-known as major symptoms in dementia and Alzheimer's disease, deficits are seen in various psychiatric disorders. Patients with ADHD exhibit characteristic deficits in working memory (associated with executive function impairments) (Martinussen *et al.* 2005). OCD patients may show deficits in working memory (Nakao *et al.* 2009) (although some argue that the deficits lie in their *confidence* of the memories (Tolin *et al.* 2001)) and actually show cognitive enhancement in procedural memory compared to normal populations (Roth *et al.* 2004). Furthermore individuals with social anxiety disorder and PTSD exhibit abnormal bias towards social-threat memories, trauma-relevant and other

related imagery, reflecting a malfunction in autobiographical memory (Coles *et al.* 2007; Williams *et al.* 2007).

Individuals with ASD tend to express conserved abilities in semantic memory, but show marked deficits in episodic memory (Goh & Peterson 2012; Gaigg *et al.* 2014). Individuals with bipolar disorder and major depression share marked deficits in episodic memory (Airaksinen *et al.* 2007; Dere *et al.* 2010) – where both show impairments in inhibiting negative components of autobiographical memory (Williams & Moulds 2008); and working memory – where automatic intrusive negative thoughts are thought to compete for attentional demands during working memory tasks (Christopher & MacDonald 2005; Gallagher *et al.* 2014). Lastly, schizophrenia disorders display the most marked deficits in memory systems with large effect sizes. These include marked deficits in spatial, digit span and verbal aspects of working memory performance; and impairments in verbal-episodic memory, especially dependent on the emotional valence of the memories recalled (Dere *et al.* 2010). Various studies have claimed that deficits also exist in semantic memory for schizophrenia (Elvevåg & Goldberg 2000). A recent meta-analysis has shown an uneven degradation in various semantic memory tests including verbal fluency, word-picture association; and categorization and priming tests (Christopher & MacDonald 2005).

1.6.1.4 – Symbolic representation / language

Arguably the most distinctive and sophisticated of human cognitive behaviours is the use of symbols and representations to convey information from individual to individual. Symbolic representation in humans is most commonly seen through the use of language (auditory or gestural) and the written, visual representations of our languages. Furthermore it is argued that humanity's development and use of language is a fundamental factor that has shaped our success as a species today. It is yet to be proven that other species acquire such complex language abilities, however, there is extensive evidence for sophisticated communications in members of the cetacean order including the Bottlenose dolphins and Orcas (Marino *et al.* 2007); and our closer relatives – Orangutans and Chimpanzees (Hobaiter & Byrne

2011; Cartmill & Byrne 2010) – that may constitute distinctive non-human languages. Some scientists have even argued that the evolution of complex language abilities in humans (and other sophisticated cognitive abilities) may be a major factor in the manifestation of our species-specific disorders like schizophrenia and ASD (Thomas Gualtieri 2014).

One of the major symptoms of schizophrenia is supposed to be formal thought disorder (FTD), characterised by a range of markers of impaired and disordered thoughts. However, most of these impairments are measured via verbal communication, where research has shown that language itself is broadly impaired in schizophrenia, meaning that many of the attributes of FTD may be more likely caused by impairments in language capabilities (Radanovic *et al.* 2013). Impairments in various forms of semantics are seen in schizophrenia, these include *glossomania* – an inability to inhibit the flow of associated subjects with a specific word (Cohen *et al.* 1974); and *approximation conduct* – to use words which do not directly address the meaning of a word but instead use an approximation or neologism (Allen *et al.* 1993). Impairments in the understanding of the structure of statements has also been observed, however, some authors have argued this is hard to distinguish from other cognitive deficits seen in schizophrenia including working memory, attention and executive function (Radanovic *et al.* 2013). The most evident deficits in language however, are the pragmatic aspects of discourse, where patients will have problems in maintaining the relevant cohesion of conversation and statements. Patients also have difficulty in introducing new information to conversation and show impairments in producing coherent, well-planned arguments or ideas (Hoffman *et al.* 1986).

ASD patients also exhibit many different types of language impairments which depend on the type and severity of their autism. These deficits can exist in all fundamental aspects of language including: lexical (Lord *et al.* 2004), pragmatic (Young *et al.* 2005) and phonetic (Kjelgaard & Tager-Flusberg 2001). Along with ASD and schizophrenia, recent meta-analyses have shown slight dysfunctions in language capabilities in bipolar patients. Recent meta-analyses have shown slight deficits in

letter fluency and marked deficits in phonemic and semantic fluency in euthymic bipolar patients (Robinson *et al.* 2006; Kurtz & Gerraty 2009). Furthermore the latter study found that patients in manic/mixed states showed marked impairment in letter fluency and semantic fluency, and during depressed states showed large deficits in phonemic fluency (Kurtz & Gerraty 2009). These differences in language capabilities were greater between depressed and euthymic states but remained similar during manic and depressed states, highlighting how differences in clinical state can affect neurocognitive performance.

1.6.1.5 – Social cognition

Social cognition describes the multidimensional psychosocial processes which facilitate how individuals understand themselves, understand their relationships with others and can calculate the feelings and intentions of others around them. The major aspects of social cognition include: self-reflection (knowing oneself as a distinct entity, and critically analysing one's own thoughts and behaviours), embodiment (the knowledge of being part of one's own body), recognition and perception of facial and body expressions (the ability to recognise other's bodily and facial expression and understand what they are communicating), social categorization (perceiving people's identifying features and forming impressions based on this information), Theory of Mind (the ability to comprehend the mental states and intentions of individuals other than yourself) and empathy (the phenomena of actually *feeling* and understanding the emotions of other individuals). These cognitive phenomena are ones which most individuals take for granted, but underpin some of the most important behavioural and spiritual attributes that make us human. And dysfunctions in these can have massive implications for the way an individual interacts with their own and other's personal well-being. These cognitive abilities can be heavily influenced by one's own immediate emotional state, their cultural and societal influences, and their own personal perceptions. These processes can be nurtured, manipulated, ignored and completely destroyed in a healthy human's life and it could be argued that most humans in their lifetime may suffer some form of impairment in social cognition (e.g. empathy) as determined by

the survival pressures of their time. However, within certain mental disorders, it has been shown that significant social cognition deficits do exist and either form the backbone of the disease, like ASD, or may be a major contributor to disease morbidity.

A number of social cognition deficits are present in schizophrenia patients. In a meta-analysis across 112 studies, Savla *et al.* showed that schizophrenia patients exhibit impairments across all areas of social cognition including social perception, Theory of Mind (ToM), emotion perception and emotional processing (Savla *et al.* 2013). Furthermore, another study (Morrison *et al.* 2013) found that patients with schizotypy performed significantly worse in facial emotion recognition tasks, ToM and emotional management tasks, compared to controls. These deficits in facial emotional processing were shown to be more significant in negative emotions for schizotypy patients (Comparelli *et al.* 2014). While these deficits may not seem as urgent as the more psychotic aspects of the disease, social cognitive deficits are actually very significant in the prediction and aggravation of various symptoms of the disorder. For example, is has been proposed that social cognition impairment may be one of the major predictors for schizophrenia in high risk, asymptomatic individuals (Barnett *et al.* 2010) and the consequences of these social impairments can lead to increased social withdrawal. This can result in a worsening of the negative aspects of schizophrenia. Furthermore the impairment in understanding another's intention or emotional state can lead to the worsening and aggravation of paranoid and delusional symptoms in patients (Brüne 2005). These social cognition impairments are also seen in other disorders but are less severe and less broad. For example: Major depressive disorder – including deficits in empathy (Schreiter *et al.* 2013) and body language perceptions (Loi *et al.* 2013); bipolar disorder – with deficits in ToM (Samamé 2013); OCD – impairments in ToM (Sayin *et al.* 2010); and ADHD with impairments in emotional facial processing (Uekermann *et al.* 2010). Lastly, ASD is mainly characterised by an individuals' poor social skills and lack of interest in other humans. This is mainly caused by severe dysfunctions and impairments in ToM (Baron-Cohen *et al.* 1994), empathy (Baron-Cohen & Belmonte 2005) and emotional facial processing (Pelphrey *et al.* 2002).

Like deficits in attentional cognitive domains, it seems social cognition is impaired in some form among all the major mental disorders. This may highlight a common aetiological source among the various disorders (apart from ASD), where problems in social cognition could aggravate an individual's mental health and lead them into worsening conditions.

1.6.1.6 – Executive function

Executive processes generally describe the unconscious and conscious 'thinking' that monitors and influences other cognitive processes in order to direct mental states and behaviour in a flexible, goal-directed manner. This allows individuals to achieve various complex tasks and outcomes that, without executive monitoring and influence, may not be achievable. These include the abilities to: break out of habitual behaviours, evaluate multiple options and then make a decision on them; plan for the future, apply deductive and inductive reasoning; adapt to new situations, and prioritise and sequence behavioural strategies. These complex mental apparatus are largely taken for granted in the healthy population but can, understandably, be detrimental to an individual when any of these processes are impaired. For this reason, much research has been put into discovering executive dysfunctions in mental illness, how they impact an individual's life and how it influences disorder progression. Outlined below is a summary of executive dysfunctions found in the major psychiatric disorders.

Bipolar patients show a number of executive dysfunctions across all phases of the disorder; these include deficits in problem solving tasks, verbal interference, and set switching tasks (Kurtz & Gerraty 2009). It has been argued that these impairments can be influenced by education status and age of onset (Robinson *et al.* 2006). However, a five year follow up study into executive dysfunction in a group of bipolar patients showed that there was little difference in performance of cognitive tasks over the five years (Santos *et al.* 2014).

In a recent meta-analysis of 15 studies, Lee *et al.* found that first-episode patients with Major Depressive Disorder (MDD) patients exhibit small to medium deficits in

all aspects of executive functioning (Lee *et al.* 2012). These deficits may all be affected by education attainment, age, antidepressant usage and other factors, however, due to small sample sizes in the studies the findings may not be completely reliable. Other studies have pointed towards mild, but broad deficits in aspects of executive functioning in MDD patients of all ages. These include cognitive flexibility, inhibition and verbal fluency (Mahurin *et al.* 2006; Stordal *et al.* 2004). This may point towards a subtle source of mental health aggravation which may contribute towards MDD aetiology. ADHD individuals also exhibit broad-based deficits in executive function but tend to be more severe than MDD (Goldstein *et al.* 2014). Many clinicians and researchers have hypothesized that the repetitive and chronic compulsions of OCD are partly because of deficits in executive functioning. This has been backed up by a recent extensive meta-analysis by Snyder *et al.* (Snyder *et al.* 2014). While previous meta-analyses produced conflicting findings on the extent of executive functioning dysfunctions in OCD (Abramovitch *et al.* 2013; Shin *et al.* 2014), Snyder *et al.* produced an extensive coverage of executive functioning dysfunctions in OCD. These include large deficits in cognitive flexibility; executive visuospatial working memory and future planning. This deficit in cognitive flexibility may help to explain the stereotypical problems OCD patients have with changing strategies to new behavioural challenges, a phenomena known as perseveration. Patients with PTSD also show mild impairments in executive functioning, which may be related to avoidance and numbing behaviours (Aupperle *et al.* 2012; Olff *et al.* 2014). ASD also exhibits a number of executive function deficits that underline a prominent component of the disease's severity. These include marked deficits in future planning and cognitive flexibility (Hill 2004).

Schizophrenia patients exhibit some of the most severe and extensive deficits in executive functioning among the major mental disorders, and is the most commonly observed cognitive deficit of the disease (Kalkstein *et al.* 2010). These deficits include poor performance in cognitive flexibility (set-shifting) tasks like the Wisconsin Card Sorting Test (WCST) (Ihara *et al.* 2000); impairments in future planning (Kalkstein *et al.* 2010) and dysfunctions in self-regulation of behaviour (Orellana & Slachevsky 2013). This underlines an important target for research into schizophrenia disease

progression, as clearly these forms of executive processes are essential for normal functioning in nearly all aspects of life. To be hindered in any of these processes may significantly affect an individual's chances of performing adequately in various platforms, including education, employment and socialising, thus increasing the risks of negative life experiences.

1.6.2 – Genetic risk factors for psychiatric disorders

Unlike other disorders of the brain, like Alzheimer's disease, there are no prominent biochemical markers in psychiatric disorders that can easily explain their aetiology and pathological progression. Furthermore there have been no real discoveries of Mendelian genetic inheritance, despite substantial evidence from heritability and familial studies indicating that the disorders have a strong genetic component. And despite the considerable work and energy put into deciphering possible genetic causes, progress has been frustratingly slow. Much of this is owed to the complex nature of human genetic study design, and the variable, subjective means of psychiatric diagnosis. However, with the enhanced sophistication and expansion of large-scale genomics investigations by groups like the Psychiatric Genomics Consortium (PGC), genetic architectures for some major disorders are arising. Multi-party collaborations within the international community on large scale genome wide association studies (GWAS) and copy number variation (CNV) studies have unveiled a number of common, rare and *de novo* risk factors in some of the major psychiatric disorders. From these discoveries the psychiatric disorders are beginning to be viewed as polygenic complex traits that are influenced by a number of genetic factors and non-genetic factors; e.g. environmental, epigenetic. These discoveries couldn't come any sooner, as two decades' worth of candidate gene and linkage studies have struggled to produce reliable or replicable evidence for genetic risk factors in the major psychiatric disorders (Sullivan *et al.* 2012; Gratten *et al.* 2014), stifling the progression of drug discovery, diagnosis and early intervention strategies.

The recent discoveries of genetic risk factors in psychiatric disorders are owed to the great advancement in technological capabilities and collaborative effort from the scientific community, which has spawned a number of unbiased and effective

methods of analysis. These are gene-wide association studies (GWAS) (or genome-wide common variant studies (CVAS)), copy number variation studies (CNVS), and rare variant association studies (RVAS). GWAS are whole genome approaches to investigate chromosomal loci regions in relation to a disorder or risk for a disorder. GWAS studies mine the genomes from extremely large datasets of cases and controls to find individual single DNA nucleotide sequence differences (known as single nucleotide polymorphisms (SNPs)) on genetic loci that may be more common in case populations whom express a particular trait. From here the frequency of specific SNPs can be associated with a specific disease. Copy number variation studies – the simultaneous analyses of multiple deletions and duplications of genomic segments – have also produced reliable risk loci in major disorders like schizophrenia and ASD. These studies have found >200kb deletions and duplications on loci that may contribute to multi-gene dysfunction. Lastly, rare variant association studies (RVAS) have made it possible to investigate rare sequence variants of high pathological penetrants, these include *de novo* mutations – which are newly arising mutations that *are not* genetically inherited (Mccarroll *et al.* 2014). Outlined below are the various findings from these new methods of investigation and how they are unveiling the genetic architecture of the major psychiatric disorders.

Despite much familial and heritability based evidence pointing towards genetic inheritance within depressive, attentive and anxiety based disorders, much of the results from whole genome studies have been patchy and inconclusive so far. This may be down to problems in misdiagnosis of disorders, an under-appreciation of the environmental impacts of heritability studies, a limited sample size, and a lack of statistical power required to delineate risk factors (McCarroll *et al.* 2014). However, CNV research and cross-disorder studies have unveiled some promising lines of evidence.

A PGC GWAS analysis into major depressive disorders found no significant genes associated with major depressive phenotypes (Ripke, Wray, *et al.* 2013). This study used a sample number of over 11,000, which in any other GWAS study with a sample size n>11,000 has produced at least one significant finding (Sullivan *et al.* 2012).

However, it has been proposed that due to MDD's high population prevalence, a successful GWAS would have to use a sample 1.8 to 2.4 times larger than equivalent studies into schizophrenia in order to detect risk variants (Wray *et al.* 2012). Recent GWASs into ADHD have yielded no risk loci as of yet (Stergiakouli *et al.* 2012; Neale *et al.* 2010). This may be down to very low sample sizes in these studies, in comparison to schizophrenia GWASs, and the variability in diagnoses of ADHD among a population. Similarly, there has been no risk loci found in OCD GWASs (Davis *et al.* 2013). However, among these disorders, rare CNV (less than 1% in the population) *de novo* (genetic mutations that are found newly in a family member, which has not been expressed in their parents or previous lineage) duplications and deletions have been found. These variations are rare in populations but have high odds ratios for exhibiting the disease (around 2 – 20) (Gratten *et al.* 2014). Using the data from an SNP data array, Degenhardt *et al.* found a number of CNVs in a population of MDD patients (Degenhardt *et al.* 2012). These include microdeletions 7p21.3, microduplications in 15q26.3; and microdeletions *and* duplications in 16p11.2. Interestingly, the 16p11.2 region has also been implicated in various psychiatric disorders including schizophrenia (McCarthy *et al.* 2009) and autism (Kumar *et al.* 2008). Furthermore research has shown that 16p11.2, among other regions, shows a reciprocal tendency – where a deletion increases the risk of autism and a duplication increases the risk of schizophrenia (Crespi *et al.* 2010). A recent study found large deletions in the locus 16p13.11 for a very small number of OCD patients (McGrath *et al.* 2014). This locus has also been implicated in seizures in epilepsy (de Kovel *et al.* 2010) and in ASD (Ullmann *et al.* 2007). There are many rare CNVs that have been implicated in ADHD too, including large duplications on 16p13.11 (Williams *et al.* 2010) and the 16p11.2 region (Lionel *et al.* 2011). Another study implicated around seventeen shared risk CNVs for ADHD and autism (Stam *et al.* 2013). This evidence supports the growing view that many psychiatric disorders share components of their genetic aetiology and will further serve to break down classical nosology of the disorders. Furthermore a genome wide analysis of SNP data for five disorders (ASD, ADHD, bi-polar disorder, MDD, and schizophrenia) using 33,332 cases and 27,888 controls found SNPs at four significant loci (Ripke *et al.* 2014). These were on regions of 3p21, 10q24 and within genes that code for two L-

type voltage-gated subunits, CACNA1C and CACNB2, implicating a possible common aetiology for these disorders rooted in calcium channel pathology. Other studies support these findings identifying CACNA1C as a risk factor for schizophrenia and major depressive disorder in Han Chinese populations (Yu *et al.* 2014; Zheng *et al.* 2014), and bipolar disorder in European populations (Green *et al.* 2010; Ferreira *et al.* 2008). This association of risk SNPs is particularly prominent between schizophrenia and bipolar disorder. This was supported by an earlier GWASs that found three loci that are associated with both disorders – CACNA1C, ANK3 and ITIH3-ITIH4 (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium 2011).

From these findings and many other studies, most psychiatric disorders are now viewed as highly polygenic traits that manifest as a conglomeration of multiple risk factors, with rare cases of a few genes producing similar traits in a population. This would make sense through an evolutionary perspective where multiple mutations with very small effect take place rather than single mutations with a large effect, which may provide less risky platforms for a genome to interact positively with its environment. No other psychiatric disorder exemplifies this complex polygenic nature like schizophrenia. It is estimated that 8300 independent loci contribute to around 50% of genetic risk in the disorder (Ripke, Dushlaine, *et al.* 2013). Where most loci carry very small risk (around 0.05) (Gratten *et al.* 2014), and others carry a much more significant risk. For example, over a decade ago a rare, and highly penetrant reciprocal chromosomal t(1:11) translocation on 1q42.1 was discovered, and was found to be strongly associated with various psychiatric disorders in a single Scottish family (St Clair *et al.* 1990; Millar *et al.* 2000). This translocation was found to directly affect the DISC1 gene – which codes for the major hub protein DISC1 – and the DISC2 gene – an antisense RNA specific gene. There has been a large body of evidence over the decade supporting DISC1 as a rare but highly penetrant risk factor for schizophrenia (Reviewed by Chubb *et al.* 2008). The DISC1 gene has been implicated in various cognitive deficits in schizophrenia including working memory. It has also been proposed that the DISC1 protein occupies a central hub position, prominent in synaptic processes (known as the DISC1-interactome) and that it

interacts with various other proteins in the regulation of cytoskeletal processes, intracellular transport and cell-cycle/division (Camargo *et al.* 2007). Some GWASs into schizophrenia have found SNPs and CNVs in genes that code for proteins that interact with the DISC1-interactome (Kirov *et al.* 2009; Need *et al.* 2009), indicating a probable network of proteins involved in schizophrenia pathology.

The most recent GWAS of 36,989 cases and 113,075 controls by the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC) identified 128 independent associations from 108 distinctive loci (Ripke *et al.* 2014). This is arguably the most successful GWAS of the major psychiatric disorders to date, yielding 83 previously undiscovered associations. The study confirmed a number of previously identified risk associations and new associations which are relevant to prominent theories of molecular aetiology and pharmacological treatment of schizophrenia. These include SNPs close to the DRD2 gene – the main target of psychotic drugs – which is involved in dopaminergic neurotransmission; GRM3, GRIN2A, and GRIA1 genes which are all components of glutamatergic pathways and synaptic plasticity; CACNA1C, CACNB2, CACNA1L, RIMS1 – components of calcium signalling; various genes involved in synaptic plasticity including KCTD13, NLGN4X, IGSF9B, CNTN4, and CNKSR2; ion channel genes – KCNB1, HCN1; neurodevelopmental genes like FXR1 and SATB2; and a component of the major histocompatibility complex (MHC) (Ripke *et al.* 2014 Hamshere *et al.* 2013;). A number of the genes identified which are involved in calcium channels, glutamatergic transmission and synaptic plasticity are associated with known rare CNV and *de novo* mutations (Purcell *et al.* 2014; Kirov *et al.* 2012). This points to a common source of pathology among common and rare genetic associations.

De novo mutations are of particular importance in delineating the non-inheritable aspects of schizophrenia too. Importantly, recent evidence has shown that offspring of older fathers have a higher chance of developing schizophrenia than younger fathers, and that paternal age at conception is positively correlated with *de novo* mutations expressed in their offspring (J. J. McGrath *et al.* 2014; Fromer *et al.* 2014). A recent study of 623 schizophrenia trios (parents and probands) found significant

enhanced enrichment of *de novo* mutations in specific synaptic genes, including ones associated with the NMDAR complex and the activity-regulated cytoskeleton-associated protein (ARC) complex. Both involved in regulating synaptic strength at glutamatergic synapses and may also be involved in cognition (Kirov *et al.* 2012). ARC is also involved in the regulation of synaptic pruning during development (Mikuni *et al.* 2013), which may be impaired in schizophrenia (Hayashi-Takagi *et al.* 2011). They also found significant cross-over between ASD and intellectual impairment for enriched CNVs; including two well established gene risk factors for ASD – SCN2A and POGZ Buxbaum *et al.* 2012). It was observed that these mutations are least severe in schizophrenia compared to ASD and intellectual impairment, and that the highest rate of loss of function mutations were correlated with the greatest intellectual cognitive impairments.

Although GWAS studies have identified a range of SNPs important in schizophrenia, there remains the possibility that other genetic variants are also important in disease mechanisms. In particular the case is strong if there is converging evidence from patient case control genetic association studies, neurobiological knowledge and post mortem tissue analysis. Two such genes that fall into this category and which are the subject of this thesis are *MAP2K7* and *FXYD6*

FXYD6 belongs to a group of proteins that are known to be involved in modulating NaKATPase activity (Béguin *et al.* 2001; Bibert *et al.* 2008; Crambert *et al.* 2005), where FXYD6 is mainly expressed in regions of the inner ear and the brain (Geering 2006, Kadowaki *et al.* 2004). There is some evidence that has indicated abnormal NaKATPase regulation within various psychiatric disorders including schizophrenia (el-Mallakh & Wyatt 1995; Renkawek *et al.* 1992, Rybakowski & Lehmann 1994). While some linkage studies have found positive associations for *FXYD6* and schizophrenia (Choudhury *et al.* 2006; Choudhury *et al.* 2007) and others have not (Iwata *et al.*, 2010; Zhang *et al.*, 2010); peripheral evidence still indicates that FXYD6 may potentially have an impact on the cognate aspects of schizophrenia. Western blots and in situ hybridisation studies have indicated expression of FXYD6 in the hippocampus and the prefrontal cortex (PFC), areas that show abnormal activity in

schizophrenia and are generally linked to higher cognitive functions (Kadowaki 2004). Furthermore, *FXYD6* is also located on area of 11q23.3 chromosome, a loci heavily implicated in schizophrenia (Grandy *et al.* 1989; Zhong *et al.* 2011). Abnormal FXYD6 expression may impair NaKATPase function in schizophrenia, and therefore, affect cognition in the PFC and hippocampal areas. As other psychiatric disorders show impaired function in NaKATPase activity, abnormal expression of FXYD6 may produce similar deficits and contribute to cognitive dysfunction in schizophrenia.

MAP2K7 has also been implicated as a risk factor for schizophrenia, particularly in cognitive symptoms. *MAP2K7* is a gene that expresses a kinase that is involved in the c-Jun N-terminal kinase (JNK) pathway, which is implicated in neuronal activity, receptor function and cortical & hippocampal plasticity (Winchester *et al.* 2014). A genetic association study found a substantial effect size for a common variant from case and control samples collected from Glasgow and Northern Europe, with a decreased expression of MAP2K7 in the PFC of post-mortem schizophrenia patients (Winchester *et al.* 2012). A study by Funk *et al.* also found decreased expression of MAP2K7 in ACC and DLPFC areas (Funk *et al.* 2012) and the antipsychotic, clozapine, has been implicated in positively regulating gene expression in the MAPK signalling pathway (Rizig *et al.* 2012). GWAS and linkage studies (Li *et al.* 2012; Ripke *et al.* 2014) have also identified a kinase - vaccinia-related kinase 2 (VRK2) - as a risk factor for schizophrenia that is involved in JNK signalling and which also interacts with MAP2K7 (Blanco *et al.* 2008).

This complex view of how rare and common mutations contribute to pathological traits by varying severity may be supported by theories from molecular systems biology. Researchers have found that certain proteins in a cell constitute major 'hubs' that interact with many other proteins in a crucial manner (Jeong *et al.* 2001). The deletion or malfunction of these hub proteins can have severe knock-on effects on the regulation and production of various other proteins and systems. This is what probably occurs with CNV and *de novo* mutations. On the other hand there are 'peripheral' proteins that cause less disruption when they undergo deletions or malfunctions. If one of these peripheral proteins is dysfunctional, the phenotypic

manifestation may be negligible. However, if many peripheral proteins are dysfunctional, impairments could be more severe. And if these mutated peripheral proteins surround a particular hub protein, it could produce similar problems seen by a single malfunction in the hub protein. This may explain why singular, rare, penetrant mutations and multiple-common, low penetrance mutations can amalgamate into very similar phenotypic traits. This notion of dysfunctional hub and peripheral nodes in molecular networks also runs in parallel with modern theories of dysfunctional neural and cortical networks giving rise to mental disorders. This could possibly constitute a 'level-up' amplification of network dysfunction from molecules to neural systems. Discussed in the following chapters are how abnormal structural and functional connectivity within and between cortical regions can give rise to the range of symptoms expressed in the major psychiatric disorders.

1.6.3 – Structural & functional connectivity

A relatively modern approach to studying and deciphering mental disorders is investigating the underlying neural circuitry that may contribute to them. This has been enabled in the wake of the development of research tools like functional magnetic resonance imaging (fMRI), positron emission tomography (PET), magnetoencephalography (MEG) electroencephalography (EEG) & diffusion tensor imaging (DTI). They are allowing researchers to more accurately probe the way that neurons, networks and brain locations communicate with each other; how their activity produces aspects of behaviour and cognition, and how their normal interactions are disrupted in certain brain disorders. This has allowed scientists to discover the multiple anatomical and functional circuitry components that interact and contribute to mental disorders – including abnormal local cortical activity; reduced intra and inter-cortical white matter connectivity; global grey matter abnormalities; hyper and hypo functional connectivity; and large-scale functional network problems (Sporns 2014). Furthermore, with the recent development of the highly sophisticated post mortem structural imaging tool – CLARITY – lies the potential to uncover the fine neural structure, anatomical connectivity and molecular properties of the brains of individuals affected by mental disorders in

intense detail (Chung *et al.* 2013). With this array of technologies, combined with the expansion of network-theory driven approaches to understanding circuit interactions – scientists are slowly uncovering intrinsic networks and neural groups that contribute to cognition and abnormal behaviours. Moreover some researchers are already beginning to utilise distinct circuit abnormalities that are proposed to be characteristic of specific mental disorders in order to develop novel neuro-imaging based diagnostic criteria. For example using abnormal hippocampal and thalamocortical oscillations during non-REM sleep as a biomarker for schizophrenia (Gardner *et al.* 2014), and using structural neuroimaging to identify patients of major depressive disorder who are likely to be resistant to conventional pharmacological treatment (Liu *et al.* 2012). Clearly, understanding of the network and circuit based aberrations that contribute to mental disorders remains one of the most important frontiers in clinical and investigative neuroscience.

Many researchers now recognise that the brain is organised into multiple scales of structurally and functionally connected networks which give rise to all behaviours, emotions and cognitive processes (Sporns *et al.* 2004; Power *et al.* 2011). These structural and functional networks are being delineated by technologies like diffusion tensor imaging (DTI) and fMRI in awake and cognitively alert humans, and – in the absence of evidence for major morphological or cellular abnormalities – has given rise to the view that the major psychiatric disorders are caused by abnormal connectivity among distributed cortical regions and large scale neural networks. This abnormal connectivity is usually characterised by problems with access, engagement and disengagement between functional networks and neural circuits, and can manifest as hyper or hypo-connectivity between regions (Menon 2011). These theories have moved on from previous ideas that mental disorders can be explained by localised abnormalities in single cortical regions; given that most mental disorders exhibit multiple symptoms spanning a diverse range of cognitive and psychological domains; which also express themselves in a variable manner over time.

1.6.3.1 – Measuring connectivity

Neural networks can be defined by their anatomical or their functional connections; where anatomical and functional connectivity do not always correlate. Anatomical connectivity is usually measured by DTI which quantifies global and local white matter projections between and within cortical regions *in vivo*. DTI determines structural connectivity by a number of measurements which are mainly quantified by white matter density and myelination. Functional connectivity is measured by the well-established neuroimaging technique functional MRI (fMRI). This technique measures temporal correlations in activity between cortical regions; implicating (but not proving) a functional connectivity between these regions. It has to be noted, however, that some studies will find strong correlation between structural and functional connectivity; where other studies will find a decoupling of these parameters. This is just one of many other contentious issues that surround connectivity studies, especially in psychiatric disorders. This is because in this area of research a huge variety of techniques and study designs are used, groups can use different parameters and definitions of the disorders that they wish to study, medication and onset of disease can also affect the results, and the technologies themselves produce inherit ambiguities and assumptions. Despite this certain patterns are emerging.

1.6.3.2 – Intrinsic connectivity networks (ICNs)

Recently researchers have been integrating graph theoretical approaches with structural and functional connectivity technologies to uncover large-scale brain networks. From extensive work into network activity correlation during task and resting states, a number of discrete functional neurocognitive networks (known as intrinsic connectivity networks (ICNs)), have been defined. The most robustly characterised is the default-mode network (DMN), a network which has been extensively studied in relation to psychiatric disorders (Fox *et al.* 2005; Greicius *et al.* 2003; Raichle *et al.* 2001). The DMN has been associated with mentation, social cognition and self-reflecting thought during quiet periods of relatively low salient stimulation (Blakemore 2008; Gusnard *et al.* 2001; Maddock 1999). The major

components of the network consist of the precuneus/posterior cingulate cortex (PCC), the medial prefrontal cortex (mPFC) and the medial, lateral and inferior parietal cortex (Greicius *et al.* 2003; Greicius *et al.* 2009; Gusnard *et al.* 2001). Activity in the DMN is strongest during quiet, rest periods (Raichle *et al.* 2001); it is inhibited but does not deactivate completely during tasks (Eichele *et al.* 2008) and shows a negative correlation in activity as task demand increases (Singh & Fawcett 2008). The study of this network has served as an anchor and baseline to investigate functional network pathology among many of the psychiatric disorders, where abnormal activation can indicate possible sources of positive symptoms (e.g. auditory hallucinations in schizophrenia), and the change in network reactivity during switching between rest and task states can unveil various conclusions on cognitive and emotional symptom aetiology. Furthermore, the discovery of the salience network (SN – a cingulo-opercular network involved in calculating the importance of, and then acting on, biologically and cognitively relevant stimuli. This network includes the dorsal-anterior cingulate cortex (dACC) and the insular cortex) (Seeley *et al.* 2007; Menon & Uddin 2010) and the central executive network (CEN – a frontoparietal network involved in executive functions. This network includes the dorso-lateral PFC and the posterior parietal cortex (PPC)) (Seeley *et al.* 2007; Bressler & Menon 2010) has shone light on how large ICNs may regulate one another in order for normal cognitive and psychological functioning, and a dysregulation in the balance of these networks can give rise to components of psychiatric disorders (van den Heuvel & Fornito 2014; Manoliu *et al.* 2014; Menon 2011). It is now being hypothesized that disequilibrium and aberrant conflict between the three major functional networks – DMN, SN and CEN – may serve as a major source of pathology in the major psychiatric disorders (Menon 2011) and that by identifying the properties of these networks, new methods of treatment and diagnosis can be developed. Described below are the current hypotheses and findings on the structural and functional connectivity of the major psychiatric disorders; how these abnormalities may exist within defined ICNs, and how these aberrant connections manifest as emotional, psychological and cognitive anomalies.

1.6.3.3 – Attention deficit hyperactivity disorder (ADHD)

The prevailing hypothesis for ADHD network pathology is major connectivity and structural deficits between and within the fronto-striatal-cerebellar system. (Bush 2010; Makris *et al.* 2009; Nakao *et al.* 2011). There is also growing evidence showing that ADHD patients exhibit marked global cortical thinning and distinct grey matter (GM) reductions in various anatomical locations including the dorsal attentional network, prefrontal regions, the cerebellum and the basal ganglia (Krain & Castellanos 2006; Batty *et al.* 2010; Proal *et al.* 2011). White matter abnormalities have also been observed in various studies of ADHD (van Ewijk *et al.* 2012), particularly between and within networks thought to be involved in attention and cognitive control. However, like many of the psychiatric disorders, there is much debate on the exact location and nature of the connective abnormalities. White matter alterations have been found in posterior parts of the corpus callosum – the isthmus and splenium, which connects bilateral parietal and temporal cortices (Dramsdahl *et al.* 2012); various fronto-striatal (Tamm *et al.* 2012) and thalamic-striatal connections (Xia *et al.* 2012), and the superior longitudinal fasciculus – a white matter tract that connects to all 4 lobes (Makris *et al.* 2008; Cubillo *et al.* 2010). A meta-analysis by Van Ewik *et al.* 2012, of 9 DTI studies into ADHD established five robust clusters of white matter abnormalities in ADHD. These were right anterior corona radiata, left cerebellar white matter; right and left interior capsule; and the right forceps minor (van Ewijk *et al.* 2012). Recent studies into white matter abnormalities in ADHD have sought to implicate specific tracts with behavioural and cognitive correlates, for example Shang *et al.* found significant correlation between white matter abnormality in the left orbitofrontal and ventrolateral tracts with severity of executive dysfunction (Shang *et al.* 2013). Wu *et al.* also found four frontol-striatal tract abnormalities in children with ADHD, where the left orbitofronto-caudate tract was associated with inattentive symptoms (Wu *et al.* 2014). Furthermore Soon-Beom Hong *et al.* found abnormal white matter connections between 23 different regions within frontal, striatal and cerebellar brain regions in ADHD patients; where a subset of these abnormal connections correlated with attentional deficits (Hong *et al.* 2014). They also found that many of the

abnormal connections are implicated in a number of established intrinsic networks including the DMN, dorsal and ventral networks and the limbic network.

Various fMRI studies have also found functional deficits in all the major lobes of the brain in ADHD (De La Fuente *et al.* 2013). Abnormal activation in ADHD patients has been observed in the orbital frontal cortex (OFC) - associated with behavioural inhibitory deficits in ADHD (Bush 2010); the dorsolateral PFC (DLPFC) – associated with sustained attention deficits in ADHD (Christakou *et al.* 2013), and impaired functional connectivity between fronto-striatal, fronto-parietal and fronto-fronto regions during set-shifting tasks (Cubillo *et al.* 2010). The dorsal-anterior cingulate cortex (dACC) also shows consistent abnormal activation in ADHD (Sun *et al.* 2012; Sato *et al.* 2012,Tian *et al.* 2006). This is significant as the dACC is thought to be extremely important in attention, motivation, inhibition and other key cognitive processes (Bush 2010). The dACC is also a key node of the cingulo-fronto-parietal (CFP) cognitive – attention network (De La Fuente *et al.* 2013). Furthermore, there is significant hyperconnectivity between the dACC and bilateral insula regions (Tian *et al.* 2006) – suggesting hyperconnectivity within the salience network. dACC – PCC connectivity is also impaired in ADHD, (Castellanos *et al.* 2008) which is a major component of the DMN. A recent study suggested that this may be due to a development delay or disruption of maturing brain networks, where ADHD adults showed impaired dACC-PCC connectivity compared to age-matched controls, but showed similar connectivity to young controls (Sato *et al.* 2012). Moreover a study of large-scale intrinsic networks of ADHD by Sripada *et al.* found that connections within the DMN and interconnections of the DMN with the frontoparietal and salience networks show a developmental lag compared to controls (C. S. Sripada *et al.* 2014a). This runs in parallel and compliments previous studies that found structural developmental delays in ADHD (Castellanos *et al.* 2002; Shaw *et al.* 2007), and may be a biological reflection of the coping mechanisms and effective treatment strategies that patients undergo as they mature. It is now being hypothesized by brain network researchers that attentional deficits in ADHD arise from impaired switching or increased interference between DMN and attentive networks like the SN and fronto-parietal network (Sonuga-Barke & Castellanos 2007; Castellanos &

Proal 2012; C. Sripada *et al.* 2014b). This is backed up by recent meta analyses and global connectivity studies that have identified impaired connectivity within particular regions of the DMN and between regions of the attentional networks in ADHD (Cortese *et al.* 2012; C. Sripada *et al.* 2014). These were namely between the posterior cingulate cortex in the DMN and the right anterior insula, a prominent component of the SN and a region linked to multiple other psychiatric disorders including anxiety disorders, depression and PTSD (Menon 2011; Paulus & Stein 2006; Sprengelmeyer *et al.* 2011; Hughes & Shin 2011).

1.6.3.4 – Post traumatic stress disorder (PTSD), major depressive disorder (MDD) and obsessive compulsive disorder (OCD)

Some researchers have suggested that hypervigilance and inhibited fear-extinction in PTSD pathology arises from a network failure to change from threat-sensitive SN to the DMN during periods of rest and low salient stimulation (Hughes & Shin 2011). This is formed from extensive evidence in neuroimaging and anatomical studies of PTSD patients compared with unaffected traumatised patients and untraumatised controls. General regional abnormalities in PTSD are all major components of the SN or DMN and exist in the form of hyperactivation in the amygdala and insular regions (Hughes & Shin 2011); abnormal activity in the dACC (Kennis *et al.* 2014); with reduced hippocampal and medial prefrontal cortex volumes (Hughes & Shin 2011). Furthermore major connectivity abnormalities exist in these components of the SN and DMN including reduced connectivity between the amygdala and putamen (Linnman *et al.* 2011); over-activity between the amygdala and insula (Rabinak *et al.* 2011); and an abnormal heightened connection between the basolateral amygala region and the pregenual anterior cingulate cortex (Brown *et al.* 2014). These abnormalities in functional connectivity between and within major intrinsic networks have also been found in MDD where it has been hypothesized that impaired switching between DMN and CEN may be correlated with enhanced negative thinking and rumination in patients (Manoliu *et al.* 2013; Hamilton *et al.* 2011). It is thought that these problems from switching from negative, rumination to task directed behaviour may be regulated by abnormal anterior insular function

(Sridharan *et al.* 2008; Manoliu *et al.* 2013), which is a characteristic abnormality seen in most MDD cases (Hamilton *et al.* 2013; Avery *et al.* 2014). Lastly, while circuit abnormalities in OCD have mostly been observed in corticostriatal regions (Beucke *et al.* 2013; Harrison *et al.* 2013) some studies have related the intense, repetitive negative ruminations expressed in OCD as problems in DMN connectivity and an intrusive DMN during cognitive tasks. (Beucke *et al.* 2014; Stern *et al.* 2012). However, this may be a secondary effect of poor corticostriatal circuitry where the DMN and CEN are affected further down the line.

1.6.3.5 – Autism spectrum disorder

ASD show great pathologies in structural and functional connectivity of the brain, which reflects the disorder's extensive and complex cognitive and behavioural pathologies. Structural aberrations include abnormally high volumes of grey and white matter (Amaral *et al.* 2008); abnormal cortical cytoarchitecture (Casanova & Trippe 2009) and disorganised white matter tracts (Barnea-Goraly *et al.* 2004). Furthermore, some researches have proposed that ASD patient's impaired ability to integrate multiple modes of cognition into a central process (known as central coherence) is to do with an abnormally high level of local connections and a distinct lack of long range, intercortical projections (Keller *et al.* 2007; Belmonte *et al.* 2004; Brambilla *et al.* 2003). Researchers proposed that this leads to highly specialised local networks which interact with each other much less than in normal subjects; leading to enhanced perceptual performance and abnormally intense focus on details in ASD. However, a recent review by Hoppenbrouwers *et al.* showed that local cortical connections are also reduced in patients with ASD (Hoppenbrouwers *et al.* 2014). They reported a presence of greater cortical integrity in very young ASD patients which may switch during synaptic pruning as the individuals develop. These local and global structural abnormalities however, have also been independently reflected in functional connectivity studies of ASD; particularly in the insula, inferior frontal gyrus, and the MTL, PFC and PCC components of the DMN (Menon *et al.* 2011). For example, Washington *et al.* (Washington *et al.* 2014) found distinctive reduced connectivity between major components of the DMN in an ASD group but

found increased local activity within the DMN nodes. They found that the long range projections across DMN nodes did not develop properly during adolescence in ASD subjects compared to controls. Furthermore Jung *et al.* found reduced connectivity between the anterior mPFC and PCC components of the DMN; which was associated with increased level of autistic traits in ASD subjects (Jung *et al.* 2014.). However, research from Lynch *et al.* found hyperconnectivity between the PCC and temporal cortex – major nodes in the DMN – arguing that this hyperconnectivity may be responsible for the social-cognitive deficits in ASD (Lynch *et al.* 2013). Other studies have also reported intra and inter cortical hyperconnectivity throughout the brain including striatal hyperconnectivity (Di Martino *et al.* 2011); posterior hyperconnectivity – related to symptom severity (Keown *et al.* 2013); and global hyperconnectivity (Supekar *et al.* 2013). This highlights the complexity of understanding the functional and structural abnormalities in ASD, where it may be more likely that the DMN goes through periods of hypo and hyper connectivity; or it may be determined by the severity and stage of the disorder. However, the hypo/hyper connectivity theory does not serve to explain the impaired white matter cross cortical connections observed in ASD groups. The anterior insula, a crucial node of the SN, also shows significant hypoactivity in ASD individuals where this abnormal activation may be responsible for ASD patient's reduced attention to social stimuli (Menon & Uddin 2010). Moreover a recent study by Uddin *et al.* found less prominent differentiation between DMN, SN and CEN when undergoing cognitive flexibility tests. (Uddin *et al.* 2014). They also found significant hyperconnectivity between these three ICNs in a previous study; arguing that this hyperconnectivity between regions blunts ASD patient's cognitive flexibility (Uddin *et al.* 2013). This research supports the growing theory of aberrant switching mechanisms between major ICNs in ASD, where dysfunctions in switching from one cognitive network to another can produce severe challenges for an ASD individual's ability to switch to another task; and deal with the task in an effective manner.

1.6.3.6 – Bipolar disorder

Structural and functional connectivity studies of bipolar have produced somewhat variable and inconsistent results over the past two decades. This is due to small sample sizes in studies, the fluctuating nature of the disorder, the subjective means of diagnosis and the high incidence of comorbidity with other disorders. For example, a recent meta-analysis of 8 studies into grey matter volume of bipolar patients found significant reductions in the left vPFC, insula, temporal cortex and claustrum (Selvaraj *et al.* 2012). Whereas an earlier voxel –based morphometry (VBM) study of bipolar patients found increased volumes in the PFC and other areas, with no reductions observed (Adler *et al.* 2005). Furthermore a longitudinal study of bipolar patients over 4 years found significant reductions in hippocampal volume but not in the PFC (Moorhead *et al.* 2007), highlighting the conflicting evidence for structural abnormalities in bipolar disorder. Studies into white matter connectivity in bipolar disorder have also produced conflicting results, with some studies indicating decreased white matter connectivity in the uncinate fasciculus, anterior thalamic radiations, and cingulum (Emsell *et al.* 2013; Lin *et al.* 2011; McIntosh *et al.* 2008), and others indicating significantly increased white matter density in the uncinate fasciculus (Houenou *et al.* 2007; Torgerson *et al.* 2013). These inconsistent results may be down to the relatively small sample sizes used in each of these studies and differing study designs. Recent large scale studies and meta-analyses have sought to limit these discrepancies and find commonality in white matter studies of bipolar disorder. A meta-analysis of ten whole brain DTI studies in bipolar disorder by Vederine *et al.* (Vederine *et al.* 2011) – indicated significant reductions in white matter in the areas of the right parahippocampal gyrus, the right ACC and the right subgenual cingulate cortex (SgCC). However, the largest white matter tractography study into bipolar disorder to-date by Sarrazin *et al.* (Sarrazin *et al.* 2014) did not find white matter reductions in these areas. They did however, find significant reductions in the body and splenium of the cingulate cortex, the left cingulum and the anterior part of the left arcuate fasciculus. They also found that reductions in the body of the cingulate cortex were more significant in bipolar disorder patients whom expressed psychotic symptoms. This large-scale tractography study supports previous

indications of the connectivity impairments in the cingulate cortex in bipolar disorder, but stands in direct contrast to what hemisphere these WM reductions are found compared to the meta-analysis by Vederine. This highlights the greater need for larger, whole brain WM studies into bipolar disorder, with the use of much larger sample sizes and more consistent techniques across studies, before any real conclusions can be drawn on white matter abnormalities in bipolar disorder.

MRI and fMRI studies into bipolar disorder have endeavoured to discover the local cortical activity and intercortical connectivity which contribute to symptomatology. Current theories propose a hypoactivity in a dorsal system including the dorsolateral PFC, ventrolateral PFC, dACC and the hippocampus. This system is involved in attention, executive function and regulation of emotional states and is thought to interact with a hyperactive ventral system. This hyperactive ventral system includes the amygdala, insula, ventral striatum, ventral ACC and the medial orbitofrontal cortex - which is thought to be involved in mediating autonomic emotional reactions, detecting emotionally relevant stimuli and producing emotional responses (Keener & Phillips 2007; Phillips *et al.* 2008). It is hypothesized that abnormal connectivity between the dorsal and ventral systems may account for unstable mood and cognitive deficits in bipolar disorder. (Kurtz & Gerraty 2009; Wessa *et al.* 2014; Strakowski *et al.* 2012). Recent resting state connectivity studies are beginning to provide evidence to back up this hypothesis and are also unveiling other network connectivity impairments that may contribute to the disorder. A resting state connectivity study of 35 bipolar disorder patients by Mamah *et al.* 2013 found decreased connectivity between cingulum and cerebellar networks; cerebellar and salience networks, and decreased connectivity within the cingulum networks (Mamah *et al.* 2013). This hypoactivity in the cingulate cortex was also backed up by a resting state study by Lui *et al.* whom found hypoactivity within the cingulate cortex and orbito-frontal cortex regions from a group of 57 bipolar disorder patients (Lui *et al.* 2014). Furthermore another resting state study found abnormal connectivity between the ACC and components of the DMN, and components of the SN. It has been hypothesized that this impaired ACC to DMN and SN connectivity could provide an abnormal balance between DMN and SN activity which could

account for the abnormal and prolonged periods of depression and mania, respectively (Magioncalda *et al.* 2014). However, it must be noted that, a review by Vargas *et al.* in 2012 into resting state functional network studies in bipolar disorder did not find consistent connectivity markers among the investigations, but did conclude that each investigation did find some abnormal connectivity between the dorsal-ventral (cortico-limbic) systems (Vargas *et al.* 2013). This suggests a more complex pathology of cortico-limbic connectivity which isn't just characterised by hypo or hyper connectivity; reflecting the dynamic nature of bipolar disorder.

1.6.3.7 – Schizophrenia

Schizophrenia is thought to represent one of the most complex of psychiatric disorders with respect to structural and functional brain network impairment and obtaining reliable and robust evidence in network structure is problematic for various reasons. The prevailing hypothesis over the past two decades is that schizophrenia arises from abnormal cortical connectivity (Friston & Frith 1995; Kochunov & Hong 2014) and that abnormal lifetime white-matter development is partly responsible for aberrations in functional connectivity. Various studies and reviews with chronic patients have reported that the most common reductions in white matter discovered in schizophrenia are in the frontal and temporal lobes, and in the connections that link these two regions; including the uncinate fasciculus, the cingulum bundle, the corpus callosum and the internal capsule (Pettersson-Yeo *et al.* 2011; Scheel *et al.* 2013; Walther *et al.* 2011; Wheeler & Voineskos 2014). Other studies, including a meta-analysis of 15 DTI studies, have found severe reductions in the left temporal and left frontal deep white matter (Ellison-Wright & Bullmore 2009), and in the left posterior parieto-occipital cortex and left frontal lobe (Pettersson-Yeo *et al.* 2011; Scheel *et al.* 2013; Walther *et al.* 2011; Wheeler & Voineskos 2014). Other studies, including a meta-analysis of 15 DTI studies, have found severe reductions in the left temporal and left frontal deep white matter (Ellison-Wright & Bullmore 2009); and in the left posterior parieto-occipital cortex and left frontal lobe (Nazeri *et al.* 2013). However, there are many other studies which have failed to find white matter abnormalities in chronic schizophrenia. For

example a review by Kanaan *et al.* found that out of 15 DTI studies investigating the same region of interest in schizophrenia; the same number of studies found no difference as those whom did find a difference (Kanaan *et al.* 2005). However, these inconsistencies have been blamed on the restrictive nature of region of interest studies. A global meta-analysis by Bora *et al.* with chronic schizophrenia patients sharpened the evidence and found reduced white matter only in interhemispheric fibres, anterior thalamic radiation, inferior longitudinal fasiculi, inferior frontal occipital fasciculi, cingulum and fornix (Bora *et al.* 2011).

However, there are still a number of possible confounding factors that may make chronic schizophrenia studies unreliable. These include the impact that long-term use of antipsychotics and other drugs may have on patient's brains; along with long-term effects of traumatic life-experiences and other long-term developments of a schizophrenia patient's brain. This is why several studies have focused on first-episode patients of schizophrenia and unaffected relatives where potential early white matter abnormalities can be identified and risk factors for unaffected individuals may be traced *before* medications and life experiences can have an impact. Generally, the positive findings of first episode studies have shown more global and diffuse aspects of white matter abnormalities in the frontal, parietal and temporal lobes compared to specific tracts seen with chronic studies (Fitzsimmons *et al.* 2013; Wheeler & Voineskos 2014). For example, a recent meta-analysis including 271 first-episode patients and 297 controls by Yao *et al.* reported significant white matter reductions in the right frontal and left temporal lobes in all first-episode patients (Yao *et al.* 2013). Another study by Filippa *et al.* found decreased grey matter volumes in the temporal, parietal and occipital regions, but also found increased white matter connectivity in the brain stem, cerebellum, interhemispheric and cortico-cortical connections, and the right anterior and posterior limb of the internal capsule (Filippi *et al.* 2014). A study into neuroinflammation and axonal degeneration of first-episode patients found that inflammation was wide spread in the white matter and grey matter of patients, but axonal degeneration was found only in central areas of the frontal lobe (Pasternak *et al.* 2012). Adding to the complexity of these findings is the range of studies that have found no white matter

abnormalities in first episode patients (Friedman *et al.* 2008; White *et al.* 2011; Zou *et al.* 2008). The studies which featured chronic patients did show white matter abnormalities compared to first-episode and controls, suggesting that either white matter abnormalities do develop as part of the disorder; or that medication and environmental factors do make an impact on white matter over a patient's lifetime. The lack of robust and replicable evidence for first-episode patients may be down to the very small sample sizes of the studies, as first-episode and unmedicated patients are much harder to recruit. Therefore, increasing sample sizes and large scale collaborations will likely lead to more robust and reliable findings on white matter integrity of early-onset schizophrenia.

Network theorists have been using structural based white matter studies to understand the network-based malfunctions that may exist in schizophrenia. A combined resting state functional imaging and DTI study by Skudlarski *et al.* 2010 discovered abnormal structural and functional connections in 27 chronic patients compared to 27 controls (Skudlarski *et al.* 2010). They found reduced connections originating from the lingual and cingulate gyri to the left inferior parietal lobule, middle and superior temporal gyri and inferior frontal gyrus. Four pairs of these showed significant functional connectivity aberrations; where three exhibited hyperconnectivity: anterior cingulate gyrus and ventromedial frontal gyrus to ventromedial gyrus, and the thalamus to left post central cortex. And one pair showed hypoconnectivity: the left middle temporal gyrus to postcentral gyrus. Furthermore they found significant functional hyperconnectivity and structural connectivity aberrations within components of the DMN and task positive networks (TPN – regions of the brain that are generally active when a patient is engaged in a task). Suggesting that structural and functional connectivity may be decoupled in some instances. An axonal fibre network study by Zalesky *et al.* 2011 found impaired connectivity in nodes of the medial frontal lobes, parietal/occipital lobes and the left temporal lobes (Zalesky *et al.* 2011). They also described sparser cortex interconnection with 20% less efficiency in schizophrenia patients. A structural network study into first-episode patients by Zhang *et al.* also found distinct interegional aberrations in connections including the fronto, parietal and occipital

regions; with increased path-length between regions and decreased network efficiency. They found the networks exhibiting decreased efficiency were mostly from the top-down sensorimotor, basal ganglia and limbic-visual systems and that psychotic symptom severity was negatively correlated with sensorimotor network efficiency.

Abnormal functional connectivity problems are also widespread and prominent in schizophrenia, many located in the prefrontal regions (Cole *et al.* 2011; Fornito *et al.* 2012; Fornito *et al.* 2013). Fronto-parietal networks associated with cognitive control systems (Repovs *et al.* 2011); the cingulo-opercular system involved in salience (Palaniyappan *et al.* 2013); and fronto-striatal and fronto-temporal systems (Dandash *et al.* 2014; Hoffman *et al.* 2011). Recent theories have also implicated a functional imbalance between DMN and CEN ICNs, caused by abnormal functioning of the SN – which is thought to control DMN – CEN switching (Menon 2011; Nekovarova *et al.* 2014; Manoliu *et al.* 2014). This has come from various lines of functional and structural studies implicating these networks in the positive, negative and cognitive aspects of schizophrenia. For example White *et al.* 2010 found aberrant SN connectivity, along with impaired SN interconnectivity with the DMN and CEN in schizophrenia patients undergoing a tactile sensation task (White *et al.* 2010). Furthermore a resting state study by Camchong *et al.* 2011 found impaired functional and anatomical connectivity in the ACC and the mPFC component of the DMN; where aberrant mPFC functional connectivity was positively associated with cognitive deficits and psychotic symptoms in schizophrenia (Camchong *et al.* 2011). Guo *et al.* found similar abnormal activity in the DMN of unmedicated first-episode patients; where hyperactivity in the PCC was observed along with hypoactivity in the mPFC. This hypoactivity was associated with intrusive thoughts and hallucinations in patients (Guo *et al.* 2014). Kasparek *et al.* 2013 found SN and DMN activation impairment in remitted first-episode schizophrenia patients during a verbal fluency task (Kasparek *et al.* 2013).

Moran *et al.* found that the right AI activity was disrupted in schizophrenia and that this served to impair CEN & DMN function. They found that this right AI disruption

was a strong predictor of cognitive dysfunction in patients (Moran *et al.* 2013). Other studies have implicated ICN dysfunction with cognitive impairment in schizophrenia. For example, a meta-analysis of 41 fMRI studies into functional connectivity and executive function in schizophrenia observed distinct impaired PFC and ACC activity which was related to executive dysfunction in patients (Minzenberg *et al.* 2009); where altered ACC activity of the salience network may induce abnormal activity of the DMN network – therefore impairing cognitive performance. Abnormal parietal and dorso-lateral prefrontal cortex activity, regularly observed in schizophrenia, has also been associated with working memory impairment in the disorder – implicating the CEN in working memory impairment of schizophrenia (Barch & Csernansky 2007). Orliac *et al.* also showed impaired DMN and SN connectivity where SN dysfunction was correlated with depressive and delusional symptoms and altered activity of the frontal regions of the DMN which was associated with dysfunctions in abstract thinking (Orliac *et al.* 2013).

1.7 – Animal models of relevance to psychiatric disorders

One of the major (if not *the* major) limitations in all aspects of biomedical research are the technical and ethical implications for researching physiological and disease mechanisms in alive humans. In our pursuit of directly understanding the neurobiological processes that contribute to our behaviours and experiences we are currently limited to studying humans with accidental brain lesions, high penetrating genetic mutations; using relatively low-spatial resolution neuroimaging technologies, and low-spatial resolution neural stimulation technologies like transcranial magnetic stimulation (TMS). There has been progress in furthering technology that can noninvasively manipulate the activation of specific brain areas in alert humans (see transcranial focused ultrasound studies (Bystritsky *et al.* 2011; Legon *et al.* 2014; Tyler *et al.* 2008)). Currently this technology does not extend to specific neural subtypes and circuits, a spatial resolution that really is needed to fully understand neural functioning *in vivo*. Because of this - with respect to psychiatric disorders where no obvious genetic or morphological aberrations have been found - a large

component of neuropsychiatric research is focused on creating and studying animal models that are relatively translatable to aspects of mental disorders.

Currently animal models in psychiatric disorders are mainly studied in rodent paradigms. This is due to their relatively low cost to maintain, the abundance of well-established behavioural and cognitive tasks available; a well-established knowledge of neurological and anatomical workings of the rodent CNS; the availability of transgenic mouse models, and the relative homology of the CNS to ourselves (although this is a subject of intense debate). This has allowed neuroscientists to manipulate specific genes, neurons and cortical locations in a highly controlled spatial and temporal manner; that enable us to uncover molecular and neural functioning in an unprecedented manner, and is serving as a useful platform for drug discovery in treating mental disorders. These discoveries are made available by technologies like transgenic mouse models, electrophysiology, optogenetics and pharmacological techniques. Aside from the clear ethical arguments about *in vivo* research with animals, there are other enormous challenges and limitations that come with modeling psychiatric disorders in other animals. This is down to the obvious problems in translating complex, multi-symptom, human conditions like depression and schizophrenia into rodent models; where finding rodent analogs for something like auditory hallucinations can be immensely challenging. Trying to find homologous brain regions in rodents is also challenging, (as the human brain has a markedly larger surface area and encephalization quotient), but important core regions that are relevant to mental disorders are shared among rodents and humans – e.g. the amygdala and hippocampus. Animal welfare is another key issue that highlight logistical and ethical problems that must be considered in the design of animal studies. For example it is known that certain types of environmental variables (e.g. laboratory noise – (Lauer *et al.* 2009)) and human contact (e.g. human male scent – (Sorge *et al.* 2014)) has shown to induce stress and abnormal physiology in rodents. If one wants to achieve robust and reliable results that are translatable to human research in psychiatric disorders, laboratories must strive for the animals to be at base-line physical *and* mental health levels. In a laboratory setting this, of course, may be hard to control for. But with appropriate foresight into experimental

design and consideration into environmental factors that affect animal mental health – it could be achievable. This further highlights the need for a constant revision of animal models that are subject to appropriate levels of scrutiny, in order to ensure the animal sacrifice in neuroscientific research provides the most robust and reliable results that can positively guide knowledge of psychiatric disorders.

1.7.1 – Measuring validity in animal models

Because of the subjective criteria that define mental disorders and the number of exclusively human phenomena that arise from them, most animal research is focused on investigating distinct components and sub-groups *of* symptoms. Allowing for the detailed probing of molecular, genetic and neural subsystems that contribute to distinct neurological phenomena, and provides an important apparatus in the pursuit of drug discovery. Although, given the inherit uncertainties and variables that come with animal models, in order for researchers to draw robust conclusions, the model must have certain validity in representing the specific disorder of interest. According to McKinney and Bunney (McKinney & Bunney 1969) an animal model is more relevant and relatable to human disorders when it satisfies as many specified parameters as possible. These parameters are aetiology, symptomatology, treatment response and pharmacological basis. There are a number of descriptors and parameters used to quantify a model's validity including face validity, construct validity, aetiological validity and predictive validity. Face validity describes how closely a symptom in the model resembles the clinical symptom being studied; but does not need to arise from the same underlying mechanism. Construct validity describes how closely the biological mechanism in the model resembles the biological mechanism being studied. Aetiological validity describes how similar the triggers are that give rise to behavioural or biological abnormalities in animal models and humans. Predictive validity describes how well performance in an animal model test can predict performance in a human clinical test, for example drug efficacy. Not only do these measures of validity provide a nomenclature for describing the validity of an animal model, but they also serve as a framework to guide the purpose of a study utilizing animals for their investigation.

For example, an investigator studying how well a drug will produce anti-depressive effects in humans using an animal model; the researcher would most likely want to have a *high predictive validity* in the study. There are numerous methodologies and approaches to investigating schizophrenia using rodent models; each with varying levels of validity in each definition. These varying levels of validity also determine how analogous a model is to the human condition being investigated. A homologous model would describe a model that has identical causes and symptomatology to the human disorder. However, within psychiatric disorders this is challenging, for reasons described above. Animal models with relevance to psychiatric disorders are more likely to be analogous, where only a small subset of the disorder is studied and the aetiology of the symptom is most likely different from the human disorder. Indeed, breaking down and investigating singular components of psychiatric disorders like working memory deficits; set-shifting abilities, and rumination; can provide valuable insights that could serve mental illness research well and neuroscience as a whole. For example, by investigating genetic and neural components of working memory in mouse models, one may be able to find a common neurobiological aetiology for working memory deficits across the spectrum of psychiatric disorders whom express working memory impairments. Breaking down the symptoms enables researchers to focus in on specific neurobiological aspects of psychiatric disorders and provides a foundation to build up a greater understanding of their aetiology, rooted in biological mechanisms. With animal models pursuing the biological aetiology of schizophrenia, various approaches have been taken, mainly from a behavioural, pharmacological or genetic perspective, with varying validity. Described below are various animal models used to decipher schizophrenia symptomatology and aetiology, the limitations that exist within them and also the value that they have in constructing a robust understanding of schizophrenia.

1.7.2 – Validity parameters in rodent models of relevance to schizophrenia

In order for animal models to be effective there must be measurable factors that validate the presence of properties that are akin to symptoms of the disorder

studied. With a multi-faceted disorder like schizophrenia this means there are a number of methods researchers use to validate the presence of schizophrenia-like symptoms in rodent models. These include electrophysiological, cognitive, locomotor, sensory discrimination and negative symptom indicators (Pratt *et al.* 2012). For example, EEG signals of sensory processing act as an electrophysiological indicator for components of schizophrenia in rodent models. This is because patients of schizophrenia have trouble detecting a change in an auditory environment, this can be detected by measuring event related potentials (ERPs) from EEG signals. It has been found that rodents exhibit analogous ERPs to humans, and therefore, exhibit analogous pathological signals to schizophrenia, and react similarly to pharmacological influence (Ehrlichman *et al.* 2008).

Cognitive deficits in rodent models can also indicate validity. Working memory can be measured by rodent behavioural apparatus like the Morris Water Maze (Vorhees & Williams 2006) and the Radial Arm Maze (Olton 1987). Novel object recognition tasks in rodents have also been used as analogs to schizophrenia deficits in visual learning and object recognition (Redrobe *et al.* 2010). However, there is much debate on how translatable many of these tasks are to human cognition (working memory tests discussed later). In order to provide valid and translatable cognitive tests for rodents, a number of factors need to be kept in mind when designing experiments. First of all, rodents do not share the exact same cognitive abilities as humans (for example the phonological loop in humans), meaning specific neuroanatomical and cognitive differences must be considered when designing cognitive tests. Furthermore, humans and other animals may utilise different neural systems for common cognitive constructs. Highlighting the challenges researchers have when deciphering analogous neural circuits and pursuing novel drug efficacy from animal behavioural paradigms. And finally, humans and animals may recruit different success strategies and components of cognition in order to complete behavioural tests.

Antipsychotic efficacy can also be measured in rodent locomotor paradigms by quantifying hyperactivity, and stereotyped grooming and sniffing following

antipsychotic administration. In effect, abnormal locomotor response to dopamine agonists or antagonists in rodents serves as a behavioural analogue to abnormal dopamine transmission seen in schizophrenia (van den Buuse 2010). Prepulse inhibition (PPI) is a phenomenon that happens when a small stimulus preceding a strong stimulus within 100ms produces a markedly reduced startle response than if there was no small prestimulus. This PPI response is expressed in various mammals - including rodents - and is also impaired in the majority of schizophrenia patients and their relatives (Braff *et al.* 1978; Cadenhead *et al.* 2000). Therefore, a component of validity can be measured by a rodent's PPI scale (Geyer *et al.* 2001), this is usually done by measuring a rodent's startle response by piezoelectric motion sensors in a tight cylindrical holder.

Negative symptoms of schizophrenia have been measured by a number of behavioural paradigms that have been designed for measuring anxiety and depression analogs. These include the sucrose preference test – a behavioural paradigm that may measure anhedonia in rodents; the elevated plus maze for measuring anxiety, and social functioning tests that quantify interspecies social interactions (Neill *et al.* 2010). However, these tests do not tap into a key feature of negative symptoms – amotivation – and hence it has been suggested that tests such as the progressive ratio task would show better translation to the human condition (Foussias and Remington 2010; Pratt *et al.* 2012).

Finally, local activity and functional connectivity can be measured in rodent brains by fMRI and 2-Deoxy-D-glucose imaging (2DG). Recent fMRI studies into anaesthetized mouse and rats brains are beginning to thoroughly characterise their resting state functional connectivity, with some analogs of human networks being identified (Sforazzini *et al.* 2014; Guilfoyle *et al.* 2013; Jonckers *et al.* 2011). 2DG imaging is a technique that is analogous to PET studies of brain metabolism which measures blood flow and energy metabolism in human subjects. 2DG imaging measures brain activity indirectly by quantifying the amount of glucose uptake in certain regions of the post-mortem brain. This technique takes advantage of 2DG's inability to undergo glycolysis when taken into the brain and has been thoroughly utilised for functional

activity studies of rodent models of schizophrenia (Dawson *et al.* 2013; Duncan *et al.* 1999; Miyamoto *et al.* 2000).

1.7.3 – Rodent models of relevance to schizophrenia

Along with quantifying validity in rodent models of schizophrenia, the other challenge is how to induce components of schizophrenia symptoms and neurobiological pathologies in rodents. Methods of inducing schizophrenia analogs are usually based on previous observations of neurobiology and pharmacological interactions in patients and are also implicated to test a hypothesis observed from human studies. Rodent models in schizophrenia predominantly manifest from developmental, genetic, lesion and pharmacological based manipulations (Pratt *et al.* 2012).

1.7.3.1 – Developmental

There is growing evidence to suggest that a major component of schizophrenia risk comes from early developmental problems during gestational and perinatal periods. These risk components arise from maternal stress, malnutrition, infection, early-social isolations and obstetric complications among others (Cannon *et al.* 2002; Lewis & Levitt 2002). Researchers have tested these hypothesis in rodent models by inducing a variety of abnormal developmental paradigms. These include post-weaning social isolation (Fone & Porkess 2008); administration of DNA alkylating agent methylazoymethanol to gestating foetus (Lodge & Grace 2008); prenatal viral or bacterial infection in rodents (Boksa 2010), and neonatal ventral hippocampal lesions (Tseng *et al.* 2009). These models have all produced valid bio-markers of affect which may be analogous to components schizophrenia; including social and cognitive impairments, sensory discrimination problems and abnormal reactivity to antipsychotics and amphetamines. However, none of the models have led to new therapies, most likely owing to the relatively low construct validity of the methods.

1.7.3.2 – Lesions

Lesion studies have also been used in specific cortical areas to reflect the impairments in cortical functioning exhibited in schizophrenia. These are done using electrophysiological, pharmacological, surgical or optogenetic methods (Lipska & Weinberger 2000). While pharmacological methods do allow for more specific control of anatomical lesions there is still limited construct validity in these models. This is because schizophrenia and other psychiatric disorders are now understood to manifest from distributed network impairments around the brain which have complex interactions of hyper and hypo activity. More sophisticated manipulation of multiple brain regions, informed from human schizophrenic network-based information, may be a source of development for lesion studies in schizophrenia. However, electrophysiological and optogenetic manipulation may provide more adaptable and less permanent solutions to modelling network dysfunctions.

1.7.3.3 – Genetic

With the introduction of large-scale genome wide studies of psychiatric disorders and the development of sophisticated transgenic mouse lines, scientists are now able to express specific genes of interest in mouse models and study their neurobiological and behavioural effect. Inserting or a manipulating a desired gene can be done in a number of ways including the 'cre/loxp' system, gene knock-in and knock-out systems using DNA vectors, and various methods of selective breeding programs. This gene manipulation paradigm allows researchers to validate findings from human genome studies; to understand the neurobiological and behavioural products of the gene, and to study gene-environment and gene-drug interactions. Many investigations have used genetic mouse models to understand how a gene of interest will produce behavioural and neurobiological analogs of schizophrenia including DISC1 (Ayhan *et al.* 2011), RELN (Laviola *et al.* 2009) and 22q.11.2 (Paylor & Lindsay 2006) gene studies. This study aimed to investigate identified potential schizophrenia gene risk factors, *FXYD6* and *MAP2K7* genes, using transgenic mouse models and neurobiological/behavioural validity markers.

1.7.3.4 – Pharmacological

Pharmacological models of schizophrenia allow researchers to probe the characteristic abnormalities of neurotransmitter and receptor expressions in schizophrenia, in a short term reversible paradigm. The two major methods of pharmacological investigation utilise either NMDA receptor antagonists, or psychostimulants that increase synaptic DA levels which stem from the glutamate and dopamine hypotheses of schizophrenia respectively. Psychostimulants like amphetamine are known to increase synaptic dopamine levels in humans (Laruelle *et al.* 2014), produce schizophrenia like positive symptoms in healthy humans, and also exacerbate symptoms in schizophrenia patients (Allen & Young 1978). NMDA-receptor antagonists, like ketamine and PCP, are known to produce schizophrenic-like positive, negative and cognitive symptoms in healthy humans. However, only the positive symptoms can be reversed using antipsychotics (Frohlich & Van Horn 2014). Furthermore, subanaesthetic ketamine and PCP tend to exacerbate symptoms in schizophrenia patients (Lahti *et al.* 1995; Lahti *et al.* 2001; Malhotra *et al.* 1997). Therefore, by using pharmacologically active compounds that manipulate the dopaminergic and glutamatergic system in rodents, researchers are able to mimic (to some extent) the neurobiological and the behavioural aspects of schizophrenia. This study takes advantage of this model by utilising ketamine to compare the profile of a genetic risk factor (using *Fxyd6*-/- mice) to that of ketamine upon regional brain activity but also to probe the role of NMDA receptors in *Fxyd6*-/- mice.

1.8 – Investigating the neurobiology of genetic risk factors and brain circuitry of schizophrenia

Of all the psychiatric disorders, schizophrenia exhibits some of the most numerous, complex and debilitating symptoms. Because the disorder affects around 1% of the human population (Andreasen 2000), the human and economic costs for society are huge. It has been reported in 2012 that the total societal cost for England alone is around £11.8 billion per year (Schizophrenia Commission 2012). Hence the need for a much greater understanding of this uniquely human disorder.

Patients with schizophrenia suffer from a number of impairments in their cognitive, emotional, perceptual and social functions. These symptoms are divided into three groups known as positive, negative and cognitive symptoms (see section 1.6.1 for further discussion). Positive symptoms constitute experiences out with normal perception including hallucinations (auditory, visual, olfactory and gustatory), disorganised speech and catatonic behaviour. Negative symptoms are mainly an attenuation in normal functioning like emotional experiences. These include impaired fluency of speech and thought (alogia), impaired motivation (avolition), reduced emotional expression (affective flattening) and social withdrawal. And as described above, cognitive deficits of schizophrenia span the whole range of cognitive domains, and are usually severe. These include deficits in working memory, episodic memory, cognitive flexibility, future planning, sustained attention, theory of mind, and language. All these symptoms can have a devastating impact on the lives of individuals with schizophrenia who have a larger risk of unemployment, homelessness, substance abuse, poor physical health, poor education and criminality compared to healthy individuals (Schizophrenia Commission 2012).

1.8.1 – Neurobiological hypotheses of schizophrenia

From decades of research into the neurobiological and molecular causes of schizophrenia the dopamine hypothesis, glutamate hypothesis and – more recently – the serotonin hypothesis have remained prominent. These hypotheses exist as a theoretical bridge between the proposed genetic/developmental causes of schizophrenia, and the consequent effects of cortical network impairment and the experiential manifestation of the disorder.

1.8.1.1 – The Dopamine Hypothesis

The dopamine hypothesis states that dysfunctional dopaminergic neurotransmission in specific cortical regions of the brain give rise to prominent components of the disorder, namely positive and cognitive symptoms (Howes & Kapur 2009). The hypothesis originally arose from observations that antipsychotic drugs blocked dopamine release from dopaminergic neurons by acting as an antagonist on D2

receptors (Creese *et al.* 1976; Seeman & Lee 1975) and from observations that drugs that increase levels of post-synaptic dopamine, like cocaine, can induce psychotic like symptoms in healthy individuals (Curran *et al.* 2004). Furthermore post-mortem and in vivo analysis of schizophrenia brains found that D2 receptors were highly expressed in the mesolimbic areas and that D2 receptors exhibited hyperfunction (Abi-Dargham *et al.* 2000; Seeman *et al.* 1984; Wong *et al.* 1986). Since D2 receptor antagonists only alleviate positive symptoms in schizophrenia (Harvey *et al.* 2005), it is thought that they are responsible only for positive symptoms and not cognitive or negative symptoms in schizophrenia. It is hypothesized that D1 receptors may be partly responsible for cognitive factors due to their abundance in the neocortex of healthy subjects (Hurd *et al.* 2001); observed abnormal upregulation of D1 binding in the DLPFC of schizophrenia patients (Abi-Dargham 2003), and post-mortem studies revealing abnormal D1 receptor expression in the PFC of schizophrenia brains (Akil *et al.* 1999). Recent GWAS studies in schizophrenia have also identified risk SNPs at 11q23.2, which is near to the D2 expressing DRD2 gene (Ripke *et al.* 2014). However, so far none have been associated with the D1 expressing gene.

1.8.1.2 – The glutamate hypothesis

The glutamate hypothesis states that glutamate transmission is permanently impaired via abnormal NMDA receptor (NMDAR) function. This theory arose from the observation that the abuse of NMDAR antagonists like ketamine and PCP, induced schizophrenia like symptoms in its users (Allen & Young 1978). Furthermore, it was observed that these drugs also exacerbated symptoms in patients with psychosis and schizophrenia (Frohlich & Van Horn 2014). Furthermore agonists of NMDAR have been found to alleviate symptoms (Millan 2002) of schizophrenia. Glycine reuptake inhibitors have also been found to alleviate negative symptoms in an early stage proof of concept study (Umbricht *et al.* 2014). NDMARs mediate the majority of excitatory transmission in the CNS and are generally made up of three subunits – NR1 (GRIN1), NR2 (GRIN2) and NR3 (GRIN3). Various studies have found an association with these subunits and schizophrenia pathology (Bitanihirwe *et al.* 2009; Galehdari *et al.* 2009; Georgi *et al.* 2007); along with recent genetic evidence

identifying the *GRIN2A* gene as a risk factor for schizophrenia, among other glutamate associated genes (Ripke *et al.* 2014). This included the GRIA1 (GLuR1) gene which is a subunit of AMPA receptors, another glutamate associated receptor which has been implicated in schizophrenia (Goff & Coyle 2001). Recent studies are beginning to indicate that the glutamate and dopamine hypotheses of schizophrenia are not mutually exclusive and may actually impact each other's systems – creating a complex and detrimental relationship that leads to schizophrenia pathology (Laruelle 2014). A recent model proposed that impaired NMDA transmission in the frontal cortex could affect DA transmission in the mesocortical system of the substantia nigra and ventral tegmental area (VTA), impacting cognitive functioning and aggravating negative symptoms (Laruelle 2014).

1.9 – *FXYD6* and *MAP2K7* as genes implicated in schizophrenia

While the prevailing neurobiological hypotheses remain valid, there remains little knowledge of the neurobiological role of recently discovered genetic risk factors. Interestingly, several genetic risk factors, such as neuregulin and *DISC1* appear to converge at the glutamate synapse (Pratt *et al.* 2012). This thesis investigates the neurobiology of two implicated genes, *FXYD6* (Unpublished) and *MAP2K7* (Morris & Pratt 2014) from a neurobehavioiural and a neural systems perspective.

1.9.1 – *MAP2K7*

It has recently been proposed that certain cognitive deficits in schizophrenia may arise due to altered synaptic plasticity in the prefrontal cortex (PFC) and the hippocampal regions. This is because research has found a lowered metabolic activity in the PFC of schizophrenia patients during cognitive tests (Hill *et al.* 2004; Molina *et al.* 2005; Tamminga *et al.* 1992) and post-mortem studies have suggested a deficit in parvalbumin containing GABAergic neurons in the temporal lobes and PFC in schizophrenia (Beasley & Reynolds 1997; Gonzalez-Burgos *et al.* 2011). Furthermore it was found that NMDA receptor antagonists produce very similar phenotypical symptoms of schizophrenia in healthy individuals, exacerbate symptoms in schizophrenics (Malhotra *et al.* 1997), achieve similar metabolic

hypofrontality (Morris *et al.* 2005; Murray 2002) and reduce parvalbumin expression in the rat PFC (Cochran *et al.* 2003). This change in cortical metabolism is now being linked to an alteration in synaptic plasticity (Canals *et al.* 2009; Lewis *et al.* 2009) which is thought to be strongly modulated by NMDA receptor signalling. A number of signalling molecules are involved in NMDA-driven long term potentiation (LTP) (Lüscher & Malenka 2012) however, many of these are linked to other neurological diseases whose symptoms don't typically arise in schizophrenia. Therefore many can be ruled out.

Recently, our research has turned to the c-Jun N-terminal kinase (JNK) pathway which is known to be involved in neuronal activity, receptor function and mediate cortical and hippocampal plasticity (Winchester *et al.* 2014). JNK is activated by the kinase MKK7 (the product of the *MAP2K7* gene) following NMDA stimulation (Centeno *et al.* 2007). Previously our lab investigated the connection between altered MKK7 expression, deficits in cognition and schizophrenia. We found decreased expression of MKK7 in the PFCs of post-mortem schizophrenic brains. A genetic association study also found a substantial effect size for a common variant from case and control samples collected from Glasgow and Northern Europe (Winchester *et al.* 2012). Yamasaki *et al.* also found MAP2K7 as an important component for axonal elongation in the developing cerebral cortex (Yamasaki *et al.* 2011). A study by Funk *et al.* also found decreased expression of MAP2K7 in ACC and DLPFC areas (Funk *et al.* 2012) and the antipsychotic – clozapine – has been implicated in positively regulating gene expression in the MAPK signalling pathway (Rizig *et al.* 2012). This supports previous evidence that antipsychotics affect MAPK pathways, from where they derive their effect (Browning *et al.* 2005; Cussac *et al.* 2002). Furthermore mice heterozygous for *Map2k7* (+/-) gene showed conserved abilities in the SHIRPA protocol (a comprehensive series of tests that quantify gross characteristics of test mice from the physical to the behavioural - including measurements of gross weight, grooming, motor function and emotional cognition (Hatcher *et al*. 2001; Rogers *et al*. 2001)) and locomotor activity but showed deficits in working memory ability in the paired-trial variable delay T-maze test (PTVTM) (Winchester *et al.* 2012). The PTVTM was developed by Aultman and Moghaddam

(Aultman & Moghaddam 2001) and consists of two parts. In the first part the mouse is forced to go down only one arm where it will be presented with a reward. It is then given an inter-trial delay and then presented with both arms again. The mouse is only rewarded if it remembers to go down the opposite arm it went down in the forced trial. This discrete test enables the study of simple, 'low-load' working memory ability in rodents where the subject must only maintain its last decision for a short period of time. However, this task does not necessarily translate well with tasks that assess working memory deficits in schizophrenia. The present experiment aimed to increase the demand on the rodents working memory and increase the proactive interference using a radial arm maze task which can be broadly conceptualised as being similar to the human n-back task (Marighetto *et al.* 2008) (the test will be referred as the n-back in this thesis). Heterozygous *Map2k7*+/- mice were used (knock-outs produced 100% infant mortality) to investigate whether Map2k7 may be involved in working memory mechanisms in mammals.

1.9.2 – *FXYD6*

Research over the years has linked many psychiatric disorders to a malfunction in the NaKATPase pump including epilepsy, bipolar disorder and depression (el-Mallakh & Wyatt 1995; Renkawek *et al.* 1992). However, evidence has shown that alterations in the modulation of the NaKATPase pump may also contribute to symptoms of schizophrenia (Rybakowski & Lehmann 1994). All of the 7 identified FXYD proteins are known to be involved in the modulation of the NaKATPase activity (Béguin *et al.* 2001; Bibert *et al.* 2008; Crambert *et al.* 2005) and are associated with the pump in a tissue-specific way; with FXYD6 mostly expressed in the brain (Kadowaki *et al.* 2004). FXYD1 for example, is mostly expressed in heart, skeletal muscle and liver; where it is proposed to have a role in heart and muscle contractility (Bibert *et al.* 2008; Bogaev *et al.* 2001; Geering 2006). FXYD2 is mainly located in the kidneys, where it is involved in electrolyte homeostasis (Therien *et al.* 1997; Geering 2006). FXYD5 is a protein related to cancerous cells and is expressed in a number of locations including the heart, intestine, lungs and spleen. It is thought to be involved in regulating E-cadherin and metastasis (Geering 2006; Ino *et al.* 2002; Lubarski *et al.* 2005). FXYD6

is mainly expressed in the CNS and the inner ear and is thought to be involved in neuronal excitability (Geering 2006; Kadowaki 2004). FXYD proteins are made up of small hydrophobic proteins with a common 35 amino transmembrane sequence (Sweadner & Rael 2000). A short sequence, proline-phenylalanine-X-tyrosine-aspartate (PFXYD), is common to all the proteins and is thought to be a major component in their modulation of the NaKATPase pump (Béguin *et al.* 2001). A subunit of the FXYD family, the γ subunit, is thought to modulate the NaKATPase pump by changing the conformation of the enzyme which would increase affinity for ATP and increase K+ antagonism (Béguin *et al.* 1997; Therien *et al.* 1999). Furthermore it has been found that FXYD modulates Na+ & K+ affinity of the pump by interacting with α/β complex of the pump. This interaction is variable between each type of FXYD protein and which α/β they interact with, furthering the idea of tissue specific associations (Cornelius & Mahmmoud 2003; Geering *et al.* 2003; Therien *et al.* 1997).

Recently, a genetic association study identified single nucleotide polymorphisms (SNPs) in the *FXYD6* gene that are potential risk factors for schizophrenia in a Caucasian population (Choudhury *et al.* 2006; Choudhury *et al.* 2007). Linkage studies in Japanese and Chinese populations have failed to indicate an association, however, there is some evidence suggesting *FXYD6* as a genetic risk factor for schizophrenia. Western blots and in situ hybridisation studies have indicated similarity in expression areas of FXYD6 and affected areas in schizophrenia, namely the hippocampus and PFC (Kadowaki 2004). Importantly, the hippocampus and PFC are areas associated with memory and working memory, and also show abnormal activation in schizophrenia patients. Further, *FXYD6* is located on the 11q23.3 chromosome which is heavily implicated in schizophrenia (Grandy *et al.* 1989; Zhong *et al.* 2011). Abnormal FXYD6 expression may impair NaKATPase function in schizophrenia, and therefore, affect cognition in the PFC and hippocampal areas. As other psychiatric disorders show impaired function in NaKATPase activity, abnormal FXYD6 expression may produce similar deficits and contribute to cognitive dysfunction in schizophrenia. It is unknown however, how altered FXYD6 expression impacts on regional brain activity, particularly in areas associated with cognitive

processing. This study aimed to investigate the activational properties of FXYD6 in the rodent brain using 2-dexoyglucose autoradiography.

1.10 – Hypothesis and Aims

From the aforementioned discussion research into psychiatric illness, particularly psychotic disorders like schizophrenia and bipolar disorder, is beginning to uncover biological factors that contribute to pathology at many levels. Scaling up from genes to molecules - to neurons, to networks – all the way to cognition and finally subjective experience. Research using animal models is currently crucial to decoding how genes and molecular systems contribute to network interactions and cognitive processes. The experiments described in this thesis aim to continue this work of linking genes to behaviours and systems level interactions using rodent models; with the aim of uncovering pathological units that contribute to the overall architecture of psychiatric disorders.

The overall hypothesis is that genetic manipulation of Fxyd6 and Map2k7 expression will disrupt cognitive processes and neural circuitry relevant to psychiatric illness, particularly schizophrenia.

The specific aims of this investigation were:

1) To assess whether *Map2k7+/-* mice exhibit deficits in working memory. This was achieved using a working memory task in an automated radial arm maze for mice which is of translational relevance to the human n-back test.
2) To determine if *Fxyd6-/-* mice show dysfunction in neural systems relevant to cognition using 2-deoxyglucose imaging.
3) To investigate a role for glutamate in relation to Fxyd6 and NaKATPase activity by assessing the local rates of cerebral glucose uptake in *Fxyd6-/-* mice treated with the NMDA receptor antagonist, ketamine.

Chapter 2: Materials and Methods

2.1 – *Map2k7* and working memory performance

2.1.1 – Rodent models of human working memory tests

Human tests of working memory generally quantify the capacity of information a subject can recall (known as memory span), along with the subject's ability to manipulate the presented information in response to a given rule. These tests generally require a real time maintenance and/or manipulation of information in an individual's mental apparatus, and can generally be distinguished from short-term memory tests which require the recall of information, but little or no manipulation of this information. The most famous short-term memory test for humans is the digit span test which involves the recall of a sequence of numbers or letters directly after presentation, in the order of presentation. Human tests of working memory include the University of Maryland Letter-Number Span Task (UMLNST) (recall a sequence of letters and numbers directly after presentation but rearranged in sequence of a given rule – involves manipulation and maintenance of information); Wechsler Memory Scale-III Spatial Span Task (WMS-III SST) (a type of visual version of the UMLNST) and the human n-back test (indicate when a stimulus matches a previous stimulus from *n* steps back - the length of *n* can be manipulated to make the test easier or harder) (Young *et al.* 2009). While human tests of working memory require the manipulation and maintenance of a span of information, many rodent models of working memory, like the T-maze delayed non-match to sample (DNMTS) test, generally require the maintenance of only one piece of information. For example in the T maze the animal is required to select one of two baited arms in a choice trial but has to remember that in the next trial presentation that only the opposite arm is baited (i.e. non match to sample rule). It has therefore been concluded that tests like the DNMTS do not require a relevant amount of online manipulation and maintenance of information, and so they may be regarded more as measures of *short-term* memory than *working* memory (Young *et al.* 2009). Where short-term memory can be described as a temporary memory storage of information that does not require conscious maintenance and manipulation. Compared to working memory which is defined as an organisational construct which requires conscious

attention, and draws from other cognitive modalities – including short-term and long-term memory – in order to manipulate and sustain information online. This discrepancy between human working memory tests and rodent models are backed up in various neuroanatomical studies of cognition (reviewed by Young *et al.* 2009). With reference to the above, animal models of working memory are generally regarded to have a more valuable construct validity and face validity (and therefore more translatable to humans); when they involve a real-time maintenance and manipulation of information, and which test the span of an animal's working memory. Rodent models that currently fit this criteria closest include the Radial Arm Maze (RAM) (Olton 1987), the Spatial Span Task (SST) (Dudchenko *et al.* 2000) and the Odor Discrimination Task (ODT) (Dudchenko *et al.* 2000; Young *et al.* 2007). A rodent analogue of the human n-back test has been developed for the RAM, the theory and set-up of the task are discussed below.

2.1.2 – Radial Arm Maze:- 'n-back Test'

The automated radial arm maze (RAM) is a rodent-based behavioural apparatus that tests spatial memory and can be modified to asses specific components of this cognitive domain. It consists of a central arena with 8 identical arms spreading from it, all separated by the exact same angle. Each arm has a reward well at the end and a vertical lifting door at the start (these are controlled by sensors and a computer program). In the original task the rodent is placed into the central domain and is free to move around the maze looking for rewards at the end of each arm. Once the rodent finds the reward at the end of each arm, the subject will no longer get a reward if it returns that arm. Therefore, in order for the rodent to be successful and expend the minimum amount of effort for maximum reward, it must maintain which arms it went down previously and which it has still yet to go down. The subject's performance in this paradigm is measured in the least amount of times a subject goes down an arm to obtain all 8 rewards – therefore, the best score for this test is 8. The RAM is considered to have a relatively robust construct validity with regards to testing short-term memory and working memory constructs. However, this form

of the test requires little manipulation of information, and therefore, is thought to be more of a test of short-term memory, rather than working memory.

Recently Marighetto (Marighetto *et al.* 2008) developed a mouse RAM task which was designed to be probe working memory abilities, and shows some analogies with the human n-back test in that it adopts a distinct level of information load and manipulation. The human n-back test is a continuous test using visual or auditory cues where a subject must indicate when a stimuli matches a previous chosen stimuli. The length and time between the chosen stimulus can be manipulated, which can increase or decrease the working memory load and manipulate the required energy put into maintaining relevant information. In the n-back adaptation of the RAM for mice, subjects are presented with 6 arms out of 8, divided into three pairs of adjoining arms (A,B,C).

In the first phase of the task the mouse is presented with each of the 3 arm pairs (both arms baited) in a pseudorandom order. On entering a particular arm the door to the other arm is closed. In the next presentation of any pair, the reward will be present in the opposite arm to that which the mouse choose previously (i.e. according to a non-match to sample rule). Therefore, the mouse must remember which arm it went down during the last presentation and correctly choose the opposite arm to obtain the reward. By actively increasing the interference between pair presentations (e.g. A→A = 0 retention intervals; A→B→A = 1 retention intervals; A→C→B→A = 2 retention intervals) the working memory demand for each subject can be increased. These retention intervals may be considered analogous to 'n- back' levels. A diagram of the RAM and an example of the n-back test is given in figure 2.1.

For each session, mice were tested up to a retention interval (referred to as n-backs in this thesis) of 5 ('n-back 0', 'n-back 1', 'n-back 2', 'n-back 3' and 'n-back 4'). A computer algorithm generated a random sequence of pair presentations each day to produce an equal number of n-backs for each session - therefore - reducing the chance of mice using their long term memory to solve the task. Mice were presented with 24 trials once a day for 5 days of the week for 6 consecutive weeks. Note that in

each session, both arms were baited in the first presentation of each of the three pairs, whereas thereafter only one arm was baited. Success was measured by the number of rewards obtained and scored as the percentage correct. Inter-trial interval lasted 20 seconds and mice were given 2 minutes at the start of each session to familiarize itself with the maze. Mice were given 30 minutes to complete the task or obtain as many rewards as possible before the test automatically ended.

As the task progresses, this demand on working is at risk of increasing, due to forms of interference known as *proactive* interference and *retroactive* interference. Proactive interference occurs when the formation of a new memory is disrupted due to an old memory which is either similar, or learned in a similar context. For example if a subject was asked to learn a list of 7 names beginning with 'M', a healthy subject would normally perform well. However, if the subject was then asked to learn another list of 7 names beginning with 'M', and then another list, and then another – the recall performance on the subsequent lists would diminish, due to proactive interference of information from the initial memory test. However, if the list to be learned was changed in an obviously different way (for example names beginning with 'Z'), proactive interference would be reduced as information from the older tests would be significantly different not to affect recall. Retroactive interference is when the recall of old information is disturbed due to interference from newly learned information. The phenomenon occurs due to new information that is similar to old information outcompeting the old information for real-time memory recall. Although the old memory still exists, competition from the new memory means that sometimes it cannot be recalled. Rodents in the RAM n-back test are at risk of proactive interference as the task progresses due to the increased incidence of subjects presented with the same pairs of doors, where old memories of previous tasks can interfere with current ones. At the same time, retroactive interference may also play a role in working memory disruption. This is because of the positive relationship between retention interval and n-back number. As the number of trials increases between the matched pair (e.g. A), the retention interval increases, giving more time for new memories to interfere and disrupt old memories (e.g. which arm did the subject go down last in pair A?).

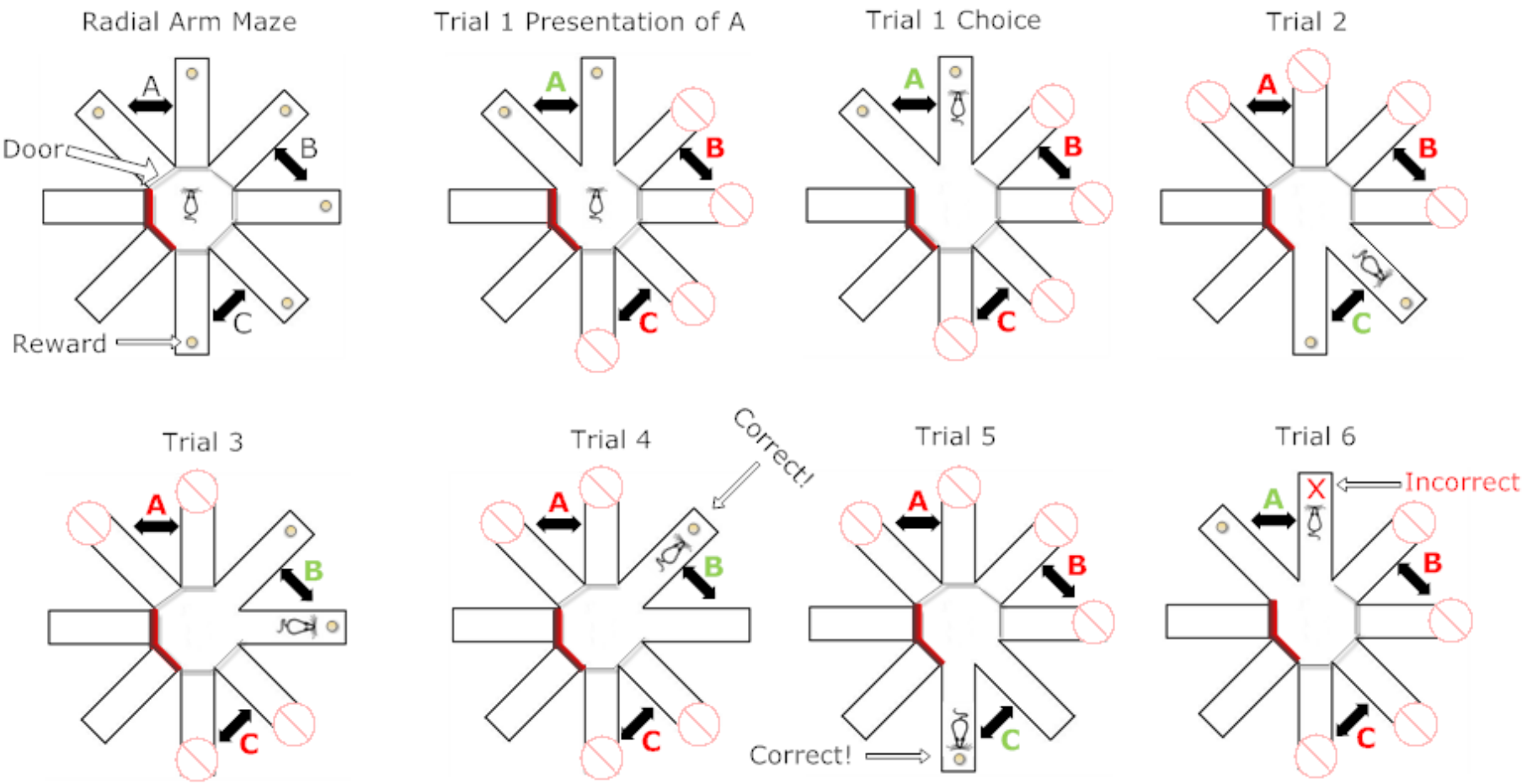
Radial Arm Maze
A
B
C
Door
Reward
Trial 1 Presentation of A
Trial 1 Choice
Trial 2
Trial 3
Trial 4
Correct!
Trial 5
Correct!
Trial 6
X
Incorrect

Figure 2.1: Radial Arm Maze (RAM) & n-back example. The radial arm maze apparatus is summarized in the top left corner, including the mouse in central chamber, the arm doors, reward in each arm (not visible to mouse); and the arm pairs labelled (A,B,C).

n-back example:

Trial 1 - Presentation of A illustrates the mouse presented with the choice of arms in Pair A (available pair in green; closed pairs in red); the mouse chooses the right arm (all arms regarded with spatial relation to the mouse anterior end of the mouse) and returns to chamber (the mouse must now remember which arm it went down in this pair in order to obtain a reward upon the next trial of A).

Trial 2: mouse presented with Pair C and chooses the left arm.

Trial 3: mouse presented with Pair B and chooses the right arm.

Trial 4: presentation of Pair B again, there are no intervening trials between this pair therefore 'n back'=0; the mouse selects the left arm of pair B which is the correct choice.

Trial 5: presentation of Pair C again – there have been two intervening trials between pair C, – therefore 'nback'=2; the mouse selects the right arm of C, which is the correct choice.

Trial 6: mouse is presented with Pair A again – there have been four intervening trials since the last presentation of Pair A – therefore 'nback'=4. In this example the mouse chooses the right arm which the mouse chose in the first presentation of Pair A therefore no reward is given and the choice is scored as incorrect.

2.1.3 – Animals

Heterozygous *Map2k7* mice were used in all experiments since *Map2k7* knockout results in 100% embryonic death. Mice were bred as heterozygous breeding pairs. Experiments were carried out in accordance with the Animals (Scientific Procedures) Act 1986.

20 mice were socially housed in cages of 2-3 mice (male and female separate) under standard conditions: 12/12 light/dark cycle at an average room temperature of 23 °C. Mice were food restricted for the duration of the experiment, making sure the mice were 85-90% of their free feeding weight on a normal growth curve and did not fall below 85% of that weight. Of the 20 mice there were 11 wild type (*Map2k7*+/+ C57BL/6, 3 female, 8 male) and 9 *Map2k7*+/- (2 female, 7 male) with an average age of 17.3 weeks upon onset of experiments

2.1.4 – Procedure

Pairs of mice were tested at the same time of the day each day (first tests typically commencing between – 9am and 9.20am, last tests between 3.30pm and 3.50 pm). The mice were given a five day acquisition period initially to familiarise themselves with the maze and understand how to solve the task successfully. After each test the whole maze was thoroughly cleaned using 50% ethanol and 50% water to eliminate or limit the chance of olfactory clues being left by mice.

2.2 – *Fxyd6* and Semi Quantitative 2DG Autoradiographic Imaging

Under normal physiological conditions, glucose is the major energy substrate that the brain utilises in order to create high energy phosphate molecules that power many important chemical reactions that support brain function. Furthermore, the metabolism of glucose is now established as a reliable indicator for functional cortical activation (Sokoloff 1981). Because of this reliable relationship between glucose metabolism and brain activation, techniques have arisen that quantify local brain activation utilising glucose analogues like 2-Deoxy-D-Glucose (2DG). Semi-quantitative 2DG autoradiographic imaging takes advantage of the different

metabolising outcomes of glucose and its analogue 2DG which possesses a hydrogen atom instead of a hydroxyl group on the second carbon of the hexose ring. Because of this slight difference, the enzyme which normally converts glucose-6-phosphate Because of this slight difference, the enzyme which normally converts glucose-6-phosphate to fructose-6-phosphate, cannot metabolise 2DG, and therefore, accumulates in the brain. The accumulation of 2DG (normally radiolabelled with 14C) can then be quantified in brain regions post-mortem and utilised as an indicator of local brain activity. In the rat, a fully quantitative measure of local cerebral glucose use can be determined through knowledge of the plasma profiles of ^{14C}DG and glucose over the course of the experiment. However, the collection of repeated plasma samples is not feasible in mice and so a semi-quantitative measure is employed (See Dawson *et al.* 2008 and section 2.2.2). This technique has become a valuable tool for analysing regional brain activity in a variety of animal models (Vyazovskiy *et al.* 2008; Dawson *et al.* 2008). The structure of chemical 2DG and schematic of how it accumulates in the cell are described in Figure 2.2.

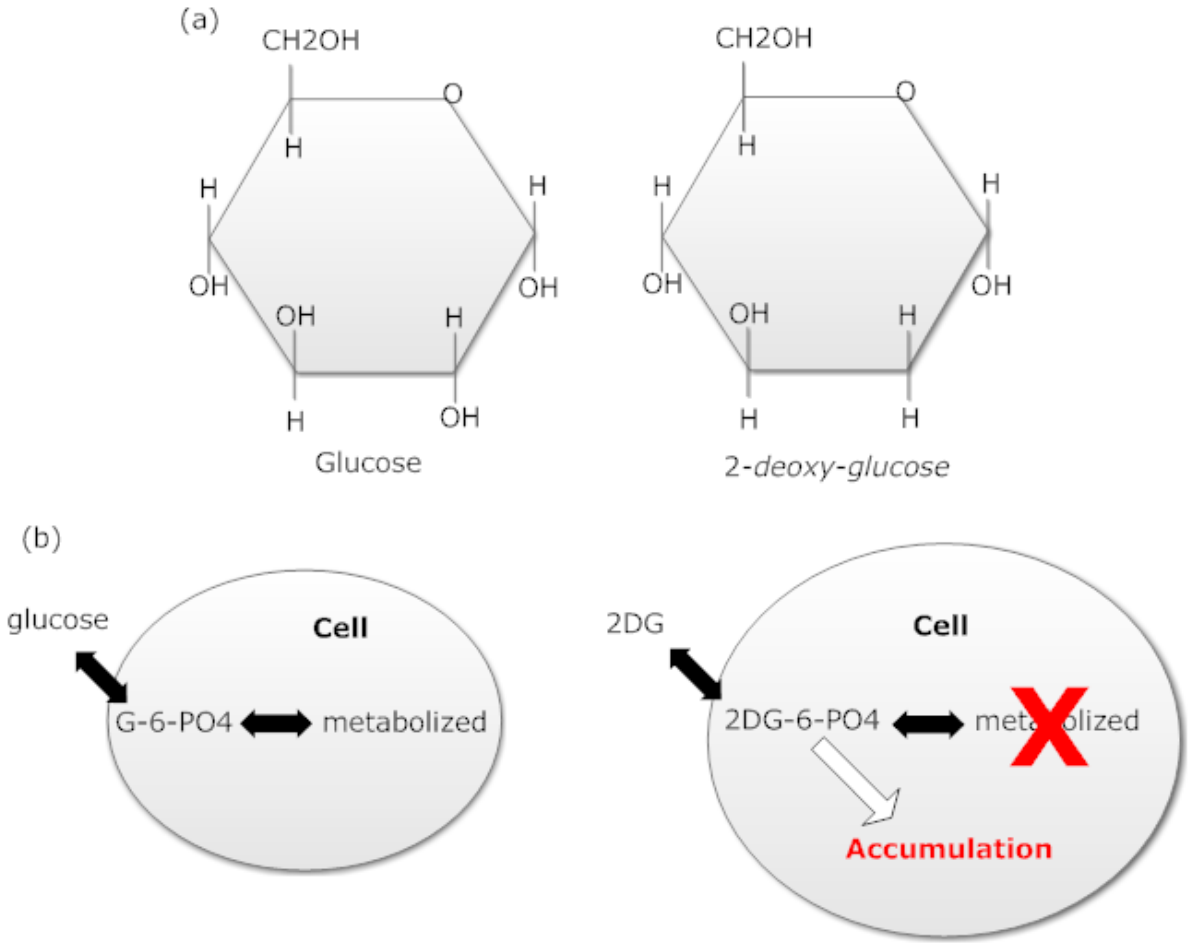

Figure 2.2: (a) Chemical structures of glucose and 2-deoxy-D-glucose (2-DG). 2-DG and glucose differ at the second carbon. (b) Schematic of glucose and 2DG metabolism. Glucose and 2DG enter the cell through a glucose transporter and are then phosphorylated by hexokinase to a 6 phosphate (6-PO4). A glucose 6 phosphatase metabolises glucose 6 phosphate but has limited capacity to metabolise 2DG-6 phosphate; therefore 2DG accumulates within the cell.

2.2.1 – Animals

Heterozygous *Fxyd6*+/- breeding pairs were bred and the offspring genotyped in the PsyRING laboratories at the University of Glasgow. Wild type and *Fxyd6-/-* mice were employed in the 2-deoxyglucose imaging experiments. Experiments were carried out in accordance with the Animals (Scientific Procedures) Act 1986.

A total of 20 male mice (12 WT C57BL/6J, 6 ketamine/6 saline; 8 KO *Fxyd6*-/-, 4 ketamine, 4 saline) aged 12-16 weeks old were used in the experiments. Mice were housed under standard 12h/12h light/dark conditions at 21°C, 45%-65% humidity. Mice went under food restriction 5 hours prior to 2DG imaging to avoid the influence of ketamine on plasma glucose levels.

2.2.2 – Semi-quantitative 2DG autoradiographic imaging

Local cerebral glucose utilization (LCGU) was determined around 1 minute after treatment with 25.mg.kg-1 of ketamine or physiological saline (2ml kg-1) interperitoneally (i.p). The dose and pretreatment time were based upon previous work by Dawson *et al.* (2011; 2014). Mice were then injected with 4.625MBq kg -1 of 14C-2-DG for 10 seconds and then returned to their cage. 45 minutes after the mice were injected, they were then decapitated and their terminal blood samples were taken via torso inversion. This time was chosen because it ensured the maximum behavioural effect of ketamine coincided with brain activity (Miyamoto *et al.* 2009). Blood samples were taken in order to assay plasma concentration of 14C-2-DG and glucose by liquid scintillation analysis (Packard) and semiautomated glucose oxidase assay (Beckman) respectively. Dr Neil Dawson carried out this part of the procedure. The brain was rapidly dissected and stored at -80°C following freezing in isopentane (-40°C). The frozen brains were then coronally sectioned (20µm) in a cryostat at -20°C. Four consecutive sections were sliced from every 60µm, thaw mounted onto slide covers and dried onto a hot plate at 70°C. These were mounted onto X-ray film for five days. The images were analysed by computer-based image analysis (MCID/M5+). The local isotope concentration for each region of interest was derived from the optical density of autoradiographic images relative to that of the

co-exposed 14C standard. Fifty-four distinct anatomical regions of interest were measured with the aid of a stereotaxic mouse brain atlas (Franklin & Paxinos *et al.* 1997) (Mouse atlas sections used to guide anatomical measurements are included in Appendix A). Uptake in each region of interest was measured by comparing the uptake with the total brain uptake, this was known as C-2-DG uptake ratio. 14C levels were determined as the average 14C concentration across all sections in which a region of interest was measured. For each region of interest the local 2DG uptake was divided by the total 2DG uptake of the whole brain area to obtain the 2DG uptake ratio which was used to represent the measurement of brain activity.

2.3 – Statistical analyses

For the radial arm maze *Map2k7*+/- experiment, data was analysed using repeated measures one way ANOVAs with post-hoc Tukey analyses to understand the effects of training period and n-back levels on each genotype group; unpaired t-tests were used to understand the differences in performance between genotype group, paired t-tests to compare effect of weekend break on each genotype group, and one-sample t-tests were used to compare genotype group performance with chance levels. For the 2DG *Fxyd6-/-* experiments the mean 2DG uptake ratio for brain regions in treatment groups (saline or ketamine) and genotype (wild type or *Fxyd6-/-)* were analysed using 2 tailed unpaired t-tests. Acceptable levels of significance were set at $p<0.05$.

Chapter 3: Determination of the effects of *Map2k7*+/- mice upon working memory performance

3.1 – Introduction and Aim

As noted in section 1.9.1, *Map2k7* has been linked to cognitive impairments and altered plasticity in schizophrenia patients. In terms of cognition, mice show deficits in a T maze task of potential relevance to working memory (Winchester *et al.* 2012). Whilst this task is widely used in the preclinical literature, it has limitations in the sense that the animals have to hold only 'one item' online (Young *et al.* 2009). Arguably a more translational task of relevance to the human 'n-back' working memory is a version of the radial arm maze task as described by Marighetto *et al.* (2008) (See section 2.1)

The aim of this experiment was therefore, to assess the effects of *Map2k7* on the spatial working memory ability of mice and assess their performance over time to perform under conditions of increased working memory load.

3.2 – Methods

The methods are described in detail in section 2.1

3.3 – Results

3.3.1 – Genotype performance for each 'n-back' level over the 30 test sessions.

Figures 3.1a – 3.1e portray the mean percentage of daily correct responses for each n-back level (n-back level 0 to n-back level 4) over the 30 test sessions in WT mice and *Map2k7*+/- mice. An overall improvement of both groups for n-back levels 0, 1 and 2 over the 30 test sessions can be observed, with little improvement in n back levels 3 and 4. For the n-back level of 0, both genotypes acquired the task rapidly, and exhibited a responding accuracy of ~70% within a test session. The average response on the first test session of n back level 0 was 80% in WT mice and 72% in *Map2k7*+/- mice, which gradually increased to an average value of 95% and 88% percent on the final test session. Notably, for 19 of the 30 sessions, the performance value of the *Map2k7*+/- mice was less than that of the WT mice. As indicated in figure 3.4, it was found that this difference was significant (p=0.004, unpaired t-test). However, a level of n-back 0 generally requires little maintenance and manipulation

of information, and is more akin to the T-maze DNMTS task. Therefore, this difference may lie more in short-term memory rather than working memory. For the n-back level of 1 mice of both genotypes appeared to acquire the task over the 30 sessions (1^{ST} session WT mice: 70%, *Map2k7*+/- mice: 50%. Last session WT mice: 86%; *Map2k7*+/- mice: 75%) and once again the *Map2k7*+/- mice showed lower performance levels than the WT mice – where WT mice outperformed *Map2k7*+/- mice in 24 out of the 30 sessions. This difference was also found to be significant as described in figure 3.4 (p=0.001, unpaired t-test). For the n-back level of 2, WT mice acquired the task rapidly on session 1 at 63%, whereas *Map2k7*+/- mice performance on day one was just above chance at 52%. The final session performance of WT mice was at 75% and *Map2k7*+/- mice at 66%. WT mice outperformed *Map2k7*+/- 17 out of the 30 sessions but this difference was not significant (p= 0.13, unpaired t-test). At the n-back level of 3, both WT mice and *Map2k7*+/- mice struggled to perform successfully over the 30 tests sessions; where both groups were still performing just above chance levels by the last session with WT mice at 59% and *Map2k7*+/- mice at 52%. However, it was found that WT mice outperformed *Map2k7*+/- mice on 21 out of 30 sessions for this n-back level, where this difference was significant (p=0.01, unpaired t-test). At the most difficult n-back level of 4, both groups showed little progress over the 30 test sessions, with both groups performing just above chance levels, with significant performance dipping and spiking throughout. *Map2k7*+/- mice performed better in the 17 out of 30 days than WT mice, however, this was not statistically significant (p=0.9, unpaired t-test).

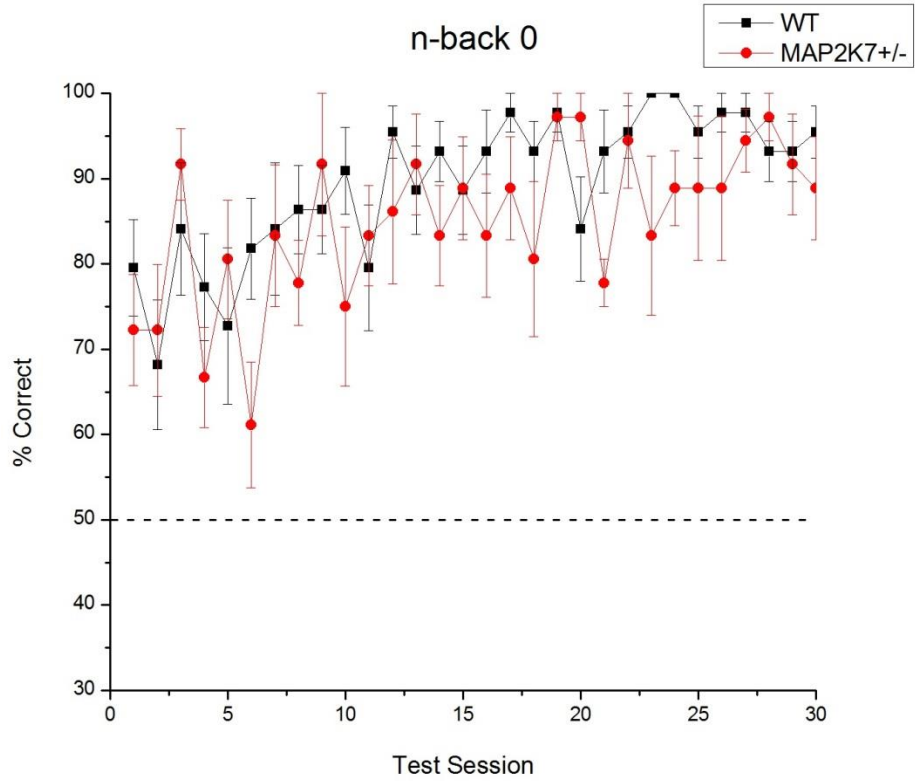

Figure 3.1a

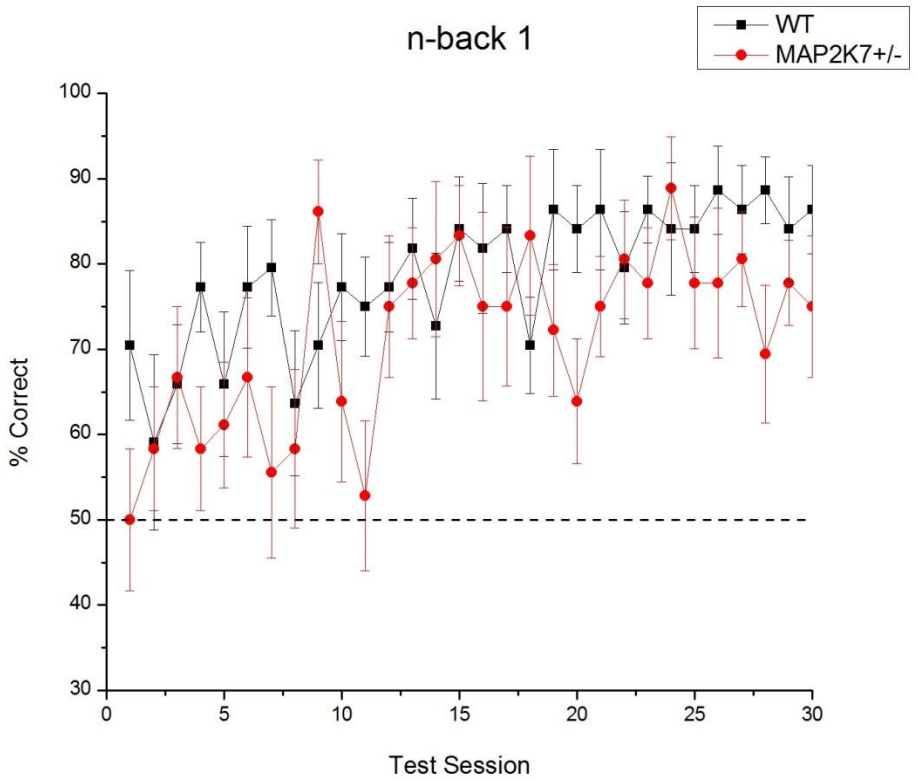

Figure 3.1b

Figure 3.1a and 3. 1b: Comparison of average performance of wild type mice and Map2k7+/- mice for n-back levels 0 and 1 over the 30 test sessions. Results are expressed as mean percentage correct for each test session +/- SEM. Dashed line represents chance levels. Sample size: WT mice = 11; Map2k7+/- mice = 9.

Figure 3.1c

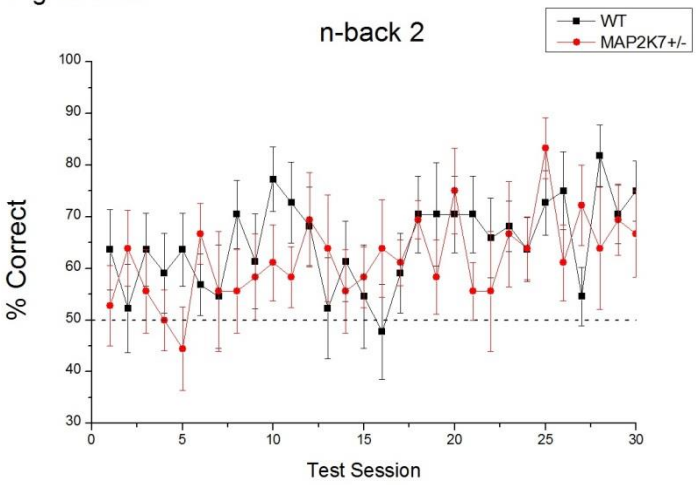

Figure 3.1d

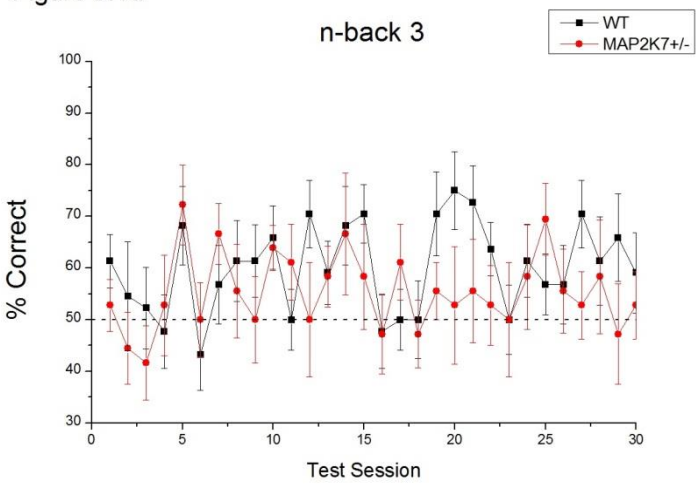

Figure 3.1e

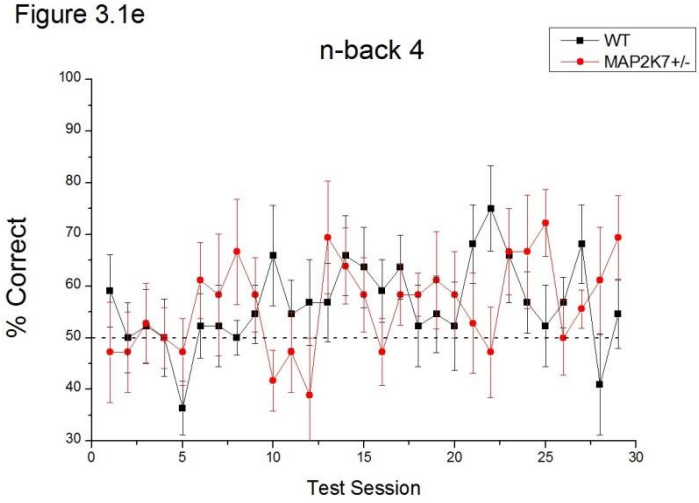

Figure 3.1c, 3.1d and 3. 1e: Comparison of average performance of wild type mice and Map2k7+/- mice for n-back levels 2, 3, and 4 over the 30 test sessions. Results are expressed as mean percentage correct for each test session +/- SEM. Dashed line represents chance levels. Sample size: WT mice = 11; Map2k7+/ mice- = 9.

3.3.2 – Investigating the effect of the 'weekend break' on performance and the effect of morning and afternoon sessions on performance.

To investigate whether the apparent dipping and spiking of performance was the result of the 2 day period (Saturday and Sunday) where the mice were not partaking in the radial arm maze, the overall performances of the animals on Fridays and consecutive Mondays for both genotypes were compared. It was hypothesised that the *Map2k7*+/- mice may perform worse on a new Monday compared to the previous Friday as they may have taken longer to re-establish the purpose of the task or may have struggled to retain the method to success, or taken longer to establish their spatial surroundings. The results are graphed in figures 3.2a and 3.2b. A comparison of performance levels for both genotypes between paired 'Fridays' and 'Mondays', (using paired t-tests) did not reveal any significant differences. ($p>0.05$ for all). The performance of both groups on Fridays and Mondays were combined and compared. However, again no significant differences were found for the 2 day gaps ($p>0.05$ for all from paired t-tests). Since mice were tested at the same time each day, some early in the morning and some in the afternoon, the overall performance of 'morning' subjects and 'afternoon' subjects were compared. This is because the mice were fed all at the same time at the end of each day, and as the food rewards of the maze acted as an incentive to perform the test correctly, subjects who were tested in the morning may not be as incentivised to obtain the reward, whereas subjects tested later in the day may well be; therefore possibly affecting the validity of the results. However an unpaired t-test comparing subjects tested in the morning (9am-12pm; n=10, *Map2k7*=5, WT=5) and subjects tested in the afternoon (12pm-2pm; n=10, *Map2k7*=4, WT=6) over all n-back levels found no significant difference between the groups ($p>0.05$; morning group = 67.35% average performance, afternoon group = 66.22% average performance).

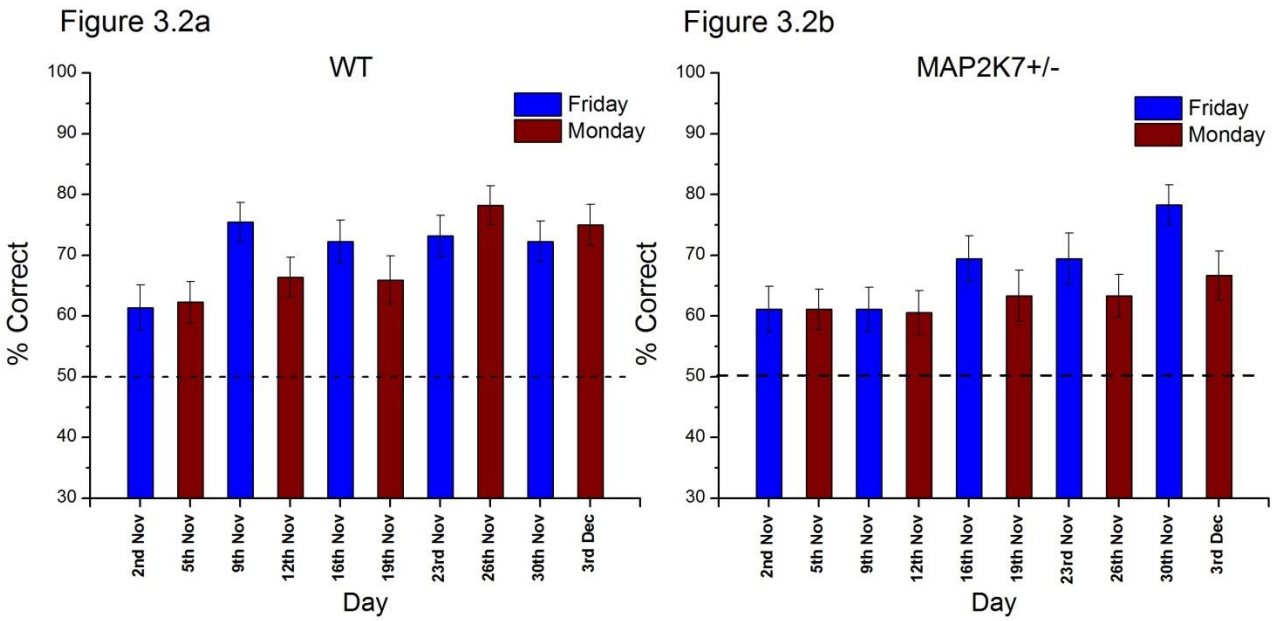

Figure 3.2a & 3.2b: Comparison of overall trial performance (average of n back levels 0-4) of WT mice and Map2k7+/- mice on consecutive Fridays and Mondays in the test period. Results are expressed as mean percentage correct for each test session +/- SEM. Dashed line represents chance levels. Sample size: WT mice = 11; Map2k7+/- mice = 9. Blue bars represent Fridays and Brown bars represent Mondays.

Figure 3.2c

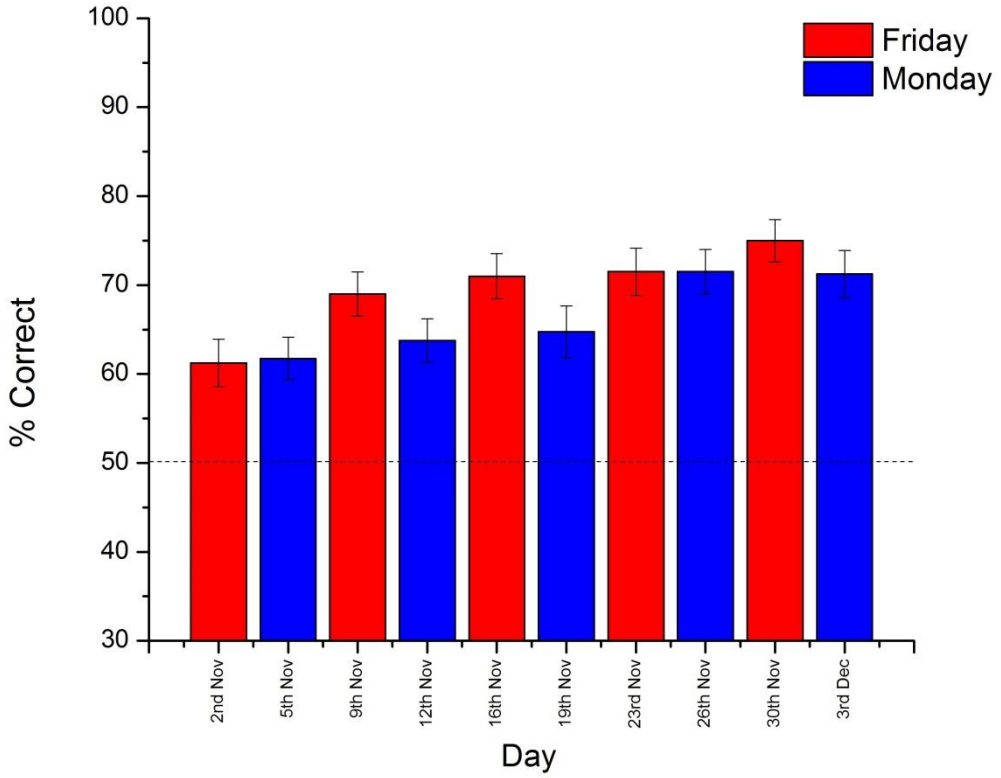

Figure 3.2c: Comparison of overall trial performance (average of n-back levels 0-4) of both groups combined on consecutive Fridays and Mondays in the test period. Results are expressed as mean percentage correct for each test session +/- SEM. Dashed line represents chance levels. Sample size: WT mice = 11; Map2k7+/- mice = 9. Red bars represent Fridays and Blue bars represent Mondays.

3.3.3 – Overall performance levels of *Map2k7*+/- mice and WT mice over ten blocks of 3 test sessions

In order to smooth out possible day to day variation the data was then compressed into 3 test session blocks (30 days = 10 blocks in total) for each genotype and then the performance was compared over the average values of all n-back levels. This procedure is widely used (e.g. Marighetto *et al.* 2008). A significant performance spike of WT mice in 'block 4' and 'block 7' was apparent compared to the *Map2k7*+/- mice. ($p= 0.0004$, $p = 0.004$ respectively for Blocks 4 and 7 [unpaired t-tests] (Figure 3.3). In order to gauge whether both genotypes were improving their overall performance over the whole 30 test sessions, one way repeated measures ANOVA were applied to compare the mean performances of each block in each genotype and then post-hoc tukey tests were used to find which blocks differed from each other. It was found that in both genotypes performance improved significantly from the first block (block 1) of sessions to the last (block 10) (WT mice F-value = 8.54, $p<0.001$; *Map2k7*+/- mice F-value = 6.19, $p<0.001$). Post-hoc Tukey analysis identified where specific differences between block performance lay. It was found that in WT mice the first three blocks differed significantly from blocks 7, 8, 9 and 10 (blocks 7,8,9 and 10 all showed significantly higher performances than 1, 2 and 3 10; all $p<0.05$); where-as block 6 did not differ significantly from blocks 1,2 and 3 (all $p>0.05$). Furthermore in WT mice the average performance in blocks 7,8, 9 and 10 were found not to be significantly different from each other. This may indicate a stabilisation of performance where WT mice reached their natural maximum overall performance.

In *Map2k7*+/- mice blocks 1 and 2 exhibited a significantly lower performance than the last three blocks of 8, 9 and 10 (all $p<0.05$); furthermore blocks 8, 9 and 10 were not found to be significantly different from each other (all $p>0.05$). Similarly to WT mice, this indicates that the mice's overall performances improved over the 30 test sessions and that their performance may have begun to stabilise towards the final 12 test sessions.

Figure 3.3

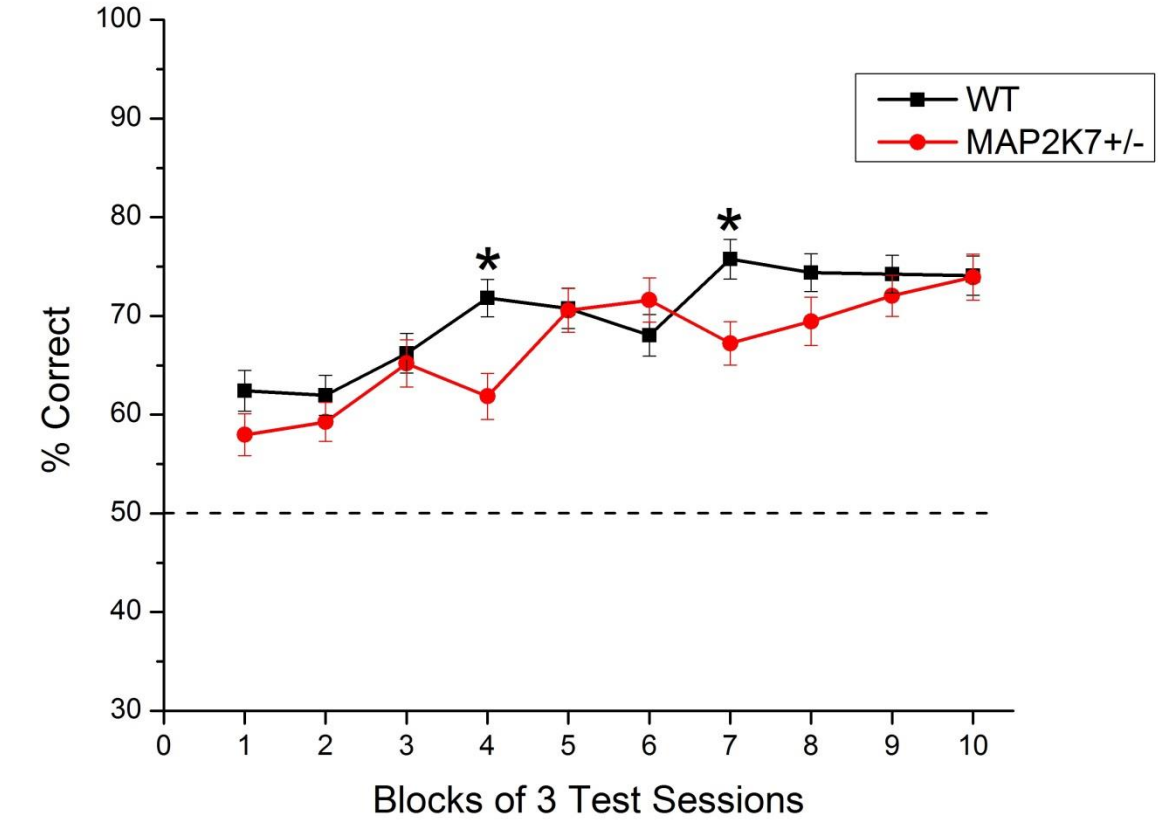

Figure 3.3: Overall performance levels of Map2k7+/- mice and WT mice over ten blocks of 3 test sessions. '' represents significant difference between genotype performance. Results are expressed as mean percentage correct for each block +/- SEM. Dashed line represents chance levels. Sample size: WT mice = 11; Map2k7+/- mice = 9.*

3.3.4 – Comparison of genotype performance at each n-back level

The average performance of WT mice and *Map2k7*+/- mice on each n-back test over a total of 30 test sessions were then compared. A significant difference in performance was found between WT mice and *Map2k7*+/- mice at the n-back level of 0 (WT mice performance= 90%; *Map2k7*+/- mice performance = 85%; p=0.004 [unpaired t-test]), n-back level of 1 (WT mice performance = 79%; *Map2k7*+/- mice performance = 71%; p<0.001 [unpaired t-test]) and the n-back level of 3 (WT mice performance = 60%; *Map2k7*+/- mice performance =55%; p=0.01). No significant difference was found between genotype group in the n-back levels of 2 and 4 (n-back level 2 performance: WT mice = 65%, *Map2k7*+/- mice = 62%; p>0.05. n-back level 4 performance: WT mice = 57%, *Map2k7*+/- mice = 56%; p>0.05). This data is portrayed in figure 3.4. In WT mice all n-back performances were significantly different from each other (repeated measures ANOVA F-value = 125.92, p<0.001; post tukey tests all p<0.01) except n-back level 3 and 4 (post-hoc Tukey, p=0.30). In *Map2k7*+/- mice all n-back performances were significantly different from each other (repeated measures ANOVA F-value = 78.10, p<0.001; post hoc Tukey all p<0.01) except n-back levels 2 and 4 (post-hoc Tukey, p=0.05) and n-back levels 3 and 4 (post-hoc Tukey, p=0.98). Finally, one sample t-tests found that all n-back level performances in both genotype groups were significantly above chance levels of 50% correct (all p<0.001).

Figure 3.4

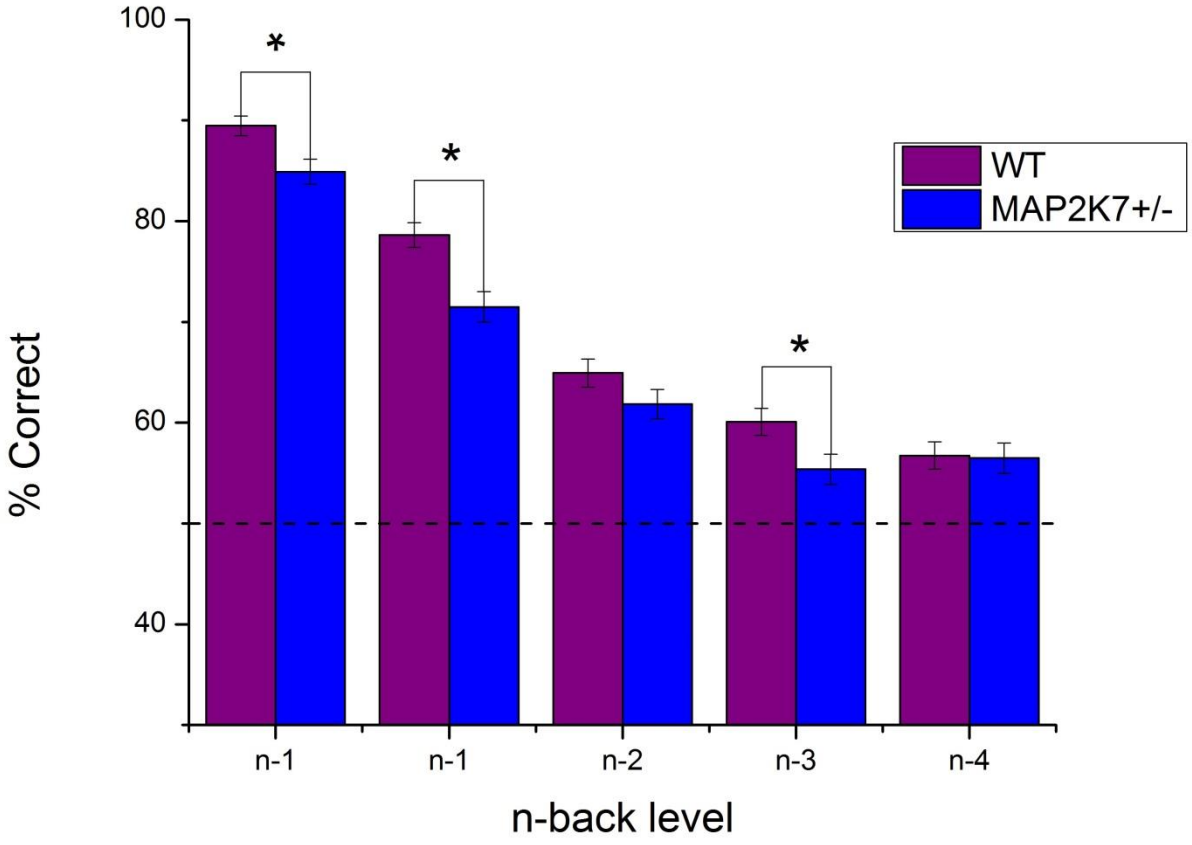

Figure 3.4: Comparison of WT mice and Map2k7+/- mice on their average percentage correct scores over 30 test sessions for each n-back level. (*) represents significant difference between genotype performance. Results are expressed as mean percentage correct for each n-back +/- SEM. Dashed line represents chance levels. Sample size: WT mice = 11; Map2k7+/- mice = 9.

3.3.5 – Comparing performance of genotype groups on all n-backs combined over the whole 30 test sessions

The overall performance of WT mice and *Map2k7*+/- mice for all the n-back levels combined, were then compared (Figure 3.5). The wild type mice exhibited a subtle, yet significantly better performance than the *Map2k7*+/- mice over the whole experiment; where WT mice exhibited an overall average performance of 70% and *Map2k7*+/- mice 66% (unpaired t-test, $p<0.001$). Thus it can be observed that *Map2k7*+/- performance was significantly different from WT performance and that *Map2k7*+/- may exhibit a subtle deficit in spatial working memory ability compared to WTs.

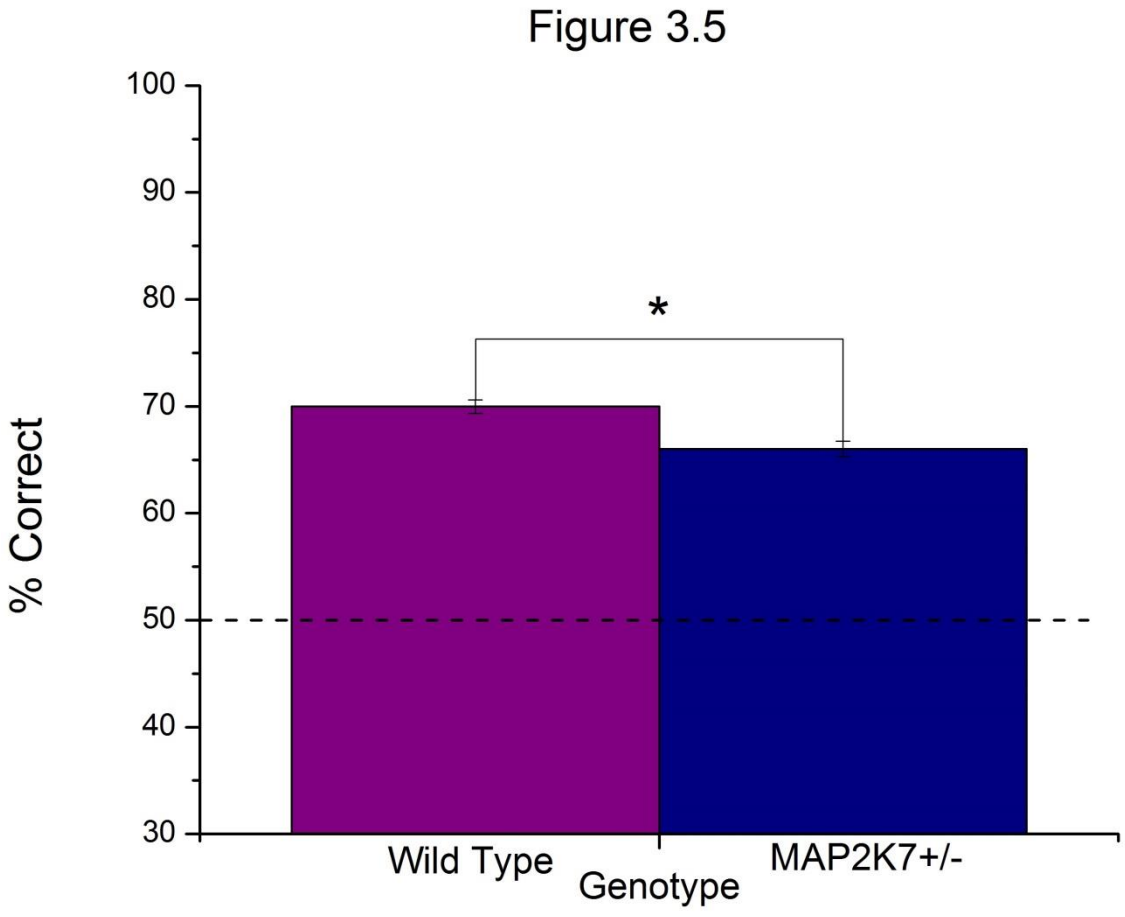

Figure 3.5: Overall performance of WT and Map2k7+/- mice in the radial arm maze with performance over all sessions and all n-backs combined. (*) indicates significant difference. Results are expressed as mean percentage correct +/- SEM. Dashed line represents chance levels. Sample size: WT mice = 11; Map2k7+/- mice = 9.

3.3.6 – Comparing overall genotype performance of n-back levels 1 to 4 over the last 4 blocks of test sessions combined

Finally, to remove the effect of initial variation in performance of both groups (where both groups may still have been acquiring the task, as described in section 3.3) and to pursue a more robust analysis of working memory performance in the groups (because it is thought that an n-back level of 0 may be more of a test of short-term memory rather than working memory), the last 4 blocks of testing and the n-back levels 1 to 4 were isolated and analysed. It was found that the overall genotype performance over the last 4 testing blocks combined (blocks 7, 8, 9 and 10), over all n-back levels (0 to 4), was again significantly different (unpaired t-test, p=0.01); where WT mice exhibited an average performance of 75% and *Map2k7*+/- mice 70%. This indicates that both genotype groups exhibited a stronger performance in the last 4 blocks of testing but WT mice still outperformed *Map2k7*+/- mice.

Overall genotype performance over n-back levels 1-4, over the whole 30 test sessions, were then compared. It was found that WT mice again outperformed *Map2k7*+/- mice over the n-back levels of 1 to 4 where WT mice performed at an average of 65% and *Map2k7*+/- mice 60% (unpaired t-test, $p<0.001$). Therefore, removing the effect of n-back level 0 on the results and further isolating the test on working memory performance.

Finally, both the first 6 blocks of testing and n-back level of 0 were removed from the analysis and the overall performance of genotype group over the last 4 blocks from n-back levels 1 to 4 were then analysed. It was found again that WT mice exhibited a significantly higher performance than *Map2k7*+/- mice (WT mice = 69%; *Map2k7*+/- mice= 64%. Unpaired t-test, p=0.002). Results are summarised in figure 3.6.

Figure 3.6

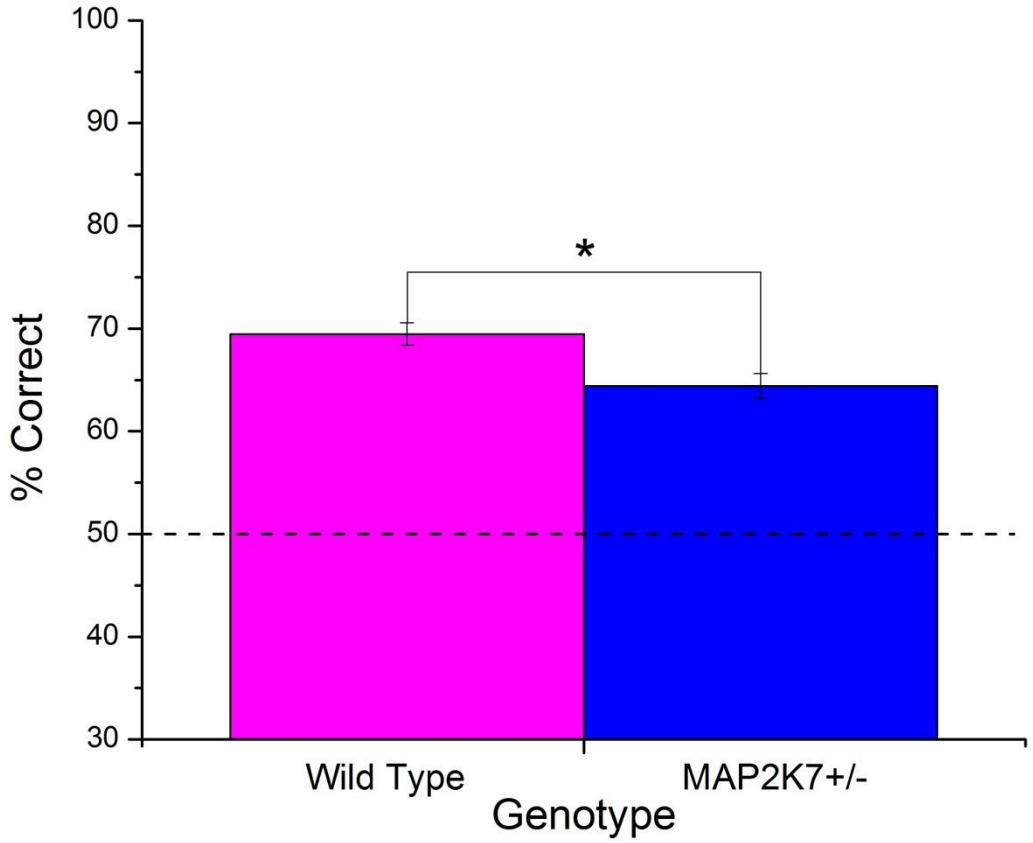

Figure 3.6: Overall performance of WT and Map2k7+/- mice in the radial arm maze with performance over the last 4 test blocks and n-back levels of 1 to 4 combined. (*) indicates significant difference. Results are expressed as mean percentage correct +/- SEM. Dashed line represents chance levels. Sample size: WT mice = 11; Map2k7+/- mice = 9.

3.4 – Discussion

This experiment has exhibited the clear benefits in utilising this adaptation of the original radial arm maze paradigm, in order to assess working memory function in mice. Over the 30 days for n-back 0 & 1 (figure 1a & 1b), WT mice showed a clear understanding of the test, beginning at average performances of 80% and 70% on the first days respectively. And then improving to 95% and 86%. At such low levels of working memory load, the n-back level of 0 is likely to represent short term memory, rather than working memory, as it generally requires a low level of real-time information maintenance. An n-back level of 0 is therefore relatively easy for healthy WT mice. However, as can be observed from figure 4, as the n-back level increases from n0 to n4, there are clear incremental reductions in performance in WT mice (and *Map2k7*+/- mice). The average performance of WT mice and *Map2k7*+/- mice over all the n-backs also existed above the 50% chance mark. These two observations give a strong indication that mice were attempting to maintain the memories of what arm they last visited in each pair, in order to gain maximum reward from the task. And as the working memory demand increased from n-back level 1 up to n-back levels of 4 (urging mice to try and actively maintain what arm they visited previously) their average performance negatively affected. But because their average performance was still significantly above chance levels at n-back levels 3 and 4 (described in section 3.4) - even if only slightly - this provides evidence that the mice were utilising their working memory in order to gain maximum reward. It would be interesting to investigate the absolute limit of rodents working memory span in this paradigm. This could be done by increasing levels to n-back levels of 5 and 6 in the RAM. This would give a good indication of the true span of rodent's spatial working memory; and would be useful in interpreting these results for translation into human working memory studies.

The results of WT performance from this experiment were also similar to the Marighetto investigation that utilised the same RAM n-back paradigm in order to study the effects of two dopamine agonists on working memory performance in aged WT mice. In the Marighetto investigation, they found that their adult aged WT (control group) mice exhibited a steady average increase in performance over a 12

day period of testing, on all n-back levels (or retention intervals as they are referred to in the paper). The average WTmice performance at the beginning of testing was at 63% correct (average performance over sessions 1-3, n=0, C57BL/6) which is very similar to the initial WT mice performance in this experiment which was 62% (average performance over sessions 1-3). Furthermore, the WT mice in the Marighetto paper improved to an average performance of 75% correct in their final blocks of testing (days 10-12); which was similar to the performance of WT mice in this experiment of 72% over sessions 10-12 (eventually plateauing around 75% from day 20). Furthermore in the Marighetto experiment WT mice exhibited a similar diminishing performance as n-back level increased – from 92-62% from n-back level 0 to n-back level 4 in the Marighetto paper – compared to 89-57% in this experiment. The WT mice also performed significantly above chance levels at all n-back levels in the Marighetto experiment. This provides good evidence of a consistent performance of WT C57BL/6 over this working memory paradigm which will inform future experiments that aim to use rodents for working memory experiments.

In this experiment it was found that *Map2k7*+/- mice exhibit a subtle yet significant deficit in their spatial memory ability compared to wild type mice in an automated spatial working memory RAM task (WT mean performance = 70%; *Map2k7*+/- mean performance = 66% p = 0.001) (figure 3.5). Furthermore, by removing the n-back level of 0 from the analysis (which tests short term memory), it can be concluded that the *Map2k7*+/- mice tested exhibited a significant deficit in working memory compared to WT mice (unpaired t-test, $p<0.001$. Described in figure 3.6). Previous work in our laboratory supports the deficit in spatial short term memory in *Map2k7*+/- mice in which mice exhibited deficits in the paired-trial variable delay T-maze test, which is analogous to the n-back level of 0 in this investigation (Winchester *et al.* 2012). This experiment expanded the experiment to test the mice's ability to successfully uphold spatial information over alternating nodes of interference which in turn produced alternating periods of time between matching tests. It was found that although wild type and *Map2k7*+/- mice improved on each n-back at a similar rate, wild types exhibited two significant performance 'bumps' over

the period of testing (blocks 4 and 7, figure 3). Furthermore it was found that wild types performed significantly better at the n-back levels of 0 and 1. Although tests at the n-back levels of 0 and 1 present a very low demand on working memory (in terms of information load, and inter-trial interval) the proactive interference is larger at these stages. As stated above, proactive interference is the psychological phenomena in which the forgetting of information occurs through the interference of other similar information learned previously (Still 1969). Therefore, if a mouse is presented with the same pairs repeatedly, the proactive interference increases and working memory maintenance may be disrupted. Could *Map2k7*+/- exhibit a dysfunction in the ability to distinguish similar contextual information? Interestingly, the ventrolateral PFC and left anterior PFC have been linked to proactive interference handling mechanisms using fMRI in and human subjects (Nee *et al.* 2007). These brain areas are usually linked to significant hypofunctionality in schizophrenia patients. This deficit could result from a downgrade in the communication from these areas to the entorhinal cortex and the hippocampus which together, encode spatial contextual information (Ainge & Langston 2012). It would be interesting to see whether there is a deficit in the functional activity of these areas in *Map2k7+/-* using the 2DG technique as used for in the *Fxyd6* experiment. A focused short term/working memory task which probes high proactive interference models predominantly utilising n-back levels 0 and 1 could be also used. This could reaffirm the deficit seen in *Map2k7*+/- mice compared to wild types. One thing that is left unexplained however, is the similar performances seen in n-back level 2 between genotypes and the differences in performance at n-back level 3. The n-back level 2 results could portray a cross-over point where the level of proactive interference is sufficiently low enough not to affect *Map2k7*+/- significantly, and the information load is not sufficiently high enough to affect them either. An n-back level of 3 could represent the point where information load begins to significantly affect *Map2k7*+/- mice compared to wild types. This speculation may be worthy of further investigation in experiments that solely focus on higher information load and lower incidence of proactive interference models.

By what mechanisms could this reduced expression of Map2k7 lead to this subtle deficit in working memory that was observed? MAP2K7 is one of two kinases (the other being MAP2K4) that regulate a component of an important mitogen-activated protein kinase (MAPK) signalling cascade – via regulation of JNK-1 (Coffey 2014). While JNKs have classically been associated with being a positive effector in cellular-stress-induced apoptosis (Liu & Lin 2005; Dhanasekaran & Reddy 2008), more recent evidence has shown that JNK plays an important role in neuronal development, axo-dentritic architecture and neuronal plasticity (Myers *et al*. 2014; Coffey 2014). Since previous studies have shown that abrogation of JNK signalling produces serious deficits in neuronal development and brain morphogenesis in rodents, one could postulate that the reduced expression of Map2k7 in this study induced abnormal neuronal development in the *Map2k7+/-* mice. However, if this was the case, one would expect the mice to perform markedly worse in the RAM test, exhibit various behavioural and motor deficits, and exhibit poor performance in other cognitive tests - which Winchester *et al*. 2012 showed was not the case. Furthermore, previous studies have indicated that MAP2K7 and MAP2K4 can play different roles in JNK signalling, where MAP2K4 has a more pronounced role in mammalian neuronal development (Asaoka & Nishina 2010; Wang & Xia 2012), therefore it could be concluded that subtle working memory deficit in *Map2k7-/-* mice was likely not because of abnormal neuronal or cortical development.

Since JNK seems to be particularly important in brain areas related to cognition, (the highest levels of JNK1 are found in the neocortex, followed by the hippocampus, the thalamus and the midbrain (Coffey 2014)), and previous research has implicated MAP2K7 & JNK in NMDA-mediated plasticity (Centeno et al. 2007; Kim et al. 2007); it could be hypothesised that reduced expression of Map2k7 downregulation produced a working memory deficit through altered synaptic plasticity in the cortical and hippocampal regions. Various evidence points towards this. For example JNK1 is activated by NMDA (Borsello *et al*. 2003; Mukherjee *et al*. 1999), which then regulates components of NMDA receptor function via a JNK-interacting scaffold protein (JIP) (Kennedy et al. 2007). This JNK activation via NMDA is mediated by MAP2K7 (Winchester et al. 2012). Therefore, reduced MAP2K7 expression may

abrogate glutamatergic NMDA-activated JNK signalling, and then in turn reduce NMDAR signal transduction from augmented JIP function. Furthermore, various substrates of JNK have been identified as important components of synaptic plasticity, including PSD95 (Kim et al. 2007; Yang *et al*. 2011) and GluR2 (Thomas et al. 2008). However, what is not clear is how this possible interference in synaptic plasticity, would interfere with spatial working memory mechanisms and not interfere in other forms of memory that rely on NMDA-mediated synaptic plasticity. Some evidence has shown that JNK plays a significant role in long-term depression (LTD), which is absent in mice JNK-/- mice and JNK-antagonised mice (Li *et al.* 2007). LTD is a process by which synapses lose their efficacy from each other for a sustained period. Recent evidence has pointed towards a role for LTD in the encoding of spatial information and spatial working memory in mice (Ge et al. 2010; Nakao *et al*. 2002). Inferring that reduced Map2k7 expression may reduce working memory capacity by interfering with specific long-term depression mechanisms within the hippocampus and neocortical areas.

This experiment therefore provides evidence that abnormal Map2k7 expression may contribute to the architecture of cognitive impairments in schizophrenia, particularly working memory. The mechanism for this may be from altered JNK signalling, which in turn would affect synaptic plasticity. Other evidence has shown that MAP2K7 is involved in axonal elongation in the developing cerebral cortex (Yamasaki *et al.* 2011), while Rizig *et al.* showed that the antipsychotic clozapine upregulates gene expression of *Map2k7* in mice (Rizig *et al.* 2012) and that MAPK signalling is involved in the efficacy of antipsychotics used to treat schizophrenia (Cussac *et al.* 2002; Browning *et al.* 2005). The MAP2K7 pathway therefore represents a potentially important area in preclinical drug discovery, which could help develop viable treatments for cognitive factors in schizophrenia. This does not mean to say that a drug that augments MAPK/JNK signalling would drastically improve working memory in schizophrenia patients, as the deficit observed was only subtle. But it would serve as an important component of discovering the complex genetic architecture that contributes to cognitive impairment in the disorder. It would therefore be important to further explore the interaction Map2k7 has with brain areas and whether

Map2k7+/- would exhibit an impaired connectivity between the PFC and hippocampal regions. This could be done by the 2DG method, as described in the *Fxyd6* experiment below. By applying methods from graph theory analysis on the data gained from 2DG (as described by Dawson *et al.* 2012; Dawson *et al.* 2014) on *Map2k7*+/-, the functional global and regional network interactions could be deciphered, enabling an insight into how *Map2k7*+/- affects working memory at a systems level.

Chapter 4: Effect of deletion of the *Fxyd6* gene upon regional brain activity in mice and the impact of NMDA-receptor antagonism on *Fxyd6-/-* regional brain activity

4.1 – Introduction and aim

As described in sections 1.9.2 *FXYD6* is a gene that may be involved in regulating NaKATPase function. Genetic association studies have also linked this gene as a possible risk factor for schizophrenia, particularly the cognitive components, due to the abnormal expression of FXYD6 discovered in the hippocampus and PFC of schizophrenia patients. It is hypothesized therefore, that abnormal FXYD6 expression may impair NaKATPase function in the PFC, hippocampus and other areas. Therefore contributing to some cognitive impairments in schizophrenia. By utilising a *Fxyd6-/-* strain of genetically modified mice and the post-mortem brain imaging technique – semi-quantitative 2DG autoradiography – this study aimed to investigate the contribution Fxyd6 has to regional brain activity. Furthermore to investigate a role for glutamate in relation to Fxyd6 and NaKATPase, regional brain activity was determined in *Fxyd6-/-* mice treated with the NMDA receptor antagonist, ketamine.

4.2 – Methods

The methods employed for the 2-DG study in *Fxyd6-/-* and WT mice are described in detail in Section 2.2

4.3 – Results

Over the next few sections the data will be referred to as the percentage difference of 2DG uptake ratio between compared groups (as outlined in tables 4b, 4c and 4d), and the p-values are sourced from the unpaired t-tests performed between the groups on the raw data of the 2DG uptake ratio (as outlined in tables 4e, 4f and 4g). A summary of the regions analysed and their corresponding abbreviations are included in table 4a.

Table 4a

Brain Regions

AMthal	*Anteromedial thalamus*	**CLthal**	*Centrolateral thalamus*	**Hab**	*Habenula*	**MG**	*Medial geniculate nucleus*	**NacC**	*Nucleus accumbens core*	**Sub**	*Subiculum*
AudC	*Auditory cortex*	**CMthal**	*Centromedial thalamus*	**HDB**	*Horizontal diagonal band*	**ML**	*Midline nuclei*	**NacS**	*Nucleus accumbens shell*	**VDB**	*Vertical diagonal band*
AVthal	*Anteroventral thalamus*	**DG**	*Dentate gyrus*	**IL**	*Infralimbic*	**MM**	*Mamillary body*	**Piri**	*Piriform cortex*	**VLthal**	*Ventrolateral thalamus*
BLA	*Basolateral amygdala*	**DLO**	*Dorsolateral orbital cortex*	**InsC**	*Insular cortex*	**MO**	*Medial oribital cortex*	**PrL**	*Anterior prelimbic area*	**VMST**	*Ventromedial striatum*
CA1	*CA1*	**DLST**	*Dorsolateral striatum*	**LO**	*Lateral orbital cortex*	**mPrL1**	*Medial prelimbic (layer 1)*	**Re**	*Reuniens*	**VMthal**	*Ventromedial thalamus*
CA2	*CA2*	**DR**	*Dorsal raphe nucleus*	**LS**	*Lateral septum*	**mPrL2**	*Medial prelimbic (layer 2)*	**RSC**	*Retrosplenial cortex*	**VO**	*Ventral orbital cortex*
CA3	*CA3*	**dRT**	*Dorsal reticular nucleus*	**M1**	*Primary motor cortex*	**mPrL3**	*Medial prelimbic (layer 3)*	**SNC**	*Substantia nigra pars compacta*	**vRT**	*Ventral reticular nucleus*
CeA	*Central amygdaloid*	**FRA**	*Frontal association*	**MDthal**	*Mediodorsal thalamus*	**MR**	*Median raphe nucleus*	**SNR**	*Substantia nigra pars reticulata*	**VTA**	*Ventral tegmental area*
Cg1	*Cingulate gyrus*	**GP**	*Globus pallidus*	**MeA**	*Medial amygdaloid*	**MS**	*Medial septum*	**SSCTX**	*Somatosensory cortex*	**VTg**	*Ventral tegmental nucleus*

Table 4a: Summary of regions analysed in the 2-DG experiment with their corresponding abbreviations

4.3.1 – Cortical regions

In the 17 cortical regions examined (Table 4b and 4e), there was no significant genotype effect on 2DG uptake, indicating that knocking out the Fxyd6 gene produced no overt effects on cortical activity. While some areas seem to exhibit large differences in glucose uptake between WT and KO mice (dorsolateral orbital (DO)=18.87%; medial prelimbic layer 1 (mPrL1)=12.52%; medial prelimbic layer 2 (mPrl2)=13.51%; medial prelimbic layer 3(mPrl3)=11.82%; somatosensory cortex (SSCTX)=-19.12%) none of these values were found to be statistically significant ($p>0.05$ for all, unpaired t-test).

Ketamine produced a significant increase in 2DG uptake in several cortical regions of WT mice, including regions of the ventral orbital (VO, 18%, p=0.01), the medial orbital (MO, 14.46%, p=0.04) the lateral orbital (LO, 14.89%, p=0.03) the infralimbic (IL,13.30%,p=0.04) and the anterior prelimbic (PrL, 18.11%, p=0.03). A significant decrease in 2DG uptake was also observed in the auditory cortex (AudC) of ketamine treated WT mice (-13.21%, p=0.01). Interestingly, the PrL region in ketamine treated *Fxyd6-/-* mice was the only cortical region to exhibit a similar effect to regions affected by ketamine in WT mice (29.58%, p=0.001) where the VO, MO, LO, IL and AudC did not show statistically significant differences in activation (all $p>0.05$; figure 4b and 4c). It should be noted that these differences in 2-DG uptake between ketamine treated WT mice and ketamine treated *Fxyd6-/-* mice were not found to be statistically significant (as shown in the fourth column of table 4b), however, this could be attributed to the lower numbers in the *Fxyd6-/-* sample.

4.3.2 – Thalamic, Hippocampal and Amygdaloid Regions

Of the regions analysed in the thalamus (table 4c and 4f), no significant genotype effect on 2-DG uptake was found. With *Fxyd6-/-* mice showing similar values to WT mice. However, there appeared to be a large difference (28%) between genotypes in the mediodorsal thalamus (MDthal) although this was not found to be statistically significant (p=0.18). Ketamine treatment in WT mice produced a marked reduction in 2-DG uptake in certain areas of the thalamus including the ventromedial thalamus (VMthal, -9.39%, p=0.01), the dorsal reticular (dRT, -23.38%, p=0.0004), the ventral

reticular (vRT, -22.75%, p=0.001) and the medial geniculate (MG, -17.54% p=0.002) (figure 4b). The only region that showed a similar effect in ketamine treated KO mice (that was statistically significant), was the vRT region, with a difference of activation of -16.21% (p=0.01) (table 4c and 4f, and figure 4c). While the far column of table 4c, describes how no statistically significant differences were observed between ketamine treatment of KO and WT mice in thalamic regions, once again this may be a result of the smaller numbers in the *Fxyd6-/-* sample (n=4 per group compared to n=6 per group in the WT groups).

Interestingly, the subiculum (sub) region of the hippocampal complex showed a marked decrease in mean 2-DG uptake in *Fxyd6-/-* mice compared to WT mice (-10.11%, p=0.02) (tables 4c, 4f and figure 4a), a region heavily linked with working memory and spatial cognition, with connections to the PFC, amygdala and entorhinal cortex.

The basolateral (BLA), central (CeA) and medial (MeA) regions of the amygdala, and the, CA1, CA2, CA3 and dentate gyrus (DG) regions of the hippocampus did not exhibit significant differences in their regional activation from any of the given variables (p>0.05 for all) (table 4c and 4f).

4.3.3 – Septal, Basal Ganglia, and Mesolimbic Regions

Both the medial septum (MS) and the lateral septum (LS) of saline treated *Fxyd6-/-* mice exhibited a significant reduction in 2-DG uptake compared to saline treated WT mice, with differences of -13.88% (p=0.05) and -13.92% (p=0.01) respectively (table 4d and 4g, figure 4a). Furthermore, both these areas were further affected in WT mice treated with ketamine, exhibiting differences of 2-DG uptake by -17.74% in the MS (p=0.001), and -15.70% in the LS (p=0.03). These regions did not show significant differences in 2DG uptake in ketamine treated *Fxyd6-/-* mice (MS, 0.43%, p=0.96; LS, -6.87% p=0.39). Similarly the differences between ketamine treated *Fxyd6-/-* and WT mice were not found to be statistically significant. However, the VDB (nucleus of the vertical limb of the diagonal band of Broca) did show a significant difference in glucose uptake between WT and *Fxyd6-/-* mice in response to ketamine treatment. WT mice treated with ketamine showed a 28.68% reduction in 2-DG uptake

compared to saline treated WT mice (-28.68%, p=0.01). Whereas ketamine-treated *Fxyd6-/-* mice did not exhibit a significant difference compared to saline treated *Fxyd6-/-* mice (18.61%, p=0.37). This difference in response to ketamine treatment between genotypes was found to be significant with a difference of 23.43%, p=0.04.

Of the basal ganglia and mesolimbic regions analysed, no difference was found between mean 2DG uptake ratio of saline treated WT mice and saline treated *Fxyd6-/-* mice. However, the globus pallidus (GP) of the basal ganglia exhibited a significant reduction in 2DG uptake in WT mice treated with ketamine (-12.98%, p=0.0001), not seen in ketamine treated *Fxyd6-/-* mice (-1.34%, p=0.89). Whilst the substantia nigra pars compacta (SNC) region of the basal ganglia was affected in ketamine treated *Fxyd6-/-* mice compared to saline treated *Fxyd6-/-* animals (-16.78%, p=0.05), a comparable effect of ketamine was not observed in WT mice (-12.48%, p=0.18).

4.3.4 – Multimodal and Neuromodulatory

There were no significant effects from any of the variables observed in the neuromodulatory regions of the dorsal raphe (DR) and median raphe (MR) (all p>0.05) (table 4d and 4g). Furthermore, the multimodal regions of the habenula (Hab) and mamillary bodies (table 4b and 4g) did not show significant differences in 2-DG uptake between saline treated *Fxyd6-/-* and saline treated WT mice (p>0.05), and the genotype groups treated with ketamine (p>0.05). While not statistically significant, it may be worth noting the large percentage difference of glucose uptake in the mammillary body in saline treated *Fxyd6-/-* mice compared to saline treated WT mice of 19.66%, a region which is heavily innervated by the subiculum and also known to be involved in components of memory.

4.4 – Discussion

In this experiment a number of brain regions in the tested mice were shown to exhibit significant hyperactivity or hypoactivity (as measured by mean 2-DG uptake ratio) in response to subanesthetic ketamine treatment or the knockout of the *Fxyd6* gene. Furthermore it was found that the presence or absence of the *Fxyd6* gene affected how certain regions responded to ketamine treatment. Figure 4a is featured

below to illustrate the brain regions that were significantly affected by the knockout of *Fxyd6*. Figures 4b and 4c are included to compare which brain regions were significantly affected by ketamine and which regions were not significantly affected by ketamine in wild type and *Fxyd6*-/- mice.

Of the 54 regions investigated, three were affected by the *Fxyd6* knockout. These were the subiculum, the medial septum and the lateral septum. Perhaps the most interesting of these results was the significant decrease in activation observed in the subiculum region of the hippocampal formation in saline treated *Fxyd6*-/- mice compared to saline treated WT mice. The subiculum is the most inferior part of the hippocampal formation, is largely innervated by inputs from the CA1, entorhinal cortical pyramidal III neurons and prefrontal cortical regions, and is the main output of the hippocampus (Witter & Groenewegen 1990; O'Mara *et al.* 2001). Furthermore the subiculum also shares reciprocal connections with subcortical regions including the ventral premammillary nucleus (connected to mammillary body, a region that showed a large increase in activity in *Fxyd6*-/-, although not statistically significant) the medial septum (which also exhibited a significant hypoactivation in *Fxyd6*-/- mice compared to WT animals), the AMthal & AVthal regions of the thalamus, and the amygdala (Canteras & Swanson 1992; Risold *et al.* 1997). Crucially, as has been described above, the subiculum and hippocampal regions are heavily associated with memory functions including memory consolidation and working memory. Evidence from studies has linked subiculum function to working memory maintenance and disambiguation of previously learned information from current information maintained in working memory (Hampson *et al.* 2000, Newmark *et al.* 2013). This may suggest an important role for the subiculum in discriminating old memories and newly formed memories in working memory, and hence pro and retro-active interference. This finding backs up the hypothesis that regions associated with cognition would be affected by knocking-out the *FXYD6* gene. Furthermore this continues the argument for FXYD6's role in the working memory impairments that are characteristic of many psychiatric disorders, including schizophrenia. Interestingly, the subiculum and septal areas are both components of the brain-motivation/reward circuit which also include the nucleus accumbens, the PFC and

the VTA (Cooper *et al.* 2006). This effect of *Fxyd6* deletion on subiculum and septal activation, therefore, may be an interesting area of behavioural investigation for *Fxyd6* knock outs, particularly in motivational and reward paradigms. This finding also highlights the need for further cognitive investigations of *Fxyd6*-/- mice, particularly for working memory. This would be the suggested next step in the discovery of a role for FXYD6 in cognition and psychiatric disorders.

It was also found that a number of regions were significantly affected by ketamine treatment in WT mice but which had a blunted response *Fxyd6*-/- mice. These included the cortical regions of the PrL, DLO, MO, VO, LO, IL and the AuC; thalamic regions of the VMThal, dRT, Vrt and MG; septal regions of the MS, LS and VDB; and the GP of the basal ganglia. Previous research by Dawson (Dawson *et al.* 2013; Dawson *et al.* 2014) into the effect of ketamine on region cortical activity in C57BL/6 mice using 2DG autoradiography produced some similarities and differences with the present data. For example, in the present study significant increases in brain metabolism were exhibited in cortical regions of the DLO, MO, VO, LO and the IL in this experiment; none of which were affected in both studies by Dawson *et al.* The PrL was the only cortical region to show significant increases in glucose metabolism across all the studies. In Dawson *et al.* 2013 and 2014, all layers of the mPrL and the entorhinal cortex exhibited hypermetabolism, which were not observed in the present study. These significant differences are hard to account for, although it may be a result of the larger number of mice used in the Dawson *et al.* experiments (9 saline treated WT mice and 9 ketamine treated WT mice in both studies); thereby increasing statistical power in the study. For example the Dawson *et al.* 2013 study showed that mPrL1, mPrL2 and mPrL3 exhibited statistically significant glucose metabolism increases of 22%, 18% and 16% in ketamine treated mice compared to saline treated, which is similar to what was found in the present experiment (mPrL1= 20.02%; mPrL2=16.70%; mPrL3=15.21%). However, it should be noted that there were various regions in the PFC which showed significance in this study, which did not come out as significant in the Dawson studies. Therefore it is hard to conclude whether increasing power would have produced similar results. It also should be noted that the dose of ketamine was slightly different in this experiment (25.mg.kg-

1) to the Dawson *et al.* experiments (30.mg.kg-1); which may also contribute to the differing results.

Interestingly, the dRT region of the thalamus showed significant hyperactivation across all the investigations in WT animals. It is thought that dRT, along with the MDThal, are components of a network which also includes the PFC and anterior thalamus (dRT-AV/MDthal-PFC) which is a crucial system that ketamine affects in order to model aspects of schizophrenia (Dawson *et al.* 2013). Whereby NMDAR antagonism of GABAergic neurons in the dRT produces a disinhibition of glutamatergic projections from the AV/MDthal to the PFC, subsequently producing a hypermetabolism of the PFC (this may explain the hypometabolism exhibited in several regions of the PFC in ketamine treated WT mice). The dRT is involved in attentional shifting (modulated by the PFC) (McAlonan *et al.* 2008; McAlonan *et al.* 2006) and auditory gating (Krause *et al.* 2003), both of which are cognitive processes known to be disrupted in schizophrenia (and various other psychiatric disorders) (Freedman *et al.* 2003; Luck & Gold 2008) and which are also disrupted by NMDAR antagonists (Dai & Carey 1994 Egerton *et al.* 2005; Ferrarelli & Tononi 2011). This highlights the dRT as a possible hub region that may affect various processes involved in attention whereby abnormal functioning of this region may contribute to attentional deficits in psychiatric disorders, including schizophrenia. This experiment did find vRT hypoactivation that was not observed in the Dawson *et al.* studies, and did not find AVthal hypometabolism, that was observed in the Dawson experiment. This discrepancy could have arisen from the experimental design differences described above. Other regions that exhibited similar reactions to ketamine across all the studies included the VDB of the diagonal band and the globus pallidus (GP) of the basal ganglia.

By comparing how ketamine affected regional glucose metabolism in WT and *Fxyd6*-/- mice, one is able to make inferences and conclusions about how Fxyd6 expression, and NaKATPase interacts and contributes to NMDA receptor antagonism & glutamate function in regional areas. In the cortical regions of both genotypes only the PrL region exhibited similar ketamine-induced hypometabolism. Importantly,

there were various regions affected by ketamine in WT mice that appeared not to be affected in *Fxyd6*-/- mice, including the hypermetabolism in the DLO, MO,VO, LO and IL of the PFC; hypometabolism in regions of the AuC, the VMthal, dRT and MG of the thalamus, and the GP of the basal ganglia (illustrated in figures 4b and 4c). It can be observed therefore, that the PFC regions of ketamine treated KO mice did not exhibit the typical hypermetabolism that would be expected, and the hypometabolism of the dRT and other thalamic regions was not observed either (in comparison to ketamine treatment of WT in this study and other studies). Hence it would appear that the *Fxyd6-/-* mice show a blunted response to ketamine in the prefrontal cortex and thalamic regions suggesting a possible dysfunction of NMDA receptors in these animals.

An alternative explanation relates to the interaction of *Fxyd6-/-* with NaKATPase. NaKATPase is known to play a role in deactivating glutamate signalling in the brain by facilitating glutamate reuptake into glia and neurons via glutamate transporters (Rose *et al.* 2009; Illarionava *et al.* 2014). Because regions of the thalamus provide GABAergic inhibition to regions of the PFC (which is inhibited by NMDAR antagonists like ketamine) this disruption in NaKATPase function (by knocking out *Fxyd6*) may indirectly compensate for the blockage of NMARs in GABAergic neurons by reducing reuptake of glutamate, and therefore, increasing post-synaptic glutamate. This could account for the unaffected prefrontal and thalamic regions in *Fxyd6*-/- mice. This could highlight an interesting area of future investigation for drug discovery for mediating prefrontal hyperactivity in the prefrontal cortex and hyperconnectivity between thalamic and PFC regions which is exhibited in schizophrenia patients. This would also depend, however, on the construct validity of NMDAR antagonism itself, and whether it actually does reflect neurobiological components of schizophrenia. It would be interesting to apply graph theory modelling using data gained from this 2DG experiment in order to decipher functional networks among the regions measured (Dawson *et al.* 2013; Dawson *et al.* 2014). This could give further insight into how FXYD6 and NaKATPase affects regional and global systems with relevance to cognition and psychiatric disorders.

It is also worth noting that the cingulate gyrus (Cg1) of ketamine treated *Fxyd6*-/- mice exhibited a significant difference as described in the fourth column of table 4b. However, as no significant difference was seen between saline treated KO mice and WT mice in the Cg1; along with very similar responses to ketamine in both groups (which were not found to be significantly different) it could be concluded that this result may be an anomaly.

Figure 4a

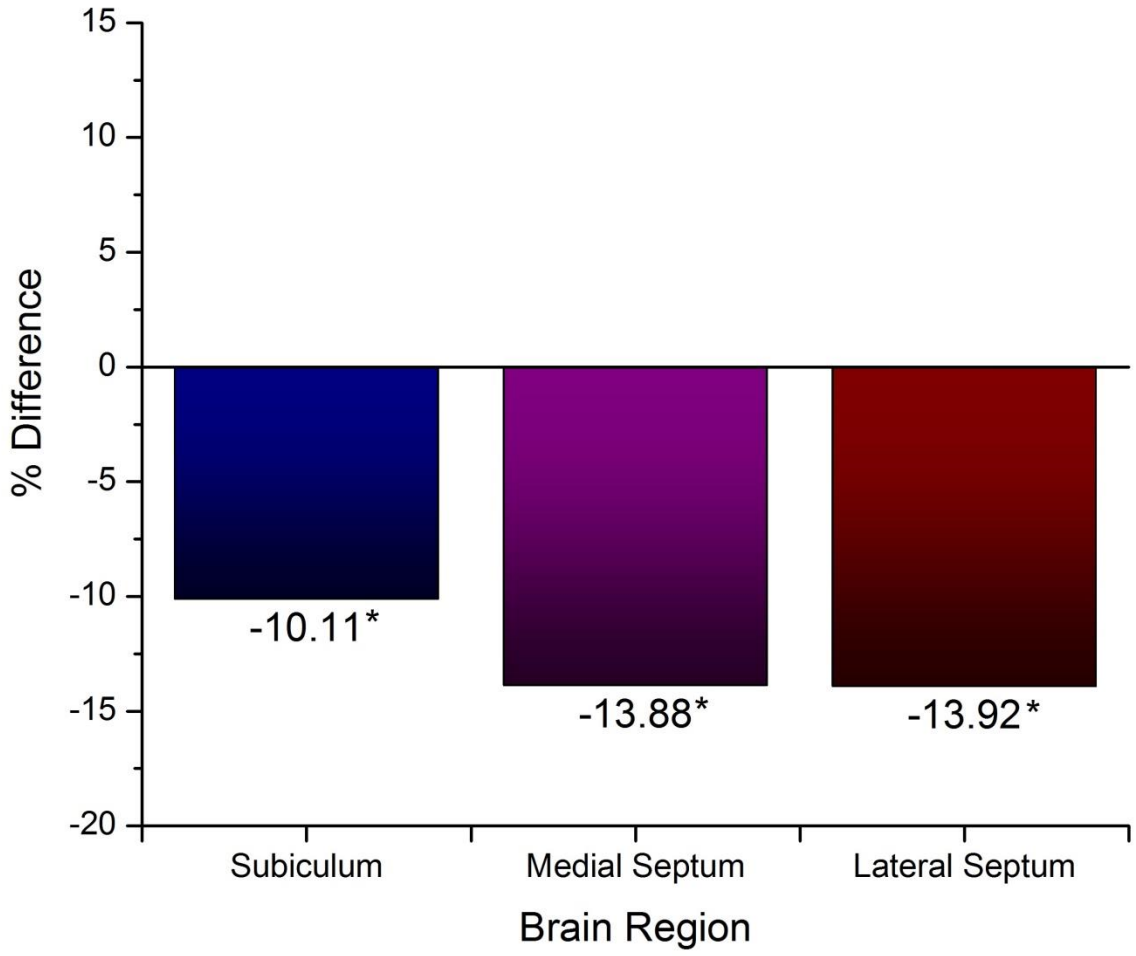

Figure 4a: Plot of regions that were significantly affected by Fxyd6 KO. Measured in % differences of 2DG uptake ratio between WT and Fxyd6-/- mice. '' denotes significant difference. Sample size: Saline WT = 6; Saline Fxyd6-/- = 4.*

Figure 4b

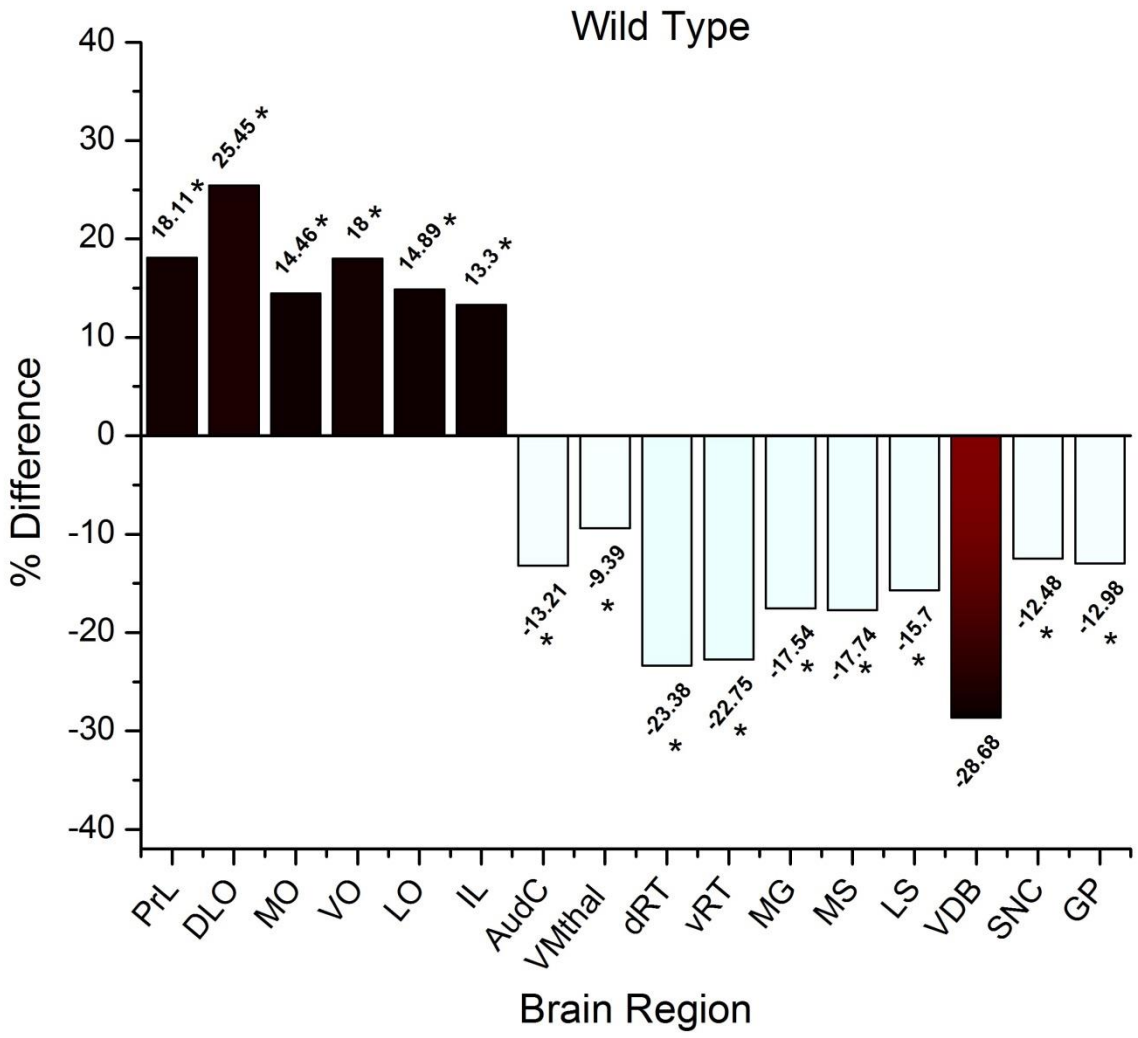

Figure 4b: Plot of brain regions of WT mice that were significantly affected by ketamine and ones not affected. '' denotes regions significantly affected by ketamine, RED bars denote regions NOT significantly affected by ketamine. Measured in % differences of 2DG uptake ratio between saline treated WT and ketamine treated WT mice. Sample size: Saline WT = 6; Ketamine WT = 6.*

Figure 4c

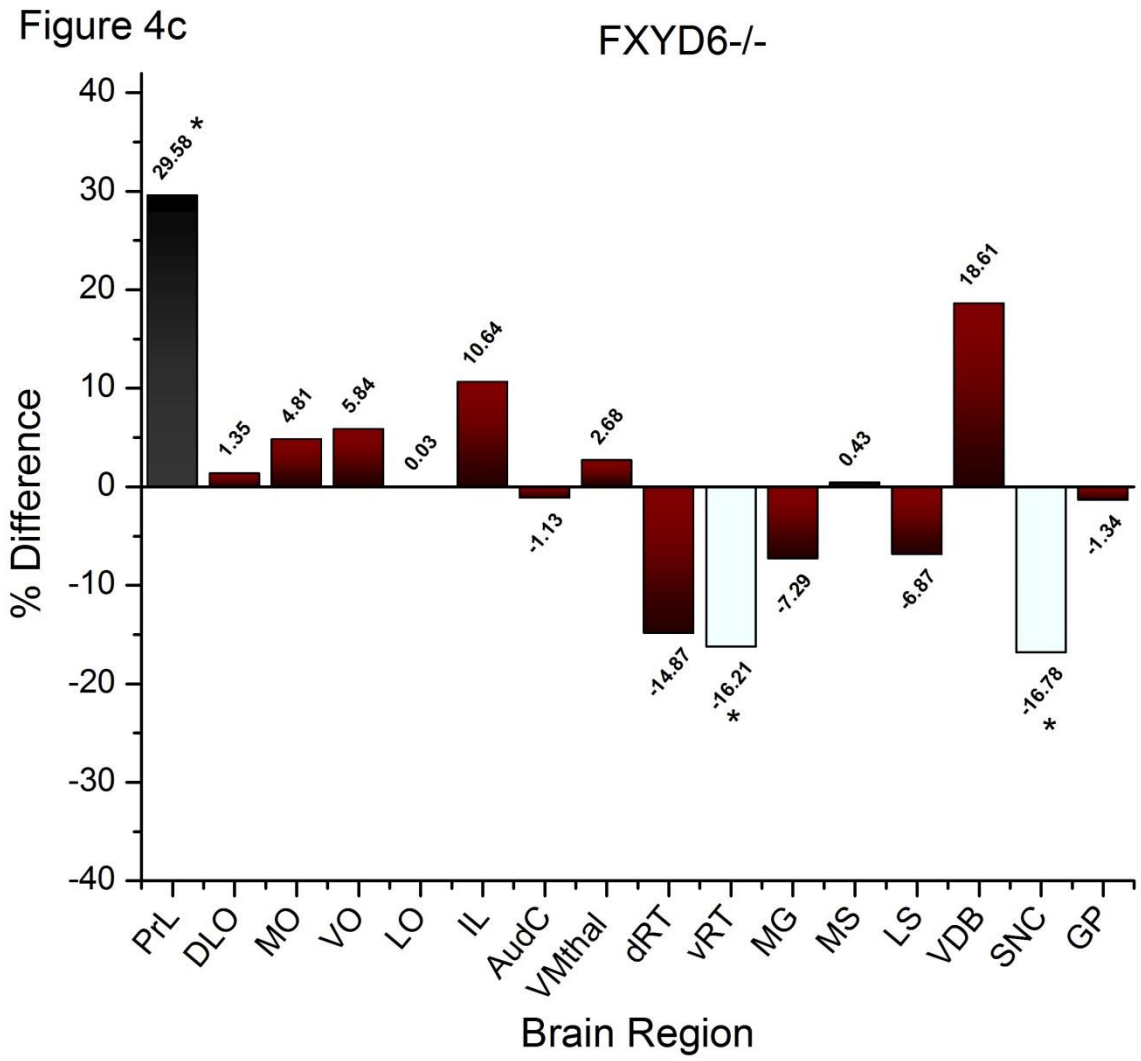

Figure 4c: Plot of brain regions of Fxyd6-/- mice that were significantly affected by ketamine and ones not affected. '' denotes significantly affected region by ketamine, RED bars denote regions NOT significantly affected by ketamine. Measured in % differences of 2DG uptake ratio between saline treated Fxyd6-/- and ketamine treated Fxyd6-/- mice. Sample size: Saline Fxyd6-/- = 4; Ketamine Fxyd6-/- = 4.*

Table 4b: Effect of genotype and ketamine on regional glucose uptake in *Fxyd6*-/- mice and wild type mice

	Genotype Effect		Treatment Effect - WT		Treatment Effect - *Fxyd6-/-*		Treatment WT * Treatment *Fxyd6-/-*	
Cortex	**%**	**p**	**%**	**p**	**%**	**p**	**%**	**p**
Anterior prelimbic (PrL)	*-2.68*	*0.71*	***18.11****	***0.03***	***29.58****	***0.001***	*6.78*	*0.30*
Frontal association (FRA)	*3.98*	*0.41*	*5.59*	*0.29*	*0.58*	*0.93*	*-0.95*	*0.89*
Dorsolateral orbital (DLO)	*18.87*	*0.08*	***25.45****	***0.03***	*1.35*	*0.92*	*-3.97*	*0.74*
Medial oribital (MO)	*9.46*	*0.14*	***14.46****	***0.04***	*4.81*	*0.59*	*0.23*	*0.98*
Ventral orbital (VO)	*10.61*	*0.20*	***18.00****	***0.01***	*5.84*	*0.43*	*-0.80*	*0.85*
Lateral orbital (LO)	*7.89*	*0.20*	***14.89****	***0.03***	*6.59*	*0.15*	*0.10*	*0.98*
Medial prelimbic (layer 1) (mPrL1)	*12.52*	*0.23*	*20.02*	*0.14*	*16.82*	*0.26*	*9.52*	*0.52*
Medial prelimbic (layer 2) (mPrL2)	*13.51*	*0.12*	*16.70*	*0.13*	*6.86*	*0.59*	*3.94*	*0.76*
Medial prelimbic (layer 3) (mPrL3)	*11.82*	*0.12*	*15.21*	*0.10*	*9.00*	*0.45*	*5.79*	*0.61*
Infralimbic (IL)	*5.79*	*0.27*	***13.30****	***0.04***	*10.64*	*0.27*	*3.31*	*0.69*
Cingulate (Cg1)	*5.91*	*0.43*	*4.33*	*0.27*	*6.31*	*0.41*	***7.91****	***0.01***
Primary motor (M1)	*0.96*	*0.85*	*-4.64*	*0.39*	*-9.77*	*0.25*	*-4.47*	*0.58*
Retrosplenial (RSC)	*-3.16*	*0.63*	*-12.78*	*0.09*	*-3.73*	*0.61*	*6.89*	*0.44*
Piriform (Piri)	*-3.79*	*0.68*	*-2.67*	*0.73*	*0.27*	*0.98*	*-0.89*	*0.91*
Insular (InsC)	*-1.60*	*0.75*	*3.10*	*0.74*	*4.19*	*0.67*	*-0.57*	*0.96*
Somatosensory Cortex (SSCTX)	*-19.12*	*0.43*	*-9.16*	*0.29*	*-2.28*	*0.95*	*-12.99*	*0.34*
Auditory Cortex (AudC)	*-4.23*	*0.62*	***-13.21****	***0.01***	*-1.13*	*0.91*	*9.10*	*0.13*
Multimodal								
Habenula (Hab)	*1.14*	*0.85*	*-3.93*	*0.61*	*-2.28*	*0.86*	*2.87*	*0.83*
Mamillary body (MM)	*19.66*	*0.33*	*24.68*	*0.11*	*-1.42*	*0.92*	*-5.38*	*0.58*

Table 4b: Comparison of alterations in regional activity in selected regions of the brains of wild type and Fxyd6-/- (KO) mice receiving ketamine or saline treatment. Data shown as % difference of mean uptake ratio between treated and control groups. 'Genotype Effect' = Comparing saline treated KO with saline WT; 'Treatment Effect – WT' = Comparing ketamine treated WT with saline WT; 'Treatment Effect – Fxyd6-/-' = Comparing ketamine treated KO with saline KO; 'Treatment WT Treatment Fxyd6-/-' = comparing ketamine treated KO with ketamine treated WTs. Saline WT n=6; Ketamine WT n=6; Saline KO n=4; Ketamine KO n=4. '*' represents significant difference. P values from two sample, unpaired t-tests. Acceptable levels of significance set at p<0.05.*

Table 4c: Effect of genotype and ketamine on regional glucose uptake in *Fxyd6*-/- mice and wild type mice

	Genotype Effect		Treatment Effect - WT		Treatment Effect - *Fxyd6-/-*		Treatment WT * Treatment *Fxyd6-/-*	
Thalamus	**%**	**p**	**%**	**p**	**%**	**p**	**%**	**p**
Anteromedial (AMthal)	*0.98*	*0.91*	*-2.18*	*0.75*	*-16.69*	*0.39*	*-14.00*	*0.37*
Anteroventral (AVthal)	*6.06*	*0.56*	*-2.55*	*0.76*	*-12.45*	*0.38*	*-14.00*	*0.69*
Mediodorsal (MDthal)	*-28.04*	*0.18*	-9.61	0.05	*36.30*	*0.33*	*8.50*	*0.27*
Centromedial (CMthal)	*-2.28*	*0.77*	*-3.44*	*0.52*	*-7.84*	*0.49*	*-6.73*	*0.38*
Centrolateral (CLthal)	*0.84*	*0.93*	*-2.13*	*0.79*	*-0.03*	*1.00*	*3.00*	*0.69*
Ventrolateral (VLthal)	*-0.37*	*0.98*	*-16.85*	*0.06*	*-14.66*	*0.30*	*2.26*	*0.83*
Ventromedial (VMthal)	*-4.43*	*0.40*	***-9.39****	***0.01***	*2.68*	*0.80*	*8.31*	*0.33*
Reuniens (Re)	*-7.58*	*0.52*	*-19.07*	*0.11*	*12.64*	*0.48*	*28.63*	*0.15*
Dorsal reticular (dRT)	*-7.74*	*0.19*	***-23.38****	***0.0004***	*-14.87*	*0.09*	*2.51*	*0.74*
Ventral reticular (vRT)	*-4.13*	*0.39*	***-22.75****	***0.001***	***-16.21****	***0.01***	*3.99*	*0.52*
Medial geniculate (MG)	*-2.86*	*0.65*	***-17.54****	***0.002***	*-7.29*	*0.37*	*9.21*	*0.17*
Midline Nuclei (ML)	*-5.11*	*0.63*	*-10.39*	*0.26*	*-13.73*	*0.10*	*-8.65*	*0.26*
Amygdala								
Basolateral (BLA)	*-8.62*	*0.22*	*-11.37*	*0.12*	*4.27*	*0.58*	*7.50*	*0.40*
Central (CeA)	*-9.15*	*0.35*	*-18.46*	*0.15*	*13.47*	*0.56*	*26.43*	*0.30*
Medial (meA)	*-0.96*	*0.95*	*-10.42*	*0.33*	*-6.20*	*0.78*	*3.71*	*0.83*
Hippocampus								
Subiculum (Sub)	***-10.11****	***0.02***	*4.38*	*0.57*	*5.93*	*0.22*	*-8.77*	*0.35*
CA1	*1.05*	*0.85*	*8.63*	*0.18*	*4.67*	*0.37*	*-2.64*	*0.68*
CA2	*-2.20*	*0.73*	*-10.71*	*0.15*	*-5.13*	*0.69*	*3.91*	*0.77*
CA3	*-8.17*	*0.58*	*-14.27*	*0.28*	*-7.85*	*0.33*	*-1.29*	*0.90*
Dentate gyrus (DG)	*-13.25*	*0.51*	*-19.87*	*0.18*	*-3.09*	*0.87*	*4.91*	*0.66*

Table 4c: Comparison of alterations in regional activity in selected regions of the brains of wild type and Fxyd6-/- (KO) mice receiving ketamine or saline treatment. Data shown as % difference of mean uptake ratio between treated and control groups. 'Genotype Effect' = Comparing saline treated KO with saline WT; 'Treatment Effect – WT' = Comparing ketamine treated WT with saline WT; 'Treatment Effect – Fxyd6-/-' = Comparing ketamine treated KO with saline KO; 'Treatment WT Treatment Fxyd6-/-' = comparing ketamine treated KO with ketamine treated WTs. Saline WT n=6; Ketamine WT n=6; Saline KO n=4; Ketamine KO n=4. '*' represents significant difference. P values from two sample, unpaired t-tests. Acceptable levels of significance set at p<0.05.*

Table 4d: Effect of genotype and ketamine on regional glucose uptake in *Fxyd6-/-* mice and wild type mice

	Genotype Effect		Treatment Effect - WT		Treatment Effect *Fxyd6-/-*		Treatment WT * Treatment *Fxyd6-/-*	
Septum/Diagonal Band of Broca	**%**	**p**	**%**	**p**	**%**	**p**	**%**	**p**
Medial septum (MS)	***-13.88****	***0.05***	***-17.74****	***0.001***	*0.43*	*0.96*	*5.14*	*0.32*
Lateral septum (LS)	***-13.92****	***0.01***	***-15.70****	***0.03***	*-6.87*	*0.39*	*-4.91*	*0.61*
Vertical DB (VDB)	*-25.77*	*0.12*	***-28.68****	***0.01***	*18.61*	*0.37*	***23.43****	***0.04***
Horizontal DB (HDB)	*-6.40*	*0.53*	*-9.39*	*0.11*	*-3.88*	*0.76*	*-0.71*	*0.91*
Basal ganglia								
Ventromedial striatum (VMST)	*-9.81*	*0.08*	*4.26*	*0.45*	*17.64*	*0.14*	*1.77*	*0.84*
Dorsolateral striatum (DLST)	*1.93*	*0.70*	*1.76*	*0.66*	*1.12*	*0.85*	*1.29*	*0.78*
Substantia nigra pars reticulata (SNR)	*-4.80*	*0.33*	*-1.76*	*0.80*	*-3.81*	*0.57*	*-6.79*	*0.45*
Substantia nigra pars compacta (SNC)	*-0.93*	*0.91*	*-12.48*	*0.18*	***-16.78****	***0.05***	*-5.80*	*0.56*
Globus pallidus (GP)	*-3.82*	*0.17*	***-12.98****	***0.0001***	*-1.34*	*0.89*	*9.04*	*0.28*
Mesolimbic								
Nucleus accumbens core (NacC)	*1.10*	*0.88*	*11.72*	*0.14*	*13.04*	*0.07*	*2.30*	*0.74*
Nucleus accumbens shell (NacS)	*-5.93*	*0.55*	*2.65*	*0.61*	*11.10*	*0.34*	*1.82*	*0.64*
Ventral tegmental nucleus (VTg)	*-1.36*	*0.82*	-11.60	0.05	*-32.14*	*0.29*	*-24.27*	*0.38*
Ventral tegmental area (VTA)	*1.65*	*0.84*	*-6.65*	*0.28*	*-14.47*	*0.10*	*-6.86*	*0.26*
Neuromodulatory								
Dorsal raphe (DR)	*-12.73*	*0.08*	*-8.81*	*0.26*	*-33.58*	*0.27*	*-36.44*	*0.16*
Median raphe (MR)	*-9.02*	*0.21*	*-12.71*	*0.13*	*-16.14*	*0.65*	*-12.59*	*0.69*

Table 4d: Comparison of alterations in regional activity in selected regions of the brains of wild type and Fxyd6 -/- (KO) mice receiving ketamine or saline treatment. Data shown as % difference of mean uptake ratio between treated and control groups. 'Genotype Effect' = Comparing saline treated KO with saline WT; 'Treatment Effect – WT' = Comparing ketamine treated WT with saline WT; 'Treatment Effect – Fxyd6-/-' = Comparing ketamine treated KO with saline KO; 'Treatment WT Treatment Fxyd6-/-' = comparing ketamine treated KO with ketamine treated WTs. Saline WT n=6; Ketamine WT n=6; Saline KO n=4; Ketamine KO n=4. '*' represents significant difference. P values from two sample, unpaired t-tests. Acceptable levels of significance set at p<0.05.*

Table 4e	WT Saline	n=6	WT Ketamine	n=6	*Fxyd6-/-* Saline	n=4	*Fxyd6-/-* Ketamine	n=4
Cortex	**Mean**	**SDEV**	**Mean**	**SDEV**	**Mean**	**SDEV**	**Mean**	**SDEV**
Anterior prelimbic (PrL)	*0.9712*	*0.1204*	*1.1471*	*0.1275*	*0.9452*	*0.0763*	*1.2248*	*0.0633*
Frontal association (FRA)	*1.0365*	*0.0871*	*1.0944*	*0.0938*	*1.0777*	*0.041*	*1.084*	*0.1379*
Dorsolateral orbital (DLO)	*1.1388*	*0.1953*	*1.4287*	*0.1939*	*1.3538*	*0.1009*	*1.372*	*0.3357*
Medial oribital (MO)	*0.9441*	*0.0765*	*1.0806*	*0.1232*	*1.0334*	*0.0957*	*1.0832*	*0.1472*
Ventral orbital (VO)	*1.3305*	*0.1594*	*1.57*	*0.0684*	*1.4716*	*0.1525*	*1.5575*	*0.1325*
Lateral orbital (LO)	*1.4632*	*0.1379*	*1.681*	*0.1599*	*1.5786*	*0.1089*	*1.6827*	*0.0601*
Medial prelimbic (layer 1) (mPrL1)	*0.8652*	*0.1446*	*1.0384*	*0.2191*	*0.9735*	*0.1002*	*1.1372*	*0.2415*
Medial prelimbic (layer 2) (mPrL2)	*0.8715*	*0.1067*	*1.0171*	*0.1863*	*0.9893*	*0.1018*	*1.0572*	*0.2147*
Medial prelimbic (layer 3) (mPrL3)	*0.8543*	*0.1017*	*0.9843*	*0.1435*	*0.9553*	*0.0671*	*1.0413*	*0.2018*
Infralimbic (IL)	*0.7852*	*0.0512*	*0.8896*	*0.0963*	*0.8306*	*0.0701*	*0.919*	*0.1271*
Cingulate (Cg1)	*1.2191*	*0.0973*	*1.2719*	*0.0533*	*1.2911*	*0.1769*	*1.3725*	*0.0437*
Primary motor (M1)	*1.135*	*0.1006*	*1.0823*	*0.1046*	*1.1459*	*0.0625*	*1.0339*	*0.1631*
Retrosplenial (RSC)	*1.48*	*0.0897*	*1.2909*	*0.2033*	*1.4332*	*0.1834*	*1.3798*	*0.0719*
Piriform (Piri)	*1.024*	*0.1474*	*0.9967*	*0.1116*	*0.9852*	*0.1215*	*0.9879*	*0.1286*
Insular (InsC)	*0.9373*	*0.0732*	*0.9664*	*0.1948*	*0.9223*	*0.0671*	*0.9609*	*0.1592*
Somatosensory Cortex (SSCTX)	*1.1903*	*0.1534*	*1.0813*	*0.1695*	*0.9627*	*0.6647*	*0.9408*	*0.2438*
Auditory Cortex (AudC)	*1.1782*	*0.1097*	*1.0226*	*0.0696*	*1.1284*	*0.1973*	*1.1156*	*0.1054*

Table 4e: Comparison of alterations in regional activity in selected regions of the brains of wild type and Fxyd6 -/- (KO) mice receiving ketamine or saline treatment. Data shown as raw mean 2DG uptake ratio for each region +/-SDEV.

Table 4f	WT Saline	n=6	WT Ketamine	n=6	*Fxyd6-/-* Saline	n=4	*Fxyd6-/-* Ketamine	n=4
Thalamus	**Mean**	**SDEV**	**Mean**	**SDEV**	**Mean**	**SDEV**	**Mean**	**SDEV**
Anteromedial (AMthal)	*1.4773*	*0.1439*	*1.4451*	*0.19*	*1.4917*	*0.2489*	*1.2428*	*0.4808*
Anteroventral (AVthal)	*1.5698*	*0.2257*	*1.5298*	*0.2108*	*1.665*	*0.2751*	*1.4578*	*0.3407*
Mediodorsal (MDthal)	*1.4226*	*0.0799*	*1.2859*	*0.127*	*1.0237*	*0.6847*	*1.3952*	*0.1629*
Centromedial (CMthal)	*1.0732*	*0.0817*	*1.0363*	*0.1069*	*1.0487*	*0.1801*	*0.9666*	*0.1284*
Centrolateral (CLthal)	*1.4185*	*0.2135*	*1.3883*	*0.1615*	*1.4304*	*0.1479*	*1.43*	*0.1461*
Ventrolateral (VLthal)	*1.336*	*0.2385*	*1.1108*	*0.0987*	*1.3311*	*0.2334*	*1.1359*	*0.2481*
Ventromedial (VMthal)	*1.406*	*0.0754*	*1.2739*	*0.079*	*1.3438*	*0.1485*	*1.3797*	*0.2361*
Reuniens (Re)	*1.107*	*0.2072*	*0.8959*	*0.1761*	*1.0231*	*0.1694*	*1.1524*	*0.3035*
Dorsal reticular (dRT)	*1.3398*	*0.0815*	*1.0265*	*0.1237*	*1.2361*	*0.1495*	*1.0523*	*0.109*
Ventral reticular (vRT)	*1.2446*	*0.0929*	*0.9614*	*0.1043*	*1.1932*	*0.08*	*0.9998*	*0.05*
Medial geniculate (MG)	*1.258*	*0.0919*	*1.0374*	*0.0923*	*1.222*	*0.1501*	*1.133*	*0.1049*
Midline Nuclei (ML)	*1.0503*	*0.1846*	*0.9412*	*0.1263*	*0.9967*	*0.1332*	*0.8598*	*0.0427*
Amygdala								
Basolateral (BLA)	*0.7857*	*0.0774*	*0.6963*	*0.1033*	*0.718*	*0.0808*	*0.7486*	*0.0676*
Central (CeA)	*0.708*	*0.1015*	*0.6343*	*0.1458*	*0.7013*	*0.2309*	*0.6578*	*0.1829*
Medial (meA)	*0.7346*	*0.1006*	*0.599*	*0.1846*	*0.6673*	*0.1146*	*0.7573*	*0.2703*
Hippocampus								
Subiculum (Sub)	*1.1858*	*0.0754*	*1.2378*	*0.2042*	*1.0659*	*0.0371*	*1.1292*	*0.0838*
CA1	*0.9994*	*0.0964*	*1.0856*	*0.111*	*1.0099*	*0.0466*	*1.057*	*0.0848*
CA2	*0.8196*	*0.0337*	*0.7318*	*0.1336*	*0.8015*	*0.1178*	*0.7604*	*0.1607*
CA3	*0.7632*	*0.2049*	*0.6543*	*0.109*	*0.7008*	*0.0571*	*0.6459*	*0.0855*
Dentate gyrus (DG)	*0.9161*	*0.2965*	*0.7341*	*0.0921*	*0.7947*	*0.2297*	*0.7701*	*0.1625*

Table 4f: Comparison of alterations in regional activity in selected regions of the brains of wild type and Fxyd6 -/- (KO) mice receiving ketamine or saline treatment. Data shown as raw mean 2DG uptake ratio for each region +/-SDEV.

Table 4g	WT Saline	n=6	WT Ketamine	n=6	*Fxyd6-/-* Saline	n=4	*Fxyd6-/-* Ketamine	n=4
Septum/Band of Broca	**Mean**	**SDEV**	**Mean**	**SDEV**	**Mean**	**SDEV**	**Mean**	**SDEV**
Medial septum (MS)	*1.0033*	*0.0796*	*0.8253*	*0.0393*	*0.864*	*0.1144*	*0.8678*	*0.0881*
Lateral septum (LS)	*0.8167*	*0.0528*	*0.6885*	*0.0958*	*0.7031*	*0.0328*	*0.6547*	*0.1001*
Vertical DB (VDB)	*0.9783*	*0.2069*	*0.6977*	*0.0779*	*0.7261*	*0.2456*	*0.8612*	*0.13*
Horizontal DB (HDB)	*0.8894*	*0.0828*	*0.8059*	*0.0818*	*0.8325*	*0.1935*	*0.8002*	*0.0681*
Basal ganglia								
Ventromedial striatum (VMST)	*1.0967*	*0.0991*	*1.1434*	*0.106*	*0.9892*	*0.0397*	*1.1636*	*0.1991*
Dorsolateral striatum (DLST)	*1.243*	*0.1109*	*1.2649*	*0.0444*	*1.2671*	*0.0498*	*1.2813*	*0.1329*
Substantia nigra pars reticulata (SNR)	*0.8084*	*0.0701*	*0.7942*	*0.1113*	*0.7696*	*0.0295*	*0.7403*	*0.093*
Substantia nigra pars compacta (SNC)	*0.9224*	*0.1386*	*0.8073*	*0.1226*	*0.9139*	*0.0738*	*0.7605*	*0.1043*
Globus palludus (GP)	*0.83*	*0.0193*	*0.7223*	*0.0396*	*0.7983*	*0.0475*	*0.7876*	*0.134*
Mesolimbic								
Nucleus accumbens core (NacC)	*0.8847*	*0.1184*	*0.9884*	*0.1038*	*0.8945*	*0.0416*	*1.0111*	*0.1001*
Nucleus accumbens shell (NacS)	*0.9141*	*0.1045*	*0.9383*	*0.0456*	*0.8599*	*0.1722*	*0.9553*	*0.068*
Ventral tegmental nucleus (VTg)	*1.098*	*0.1028*	*0.9706*	*0.0236*	*1.083*	*0.0375*	*0.735*	*0.4919*
Ventral tegmental area (VTA)	*1.1274*	*0.143*	*1.0524*	*0.0723*	*1.146*	*0.1223*	*0.9802*	*0.1197*
Neuromodulatory								
Dorsal raphe (DR)	*1.0126*	*0.0643*	*0.9234*	*0.1289*	*0.8837*	*0.0952*	*0.5869*	*0.3952*
Median raphe (MR)	*1.0695*	*0.0991*	*0.9336*	*0.1192*	*0.9731*	*0.07*	*0.8161*	*0.545*
Multimodal								
Habenula (Hab)	*1.222*	*0.0871*	*1.1739*	*0.2048*	*1.2359*	*0.1417*	*1.2077*	*0.2654*
Mamillary body (MM)	*1.5347*	*0.4481*	*1.9133*	*0.2938*	*1.8364*	*0.4622*	*1.8104*	*0.2466*

Table 4g: Comparison of alterations in regional activity in selected regions of the brains of wild type and Fxyd6 -/- (KO) mice receiving ketamine or saline treatment. Data shown as raw mean 2DG uptake ratio for each region +/-SDEV.

Chapter 5: Conclusions

This study endeavoured to investigate the neurobiology of two genes which have been implicated as risk factors for schizophrenia, in order to better inform how genes and molecular systems contribute to cognitive systems that are impaired in the disorder and other psychiatric illnesses. It also aimed to reinforce previous work in establishing new methods of assessing working memory span in rodent paradigms by using an adapted version of the human n-back test in the rodent's radial arm maze apparatus.

This investigation provided strong evidence that the adapted n-back test for rodents using the radial arm maze is a robust and adaptable paradigm for measuring rodent's spatial working memory ability; supporting previous findings by Marighetto *et al.* 2008. The n-back RAM paradigm for rodents exhibits advantages over previously used methods for testing working memory, by increasing information loads and actively encouraging rodents to maintain and manipulate the given information – in order to maximise their chances of reward. Previous methods used to test working memory in rodents, including the DNMTS T-maze task, have not required the rodents to maintain more than one unit of information at a time, therefore not fully engaging the rodent's working memory span. Furthermore because only one unit of information needs to be maintained, the information does not need to be actively manipulated in the rodent's working memory. The n-back RAM paradigm overcomes this by encouraging the rodents to learn choices they made 2, 3 and even 4 steps back, which means the rodent must actively maintain and manipulate subsequent pieces of information in order to gain maximum reward. This investigation demonstrates rodent's engagements with the test by confirming performances significantly above chance levels even in the more difficult levels of n3 and n4. This paradigm could also be modulated easily to probe different aspects of working memory by focusing in on specific n-back levels (e.g. trials with only n-back 3), increasing the intertrial interval and increasing the number of n-back levels in the trial (test upper limit of working memory load).

This investigation, for the first time, also provides evidence that *Map2k7* may play a role in working memory maintenance in rodents. This was confirmed through

rigorous testing of WT and *Map2k7*+/- mice in the n-back RAM paradigm, where *Map2k7*+/- exhibited a subtle, yet significant deficit in their spatial working memory ability. MAP2K7 is a gene involved in JNK signalling which is involved in cortical and hippocampal plasticity. By down regulating expression of Map2k7, this could therefore have had subtle effects on cortical and hippocampal plasticity, therefore affecting cognitive systems. It would therefore be highly desirable to investigate what effect *Map2k7*+/- would have on regional brain activity. This could be achieved by 2DG methods and EEG techniques adapted for mice. Such investigations may indicate what regions may contribute to this working memory deficit. From previous work it could be hypothesized that the hippocampal regions and the PFC may be involved. In order to determine reproducibility, it would be desirable to repeat the *Map2k7* tests in the RAM but focus on the higher working memory load levels of n2, n3 and n4 (possibly even n5) with larger group sizes. By doing so it will provide greater confidence for a working memory deficit in these MAP27+/- mice.

The 2DG investigation of Fxyd6's role in regional cerebral metabolism identified three regions that exhibited a significant difference in activity compared to WT mice. These regions were the subiculum, medial septum and the lateral septum, all of which exhibited a reduced glucose metabolism compared to wild types. This finding confirms the hypothesis that a hippocampal region would be significantly affected by knocking out the *Fxyd6* gene and provides tantalising evidence that could link FXYD6 and NaKATPase to regions that regulate memory systems, including working memory. Furthermore the subiculum is thought to be involved in the discrimination of previously learned information to current information in working memory. This means it would be very interesting to see how *Fxyd6*-knock-out mice would perform in the n-back RAM test. It could be hypothesized that these mice would underperform in this paradigm compared to wild-types, due to hypoactivity in the subiculum. Furthermore it would also be interesting to analyse how the mice perform in tasks with a high level of proactive and retro-active interference, as an abnormally active subiculum could affect performance on the disambiguation of previously formed memories with new ones. The subiculum, medial septum and lateral septum are also all components of the brain-reward/motivation circuit. This

highlights a potential area of investigation for Fxyd6 involvement in behavioural paradigms that measure motivation and reward valuation.

Finally, it was found that the effect of NMDAR antagonism using ketamine on *Fxyd6*-/- mice produced unforeseen results. Generally regions which were affected by ketamine in WT mice including PFC, thalamic and septal regions, were not affected in *Fxyd6*-/- mice. It is hypothesized that this may be down to a compensatory effect that knocking-out *Fxyd6* may have on glutamate reuptake. Because NaKATPase is involved in glutamate reuptake into glia and neurons, the blockage of NMDA receptors may have less effect due to a reduction in glutamate reuptake, and therefore, higher than normal postsynaptic glutamate concentrations. This highlights an interesting lead for further investigation into the relationship between NaKATPase and glutamate signalling. This, however, is merely speculative, where more robust investigation with larger sample sizes would provide more reliable insight into these observations.

In conclusion, this thesis has investigated the neurobiology and behavioural components of two genes that have been linked to schizophrenia risk. By investigating how genes contribute to discrete components of psychiatric disorders from the bottom up, the clinical and scientific community will be able to construct reliable and intelligent models of psychiatric disorders that are based on measurable and robust biological components, rather than subjective criteria. This will, undoubtedly improve the reliability of diagnoses of psychiatric disorders and provide a more robust grounding in the process of drug discovery. Furthermore, the process of breaking down complex symptoms of psychiatric disorders into the investigation of singular components of pathology via their genes, neurobiology and neural connectivity, will create an invaluable insight into how all of these systems contribute to the workings of the human brain and ultimately how the combined interaction of all these systems amalgamate into our own human experiences and behaviours.

Appendix A: Figures used to guide 2-DG brain region analysis in *Fxyd6* experiment.

All figures sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

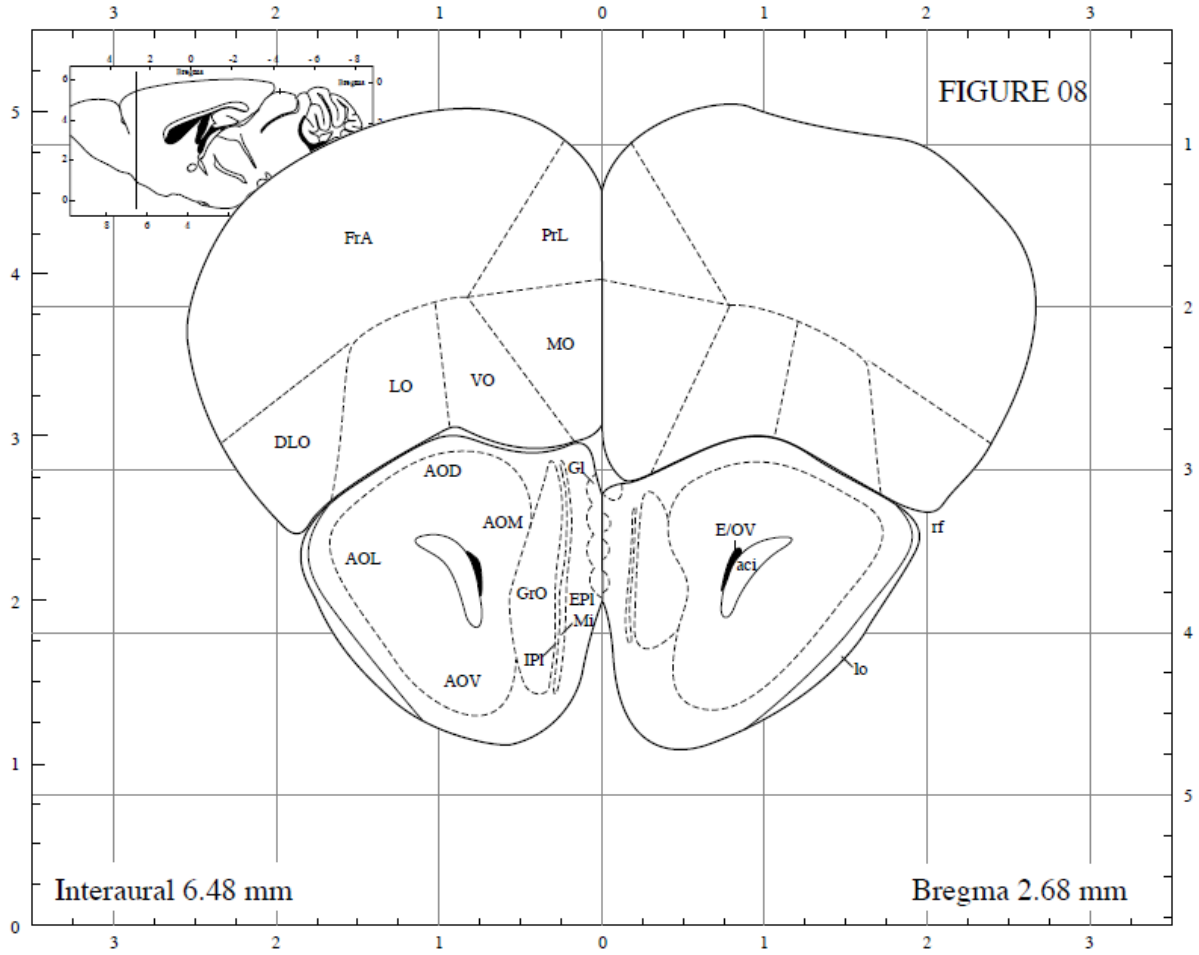

Figure A1: Regions measured: dorsolateral orbital cortex (DLO), lateral orbital (LO), ventral orbital (VO), medial orbital (MO), anterior prelimbic area (PrL), frontal association (FrA), medial prelimbic area (layer1) (mPrL1), medial prelimbic area (layer2) (mPrL2), medial prelimbic area (layer3) (mPrL3). Measured in both hemispheres.

"Figure 08" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

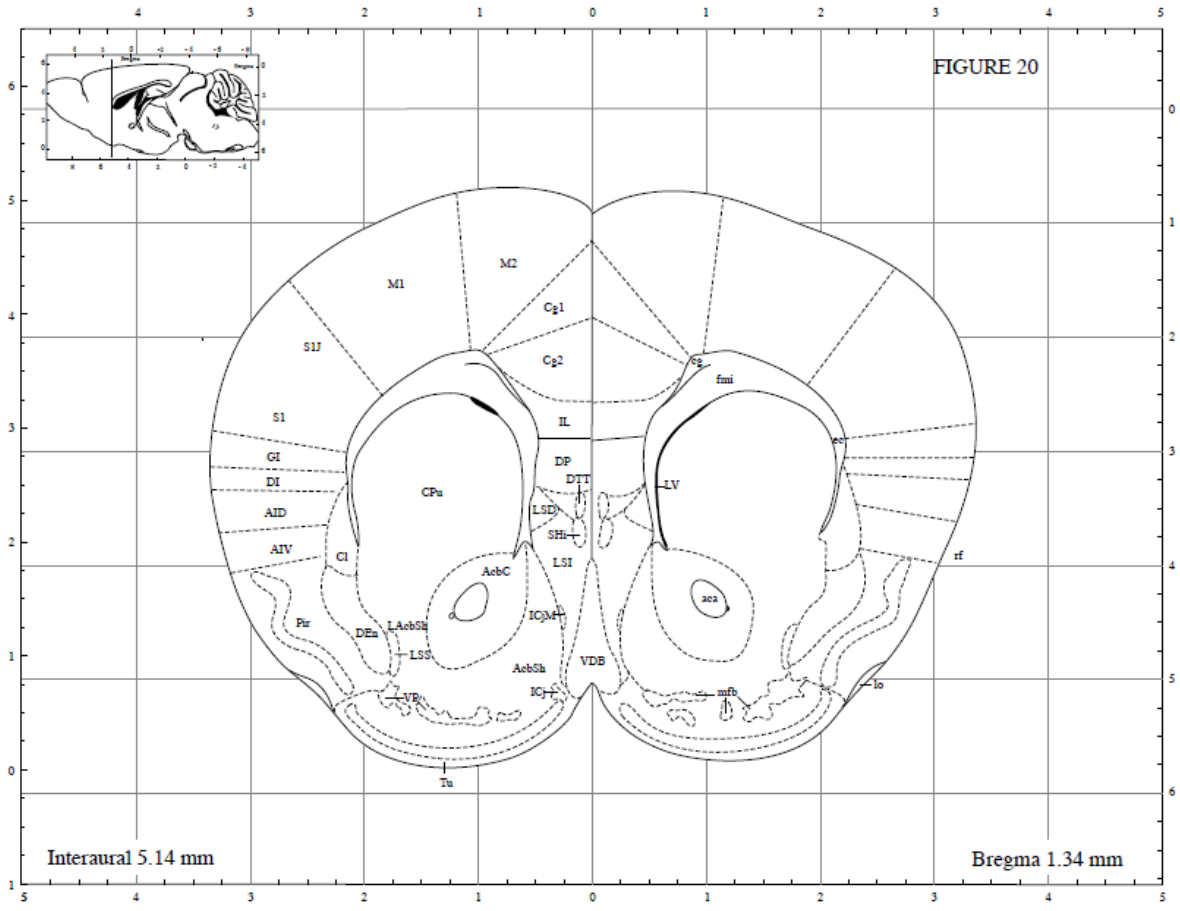

Figure A2: Regions measured: Cingulate gyrus (Cg1), primary motor cortex (M1), insular cortex (InsC), infralimbic (IL). Measured in both hemispheres.

"Figure 20" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

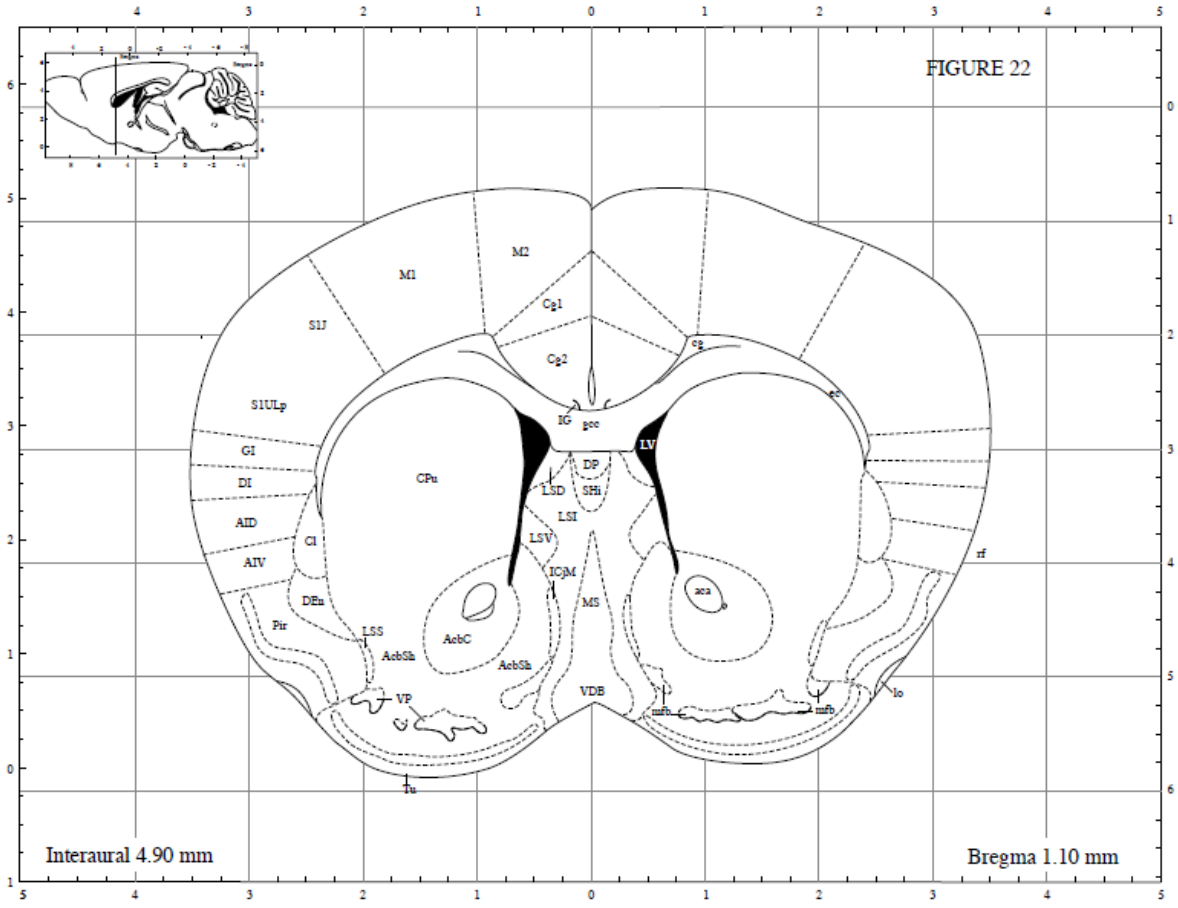

Figure A3: Regions measured: nucleus accumbens core (NacC), nucleus accumbens shell (NacSh), medial septum (MS), lateral septum (LS), vertical diagonal band (VDB). Measured in both hemispheres.

"Figure 22" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

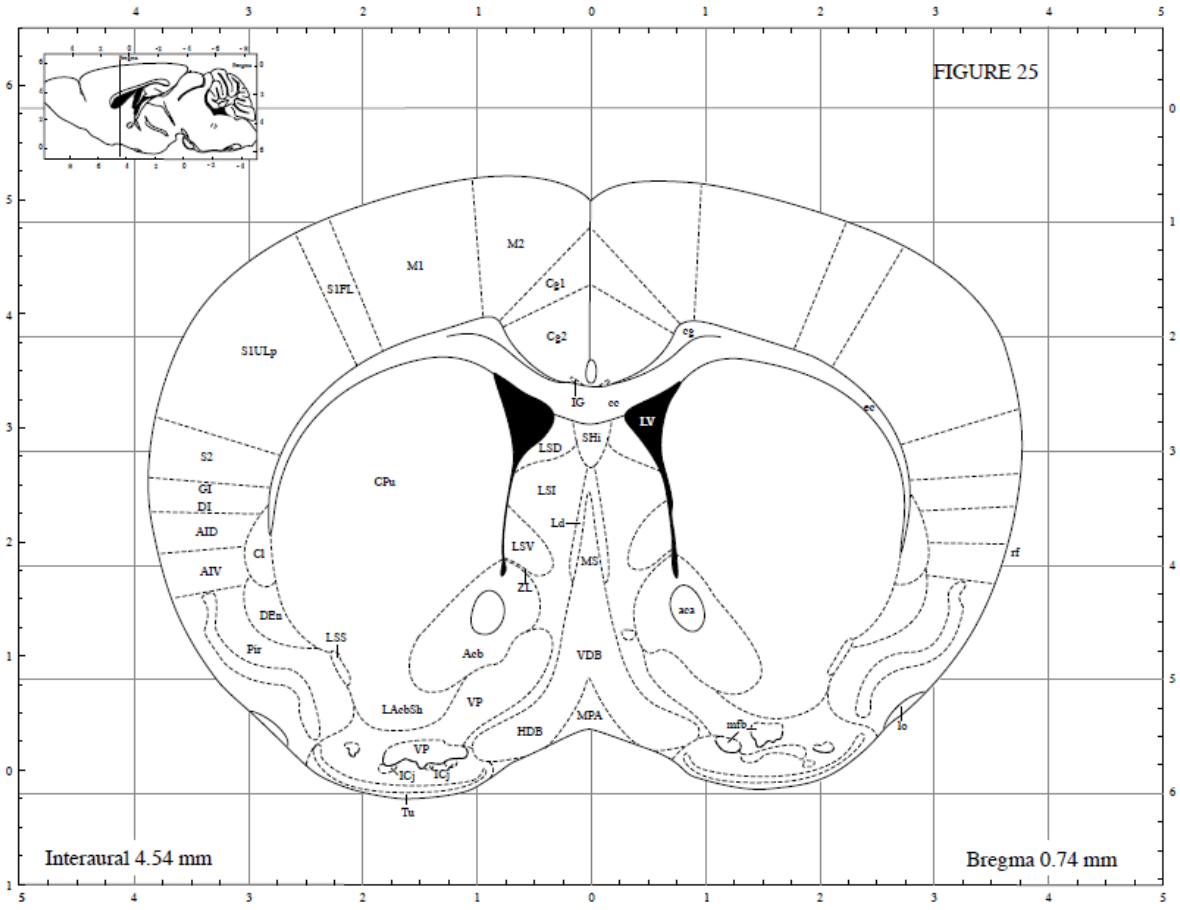

Figure A4: Regions measured: horizontal diagonal band (HDB). Measured in both hemispheres.

"Figure 25" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

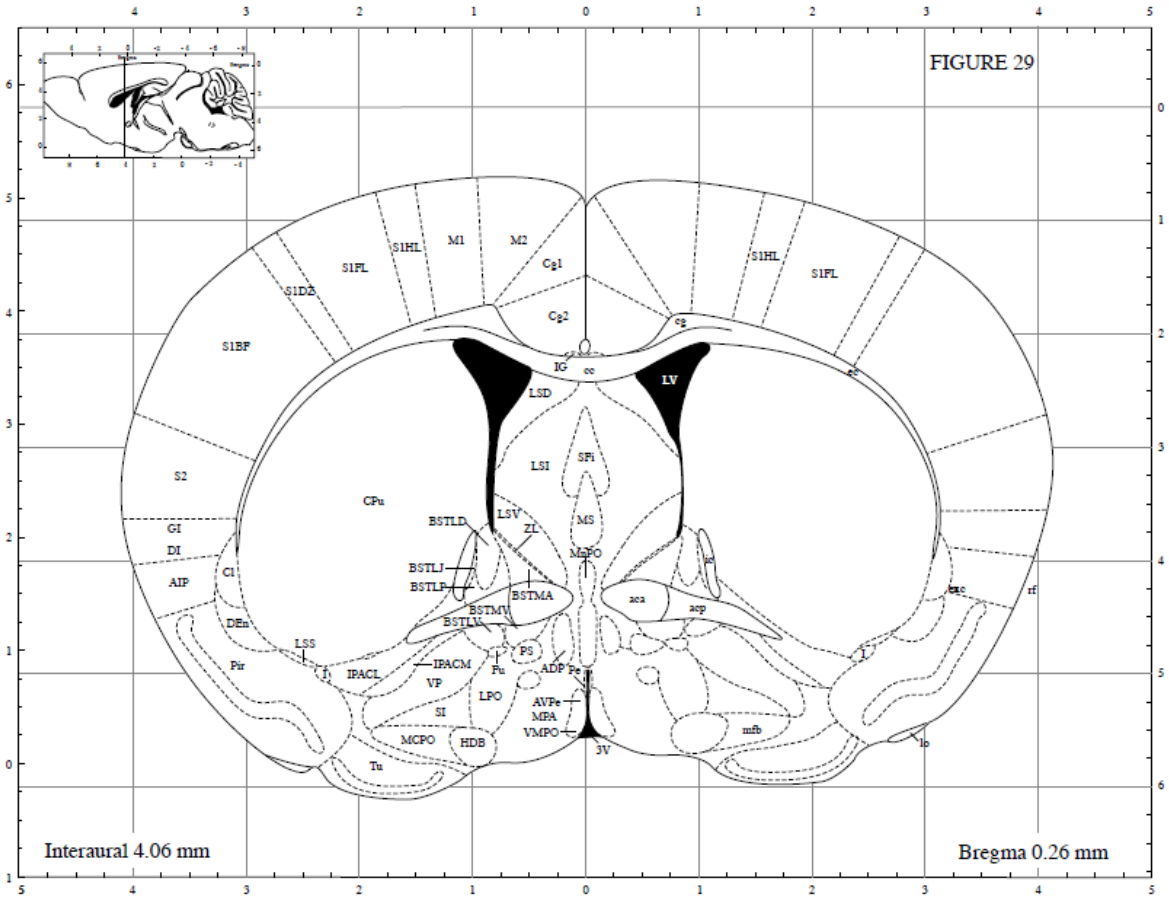

Figure A5: Regions measured: dorsolateral striatum (DLST), ventromedial striatum (VMST). Measured in both hemispheres.

"Figure 29" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

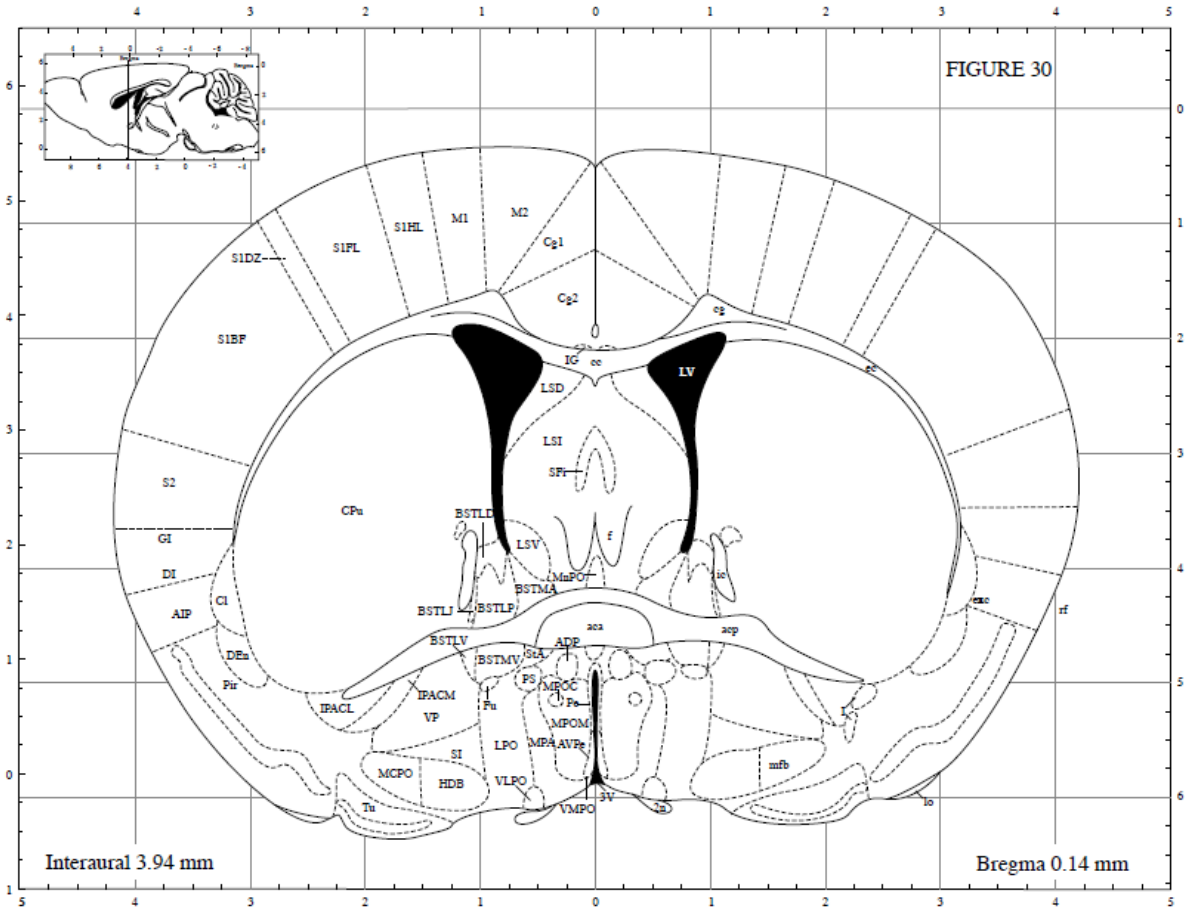

Figure A6: Regions measured: somatosensory cortex (SSCTX) Measured in both hemispheres.

"Figure 30" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

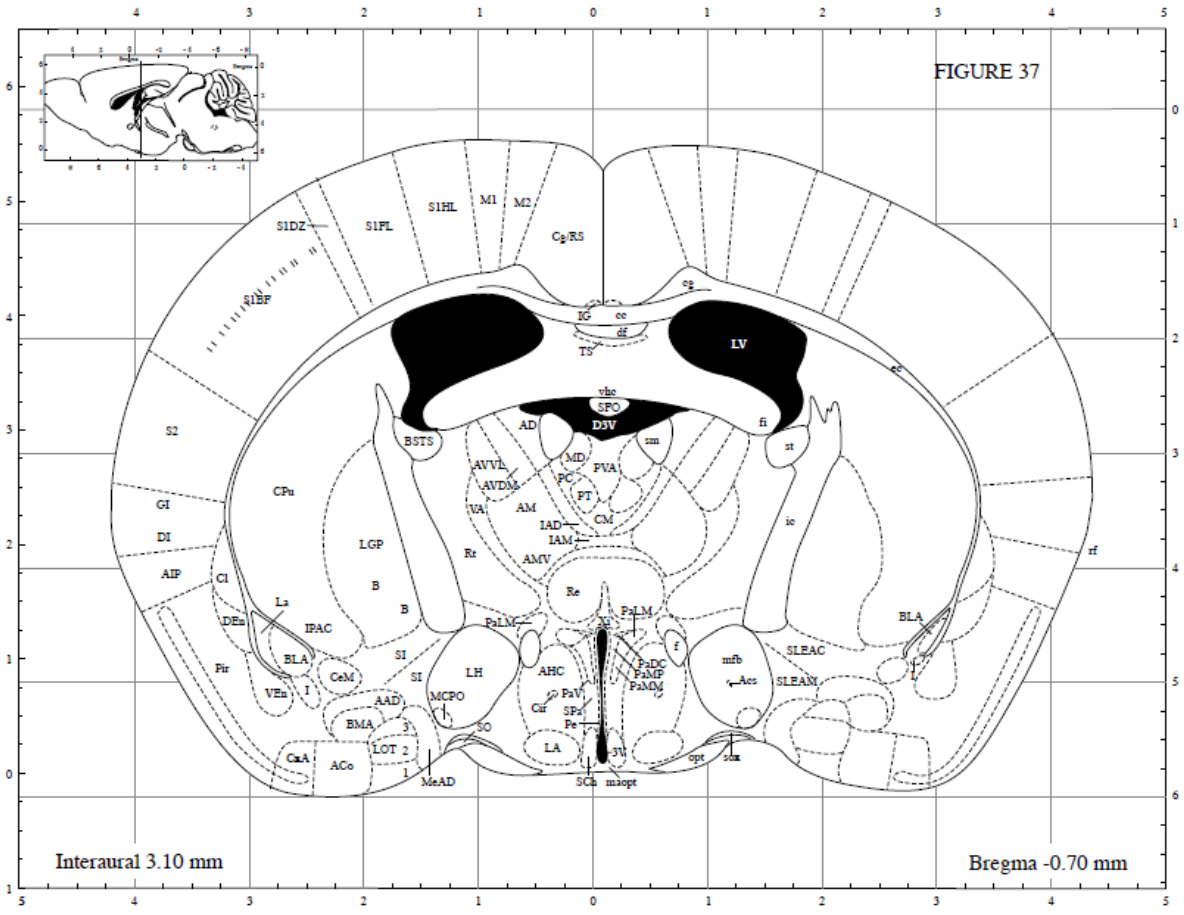

Figure A7: Regions measured: anteromedial thalamus (AMthal), basolateral amygdala (BLA), dorsal reticular nucleus (dRT), ventral reticular nucleus (vRT). Measured in both hemispheres.

"Figure 37" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

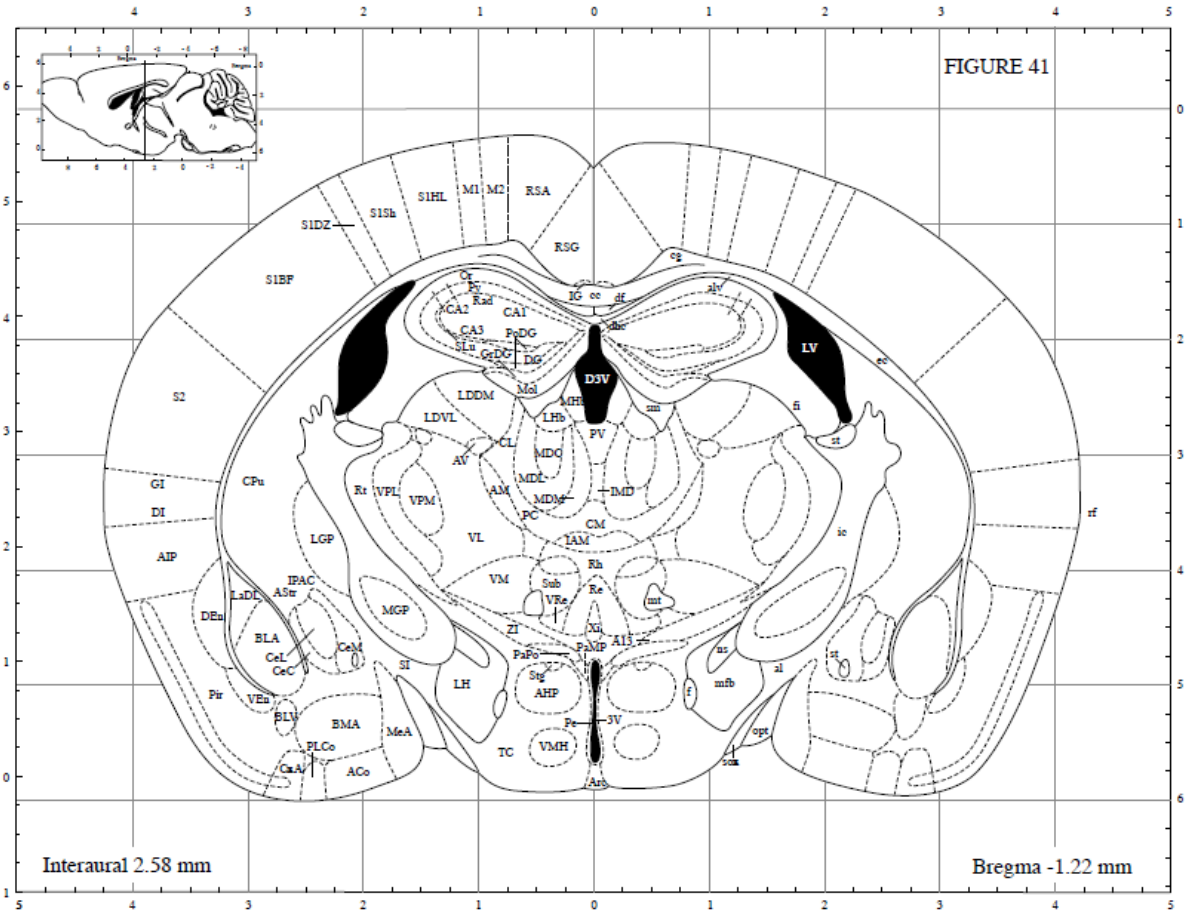

Figure A8: Regions measured: retrosplenial cortex (RSC), ventrolateral thalamus (VLthal), ventromedial thalamus (VMthal), anteroventral thalamus (AVthal), reuniens (Re), central amygdaloid (CeA), globus pallidus (GP). Measured in both hemispheres.

"Figure 41" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

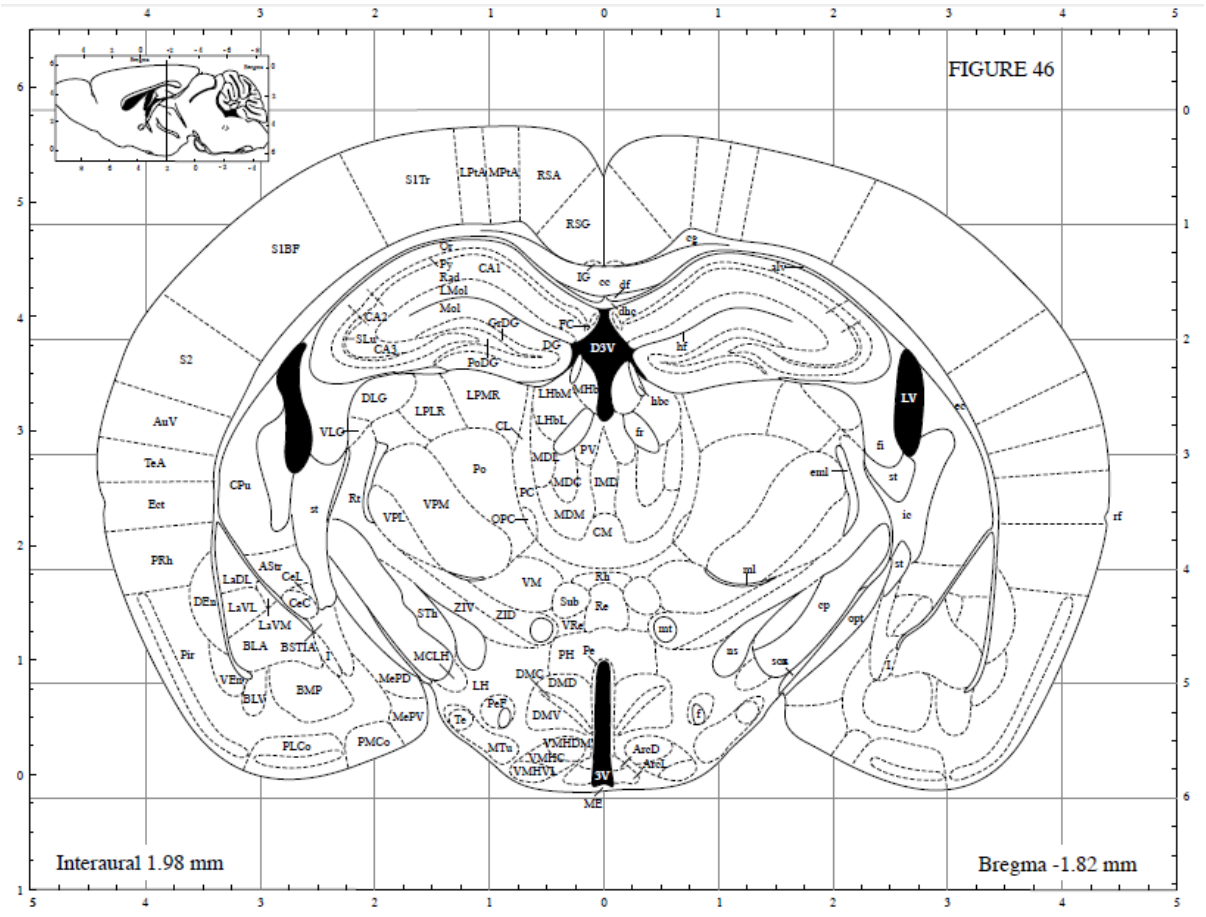

Figure A9: Regions measured: medialdorsal thalamus (MDthal), centromedial thalamus (CMthal), centrolateral thalamus (CLthal). Measured in both hemispheres.

"Figure 41" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

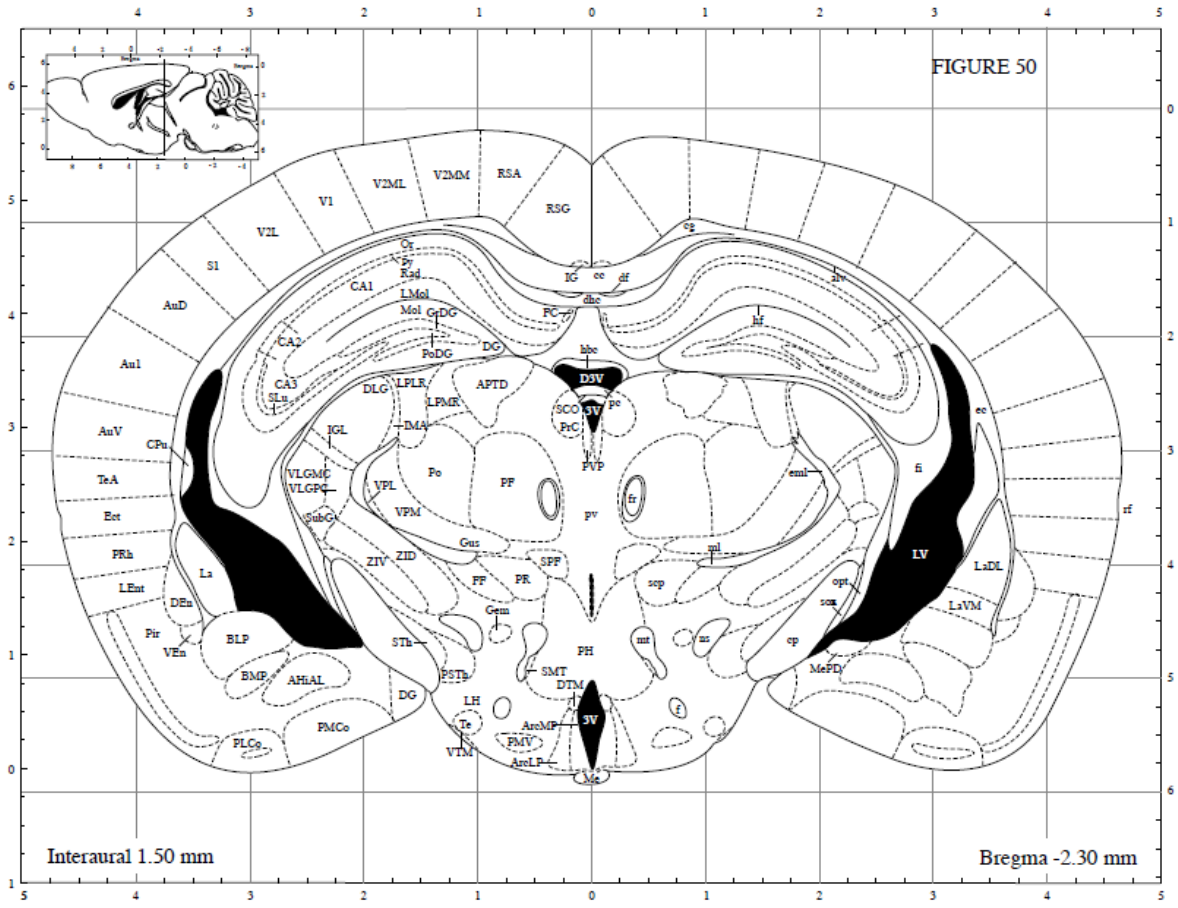

Figure A10: Regions measured: habenula (Hab), medial amygdaloid (MeA). Measured in both hemispheres.

"Figure 50" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

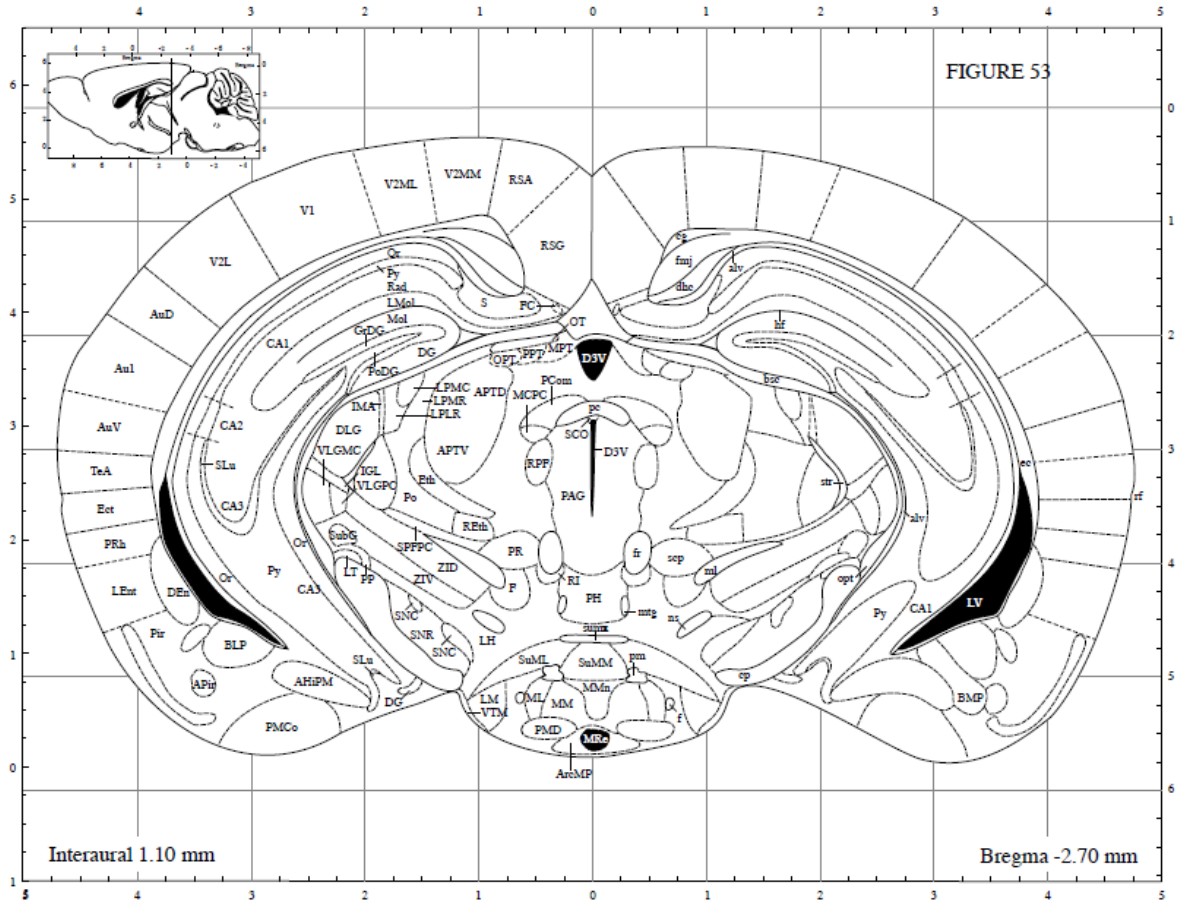

Figure A11: Regions measured: piriform cortex (Piri), auditory cortex (AudC), midline nuclei (ML). Measured in both hemispheres.

"Figure 53" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

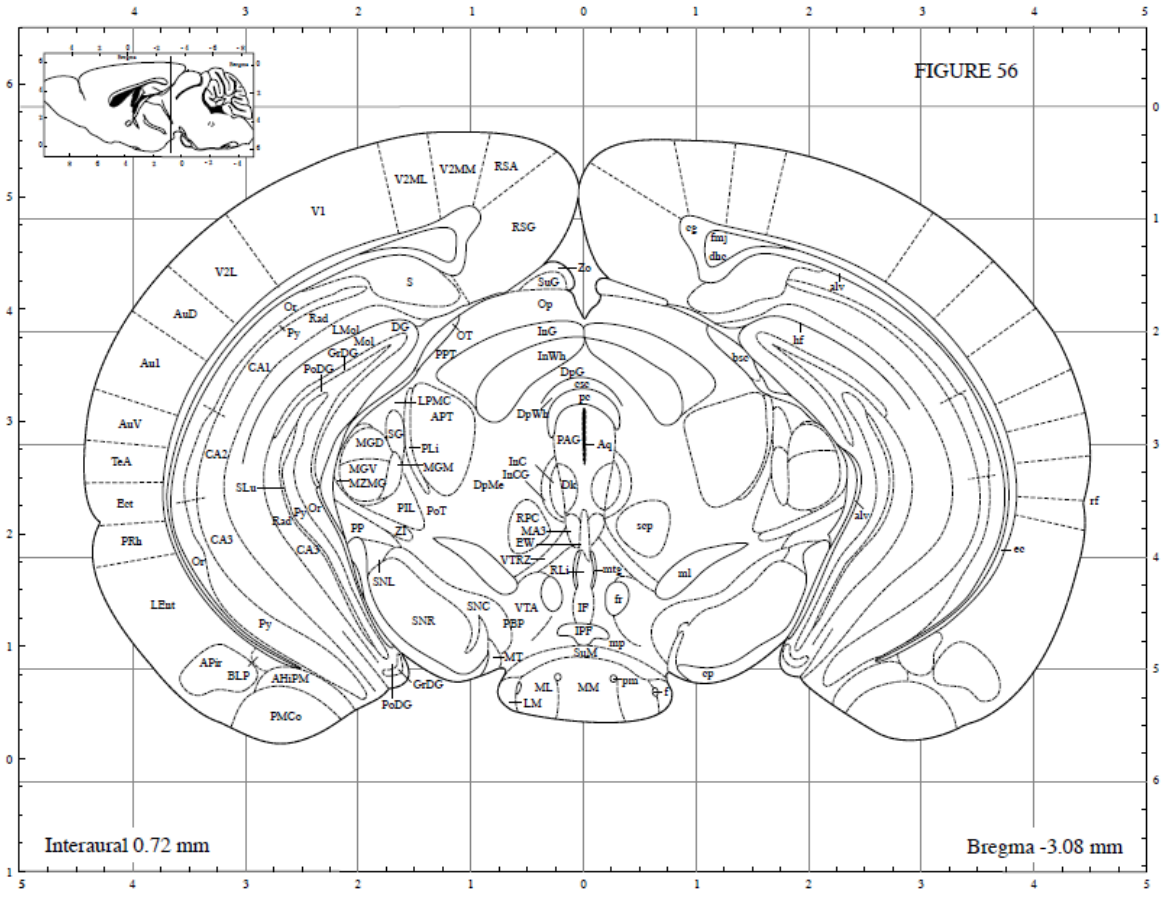

Figure A12: Regions measured: Substantia nigra pars compacta (SNC), substantia nigra pars reticulata (SNR), CA2, dendate gyrus (DG), subiculum (Sub). Measured in both hemispheres.

"Figure 56" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

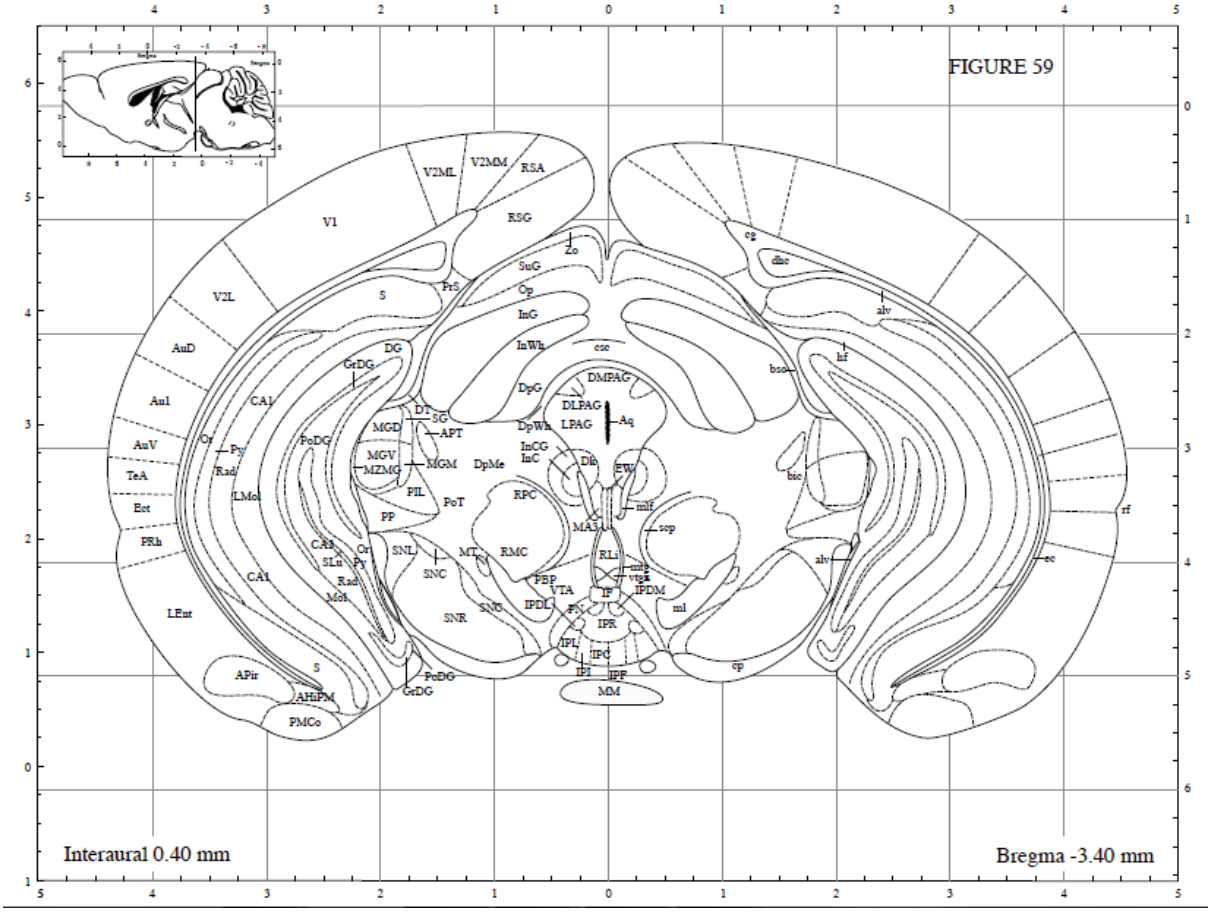

Figure A13: Regions measured: CA1, CA3, mamillary body (MM), ventral tegmental area (VTA). Measured in both hemispheres.

"Figure 59" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

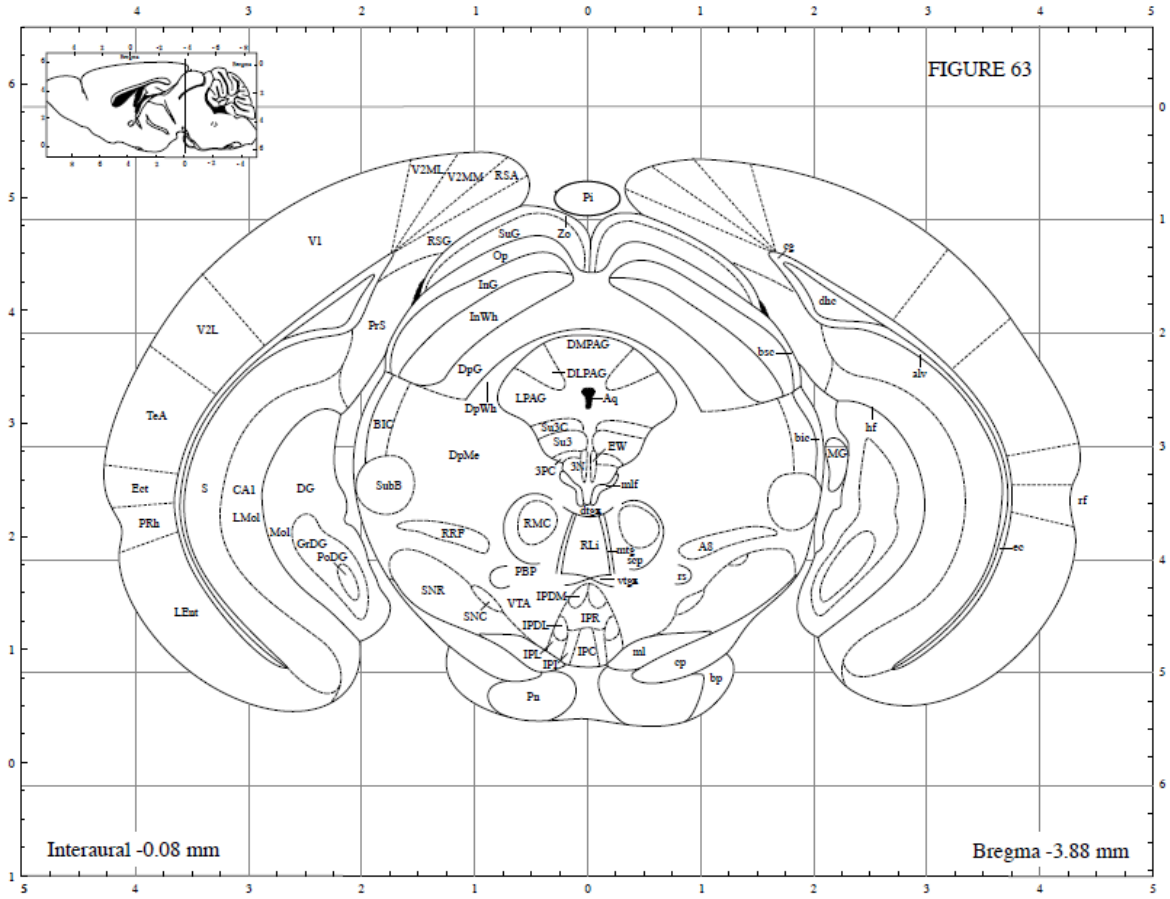

Figure A14: Regions measured: medial geniculate nucleus (MG). Measured in both hemispheres.

"Figure 63" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

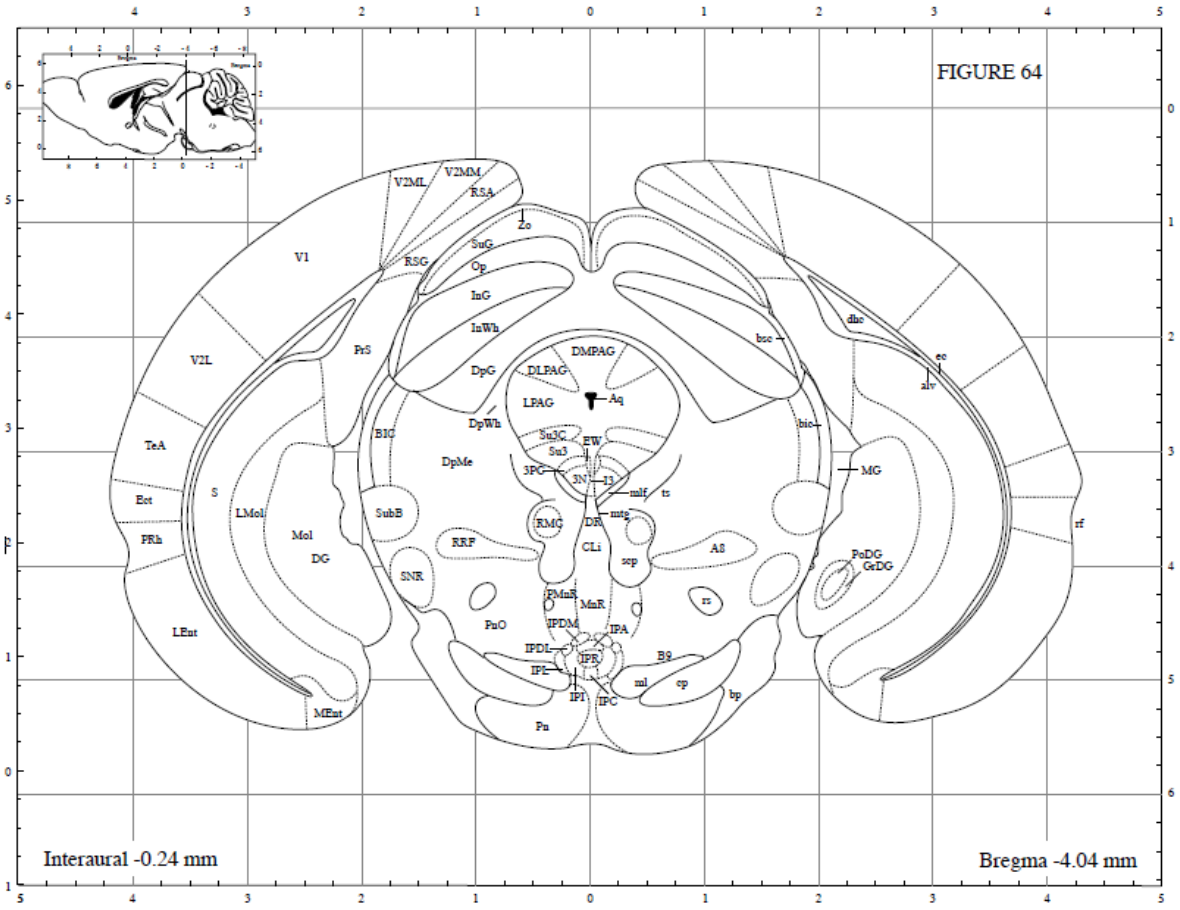

Figure A15: Regions measured: dorsal raphe nucleus (DR), median raphe nucleus (MR). Measured in both hemispheres.

"Figure 64" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

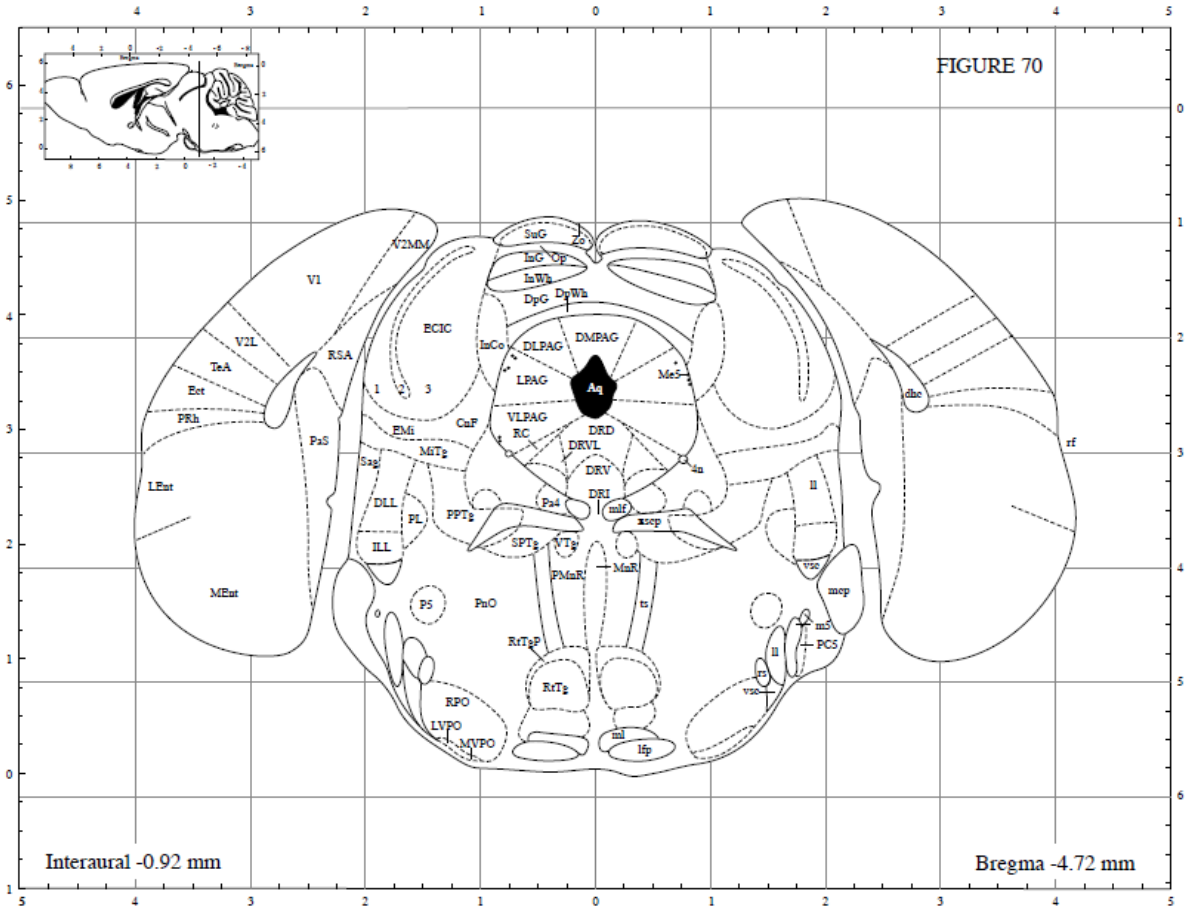

Figure A16: Regions measured: ventral tegmental nucleus (VTg) Measured in both hemispheres.

"Figure 70" Sourced from: Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

Chapter 6: Reference List

Abed, R.T. & Abbas, M.J., 2011. A reformulation of the social brain theory for schizophrenia: the case for out-group intolerance. *Perspectives in biology and medicine*, 54(2), pp.132–51.

Abed, R.T. & Abbas, M.J., 2014. Can the new epidemiology of schizophrenia help elucidate its causation? *Irish Journal of Psychological Medicine*, 31(01), pp.1–5.

Abi-Dargham, A. *et al.*, 2000. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America*, 97(14), pp.8104–9.

Abi-Dargham, A., 2003. Probing cortical dopamine function in schizophrenia: what can D1 receptors tell us? *World psychiatry : official journal of the World Psychiatric Association (WPA)*, 2(3), pp.166–71.

Abramovitch, A., Abramowitz, J.S. & Mittelman, A., 2013. The neuropsychology of adult obsessive-compulsive disorder: a meta-analysis. *Clinical psychology review*, 33(8), pp.1163–71.

Adam, D., 2013. Mental health: On the spectrum. *Nature*, 496(7446), pp.416–8.

Adler, C.M. *et al.*, 2005. Changes in gray matter volume in patients with bipolar disorder. *Biological psychiatry*, 58(2), pp.151–7.

Adolphs, R., Sears, L. & Piven, J., 2001. Abnormal processing of social information from faces in autism. *Journal of cognitive neuroscience*, 13(2), pp.232–40.

Ainge, J.A. & Langston, R.F., 2012. Ontogeny of neural circuits underlying spatial memory in the rat. *Frontiers in neural circuits*, 6, p.8.

Airaksinen, E. *et al.*, 2007. Low episodic memory performance as a premorbid marker of depression: evidence from a 3-year follow-up.

Akil, M. *et al.*, 1999. Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects.

Allen, H.A., Liddle, P.F. & Frith, C.D., 1993. Negative features, retrieval processes and verbal fluency in schizophrenia. *The British journal of psychiatry : the journal of mental science*, 163, pp.769–75.

Allen, R.M. & Young, S.J., 1978. Phencyclidine-induced psychosis. *The American journal of psychiatry*, 135(9), pp.1081–4.

Amaral, D.G., Schumann, C.M. & Nordahl, C.W., 2008. Neuroanatomy of autism. *Trends in neurosciences*, 31(3), pp.137–45.

Andreasen, N.C., 2000. Schizophrenia: the fundamental questions. *Brain research. Brain research reviews*, 31(2-3), pp.106–12.

American Psychiatric Association,. 1952. Diagnostic and statistical manual of mental disorders (1st ed.,text rev.)

American Psychiatric Association,. 2000. Diagnostic and statistical manual of mental disorders (4th ed.,text rev.)

Arnsten, A.F.T., 2006. Fundamentals of attention-deficit/hyperactivity disorder: circuits and pathways. *The Journal of clinical psychiatry*, 67 Suppl 8, pp.7–12.

Asaoka, Y. & Nishina, H., 2010. Diverse physiological functions of MKK4 and MKK7 during early embryogenesis. *Journal of biochemistry*, 148(4), pp.393–401.

Aultman, J.M. & Moghaddam, B., 2001. Distinct contributions of glutamate and dopamine receptors to temporal aspects of rodent working memory using a clinically relevant task. *Psychopharmacology*, 153(3), pp.353–64.

Aupperle, R.L. *et al.*, 2012. Executive function and PTSD: disengaging from trauma. *Neuropharmacology*, 62(2), pp.686–94.

Avery, J.A. *et al.*, 2014. Major depressive disorder is associated with abnormal interoceptive activity and functional connectivity in the insula. *Biological psychiatry*, 76(3), pp.258–66.

Ayhan, Y. *et al.*, 2011. Differential effects of prenatal and postnatal expressions of mutant human DISC1 on neurobehavioral phenotypes in transgenic mice: evidence for neurodevelopmental origin of major psychiatric disorders. *Molecular psychiatry*, 16(3), pp.293–306.

Barch, D.M. & Csernansky, J.G., 2007. Abnormal parietal cortex activation during working memory in schizophrenia: verbal phonological coding disturbances versus domain-general executive dysfunction. *The American journal of psychiatry*, 164(7), pp.1090–8.

Barnea-Goraly, N. *et al.*, 2004. White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biological psychiatry*, 55(3), pp.323–6.

Barnett, J.H. *et al.*, 2010. Assessing cognitive function in clinical trials of schizophrenia. *Neuroscience and biobehavioral reviews*, 34(8), pp.1161–77.

Baron-Cohen, S. & Belmonte, M.K., 2005. Autism: a window onto the development of the social and the analytic brain. *Annual review of neuroscience*, 28, pp.109–26.

Baron-Cohen, S., Tager-Flusberg, H. & Cohen, D.J., 1994. *Understanding other minds: Perspectives from autism.*, Oxford University Press. xiii 515 pp.

Batty, M.J. *et al.*, 2010. Cortical gray matter in attention-deficit/hyperactivity disorder: a structural magnetic resonance imaging study. *Journal of the American Academy of Child and Adolescent Psychiatry*, 49(3), pp.229–38.

Bayles, B.P. & Katerndahl, D.A., 2009. Culture-bound syndromes in Hispanic primary care patients. *International journal of psychiatry in medicine*, 39(1), pp.15–31.

Bauman, Z., 2001 - The Individualized Society. Cambridge: Polity Press.

Beasley, C. & Reynolds, G., 1997. Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. *Schizophrenia Research*, 24(3), pp.349–355.

Béguin, P. *et al.*, 2001. CHIF, a member of the FXYD protein family, is a regulator of Na,K-ATPase distinct from the gamma-subunit. *The EMBO journal*, 20(15), pp.3993–4002.

Béguin, P. *et al.*, 1997. The gamma subunit is a specific component of the Na,K-ATPase and modulates its transport function. *The EMBO journal*, 16(14), pp.4250–60.

Belmonte, M.K. *et al.*, 2004. Autism and abnormal development of brain connectivity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 24(42), pp.9228–31.

Beucke, J.C. *et al.*, 2013. Abnormally high degree connectivity of the orbitofrontal cortex in obsessive-compulsive disorder. *JAMA psychiatry*, 70(6), pp.619–29.

Beucke, J.C. *et al.*, 2014. Default mode network subsystem alterations in obsessive-compulsive disorder. *The British journal of psychiatry : the journal of mental science*.

Bibert, S. *et al.*, 2008. Phosphorylation of phospholemman (FXYD1) by protein kinases A and C modulates distinct Na,K-ATPase isozymes. *The Journal of biological chemistry*, 283(1), pp.476–86.

Bitanihirwe, B.K.Y. *et al.*, 2009. Glutamatergic deficits and parvalbumin-containing inhibitory neurons in the prefrontal cortex in schizophrenia. *BMC psychiatry*, 9(1), p.71.

Blanco, S. *et al.*, 2008. Modulation of interleukin-1 transcriptional response by the interaction between VRK2 and the JIP1 scaffold protein. PloS one, 3(2), p.e1660.

Bogaev, R.C. *et al.*, 2001. Gene structure and expression of phospholemman in mouse. *Gene*, 271(1), pp.69–79.

Boksa, P., 2010. Effects of prenatal infection on brain development and behavior: a review of findings from animal models. *Brain, behavior, and immunity*, 24(6), pp.881–97.

Bora, E. *et al.*, 2011. Neuroanatomical abnormalities in schizophrenia: a multimodal voxelwise meta-analysis and meta-regression analysis. *Schizophrenia research*, 127(1-3), pp.46–57.

Borsello, T. et al., 2003. A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. *Nature medicine*, 9(9), pp.1180–6.

Bourque, F., van der Ven, E. & Malla, A., 2011. A meta-analysis of the risk for psychotic disorders among first- and second-generation immigrants. *Psychological medicine*, 41(5), pp.897–910.

Boydell, J. *et al.*, 2001. Incidence of schizophrenia in ethnic minorities in London: ecological study into interactions with environment. *BMJ (Clinical research ed.)*, 323(7325), pp.1336–8.

Braff, D. *et al.*, 1978. Prestimulus Effects on Human Startle Reflex in Normals and Schizophrenics. *Psychophysiology*, 15(4), pp.339–343.

Brambilla, P. *et al.*, 2003. Brain anatomy and development in autism: review of structural MRI studies. *Brain research bulletin*, 61(6), pp.557–69.

Bresnahan, M. *et al.*, 2007. Race and risk of schizophrenia in a US birth cohort: another example of health disparity? *International journal of epidemiology*, 36(4), pp.751–8.

Bressler, S.L. & Menon, V., 2010. Large-scale brain networks in cognition: emerging methods and principles. *Trends in cognitive sciences*, 14(6), pp.277–90.

Brown, V.M. *et al.*, 2014. Altered resting-state functional connectivity of basolateral and centromedial amygdala complexes in posttraumatic stress disorder. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 39(2), pp.351–9.

Browning, J.L. *et al.*, 2005. Clozapine and the mitogen-activated protein kinase signal transduction pathway: implications for antipsychotic actions. *Biological psychiatry*, 57(6), pp.617–23.

Brüne, M., 2005. "Theory of mind" in schizophrenia: a review of the literature. *Schizophrenia bulletin*, 31(1), pp.21–42.

Burdick, K.E. *et al.*, 2008. Neurocognitive profile analysis in obsessive-compulsive disorder. *Journal of the International Neuropsychological Society : JINS*, 14(4), pp.640–5.

Bush, G., 2010. Attention-deficit/hyperactivity disorder and attention networks. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 35(1), pp.278–300.

Van den Buuse, M., 2010. Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. Schizophrenia bulletin, 36(2), pp.246–70.

Buxbaum, J.D. *et al.*, 2012. The autism sequencing consortium: large-scale, high-throughput sequencing in autism spectrum disorders. *Neuron*, 76(6), pp.1052–6.

Bystritsky, A. *et al.*, 2011. A review of low-intensity focused ultrasound pulsation. *Brain stimulation*, 4(3), pp.125–36.

Cadenhead, K.S. *et al.*, 2000. Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. *The American journal of psychiatry*, 157(10), pp.1660–8.

Canals, S. *et al.*, 2009. Functional MRI evidence for LTP-induced neural network reorganization. *Current biology : CB*, 19(5), pp.398–403.

Cannon, M., Jones, P.B. & Murray, R.M., 2002. Obstetric complications and schizophrenia: historical and meta-analytic review. *The American journal of psychiatry*, 159(7), pp.1080–92.

Canteras, N.S. & Swanson, L.W., 1992. Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. The Journal of comparative neurology, 324(2), pp.180–94.

Cantor-Graae, E. & Selten, J.-P., 2005. Schizophrenia and migration: a meta-analysis and review. *The American journal of psychiatry*, 162(1), pp.12–24.

Carlisle, S. & Hanlon, P., 2007. Well-being and consumer culture: a different kind of public health problem? *Health promotion international*, 22(3), pp.261–8.

Camargo, L.M. *et al.*, 2007. Disrupted in Schizophrenia 1 Interactome: evidence for the close connectivity of risk genes and a potential synaptic basis for schizophrenia. Molecular psychiatry, 12(1), pp.74–86.

Cartmill, E.A. & Byrne, R.W., 2010. Semantics of primate gestures: intentional meanings of orangutan gestures. *Animal cognition*, 13(6), pp.793–804.

Casanova, M. & Trippe, J., 2009. Radial cytoarchitecture and patterns of cortical connectivity in autism. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 364(1522), pp.1433–6.

Casey, B.J. *et al.*, 2013. DSM-5 and RDoC: progress in psychiatry research? *Nature reviews. Neuroscience*, 14(11), pp.810–4.

Castaneda, A.E. *et al.*, 2008. A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. *Journal of affective disorders*, 106(1-2), pp.1–27.

Castellanos, F.X. *et al.*, 2008. Cingulate-precuneus interactions: a new locus of dysfunction in adult attention-deficit/hyperactivity disorder. *Biological psychiatry*, 63(3), pp.332–7.

Castellanos, F.X. *et al.*, 2002. Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *JAMA*, 288(14), pp.1740–8.

Castellanos, F.X. & Proal, E., 2012. Large-scale brain systems in ADHD: beyond the prefrontal-striatal model. *Trends in cognitive sciences*, 16(1), pp.17–26.

Centeno, C. *et al.*, 2007. Role of the JNK pathway in NMDA-mediated excitotoxicity of cortical neurons. *Cell death and differentiation*, 14(2), pp.240–53.

Choudhury, K. *et al.*, 2006. A genetic association study implicates the chromosome 11q23.3 gene FXYD6 encoding phopshohippolin in susceptibility to schizophrenia in University College London and Aberdeen case control samples. *The American Journal of Human Genetics*.

Choudhury, K. *et al.*, 2007. A genetic association study of chromosome 11q22-24 in two different samples implicates the FXYD6 gene, encoding phosphohippolin, in susceptibility to schizophrenia. *American journal of human genetics*, 80(4), pp.664–72.

Christakou, A. *et al.*, 2013. Disorder-specific functional abnormalities during sustained attention in youth with Attention Deficit Hyperactivity Disorder (ADHD) and with autism. *Molecular psychiatry*, 18(2), pp.236–44.

Christopher, G. & MacDonald, J., 2005. The impact of clinical depression on working memory. *Cognitive neuropsychiatry*, 10(5), pp.379–99.

Chubb, J.E. *et al.*, 2008. The DISC locus in psychiatric illness. Molecular psychiatry, 13(1), pp.36–64.

Chung, K. *et al.*, 2013. Structural and molecular interrogation of intact biological systems. *Nature*, 497(7449), pp.332–7.

Clayton, I.C., Richards, J.C. & Edwards, C.J., 1999. Selective attention in obsessive-compulsive disorder. *Journal of abnormal psychology*, 108(1), pp.171–5.

Cochran, S.M. *et al.*, 2003. Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: differential modulation by antipsychotic drugs. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 28(2), pp.265–75.

Coffey, E.T., 2014. Nuclear and cytosolic JNK signalling in neurons. *Nature reviews. Neuroscience*, 15(5), pp.285–99.

Cohen, B. D., Nachmani, G., & Rosenberg, S. (1974). Referent communication disturbances in acute schizophrenia. *Journal of Abnormal Psychology*, *83*(1), 1–13.

Cole, M.W. *et al.*, 2011. Variable global dysconnectivity and individual differences in schizophrenia. *Biological psychiatry*, 70(1), pp.43–50.

Coles, M.E., Turk, C.L. & Heimberg, R.G., 2007. Memory bias for threat in generalized anxiety disorder: the potential importance of stimulus relevance. *Cognitive behaviour therapy*, 36(2), pp.65–73.

Comparelli, A. *et al.*, 2014. Symptom correlates of facial emotion recognition impairment in schizophrenia. *Psychopathology*, 47(1), pp.65–70.

Cooper, D.C. *et al.*, 2006. A role for the subiculum in the brain motivation/reward circuitry. Behavioural brain research, 174(2), pp.225–31.

Cornelius, F. & Mahmmoud, Y.A., 2003. Functional modulation of the sodium pump: the regulatory proteins "Fixit". *News in physiological sciences : an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society*, 18, pp.119–24.

Cortese, S. *et al.*, 2012. Toward systems neuroscience of ADHD: a meta-analysis of 55 fMRI studies. *The American journal of psychiatry*, 169(10), pp.1038–55.

Craddock, N. & Owen, M.J., 2010. The Kraepelinian dichotomy - going, going... but still not gone. *The British journal of psychiatry : the journal of mental science*, 196(2), pp.92–5.

Crambert, G. *et al.*, 2005. FXYD3 (Mat-8), a new regulator of Na,K-ATPase. *Molecular biology of the cell*, 16(5), pp.2363–71.

Creese, I., Burt, D. & Snyder, S., 1976. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science*, 192(4238), pp.481–483.

Crespi, B., Stead, P. & Elliot, M., 2010. Evolution in health and medicine Sackler colloquium: Comparative genomics of autism and schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America*, 107 Suppl (suppl_1), pp.1736–41.

Cubillo, A. *et al.*, 2010. Reduced activation and inter-regional functional connectivity of fronto-striatal networks in adults with childhood Attention-Deficit

Hyperactivity Disorder (ADHD) and persisting symptoms during tasks of motor inhibition and cognitive switching. *Journal of psychiatric research*, 44(10), pp.629–39.

Curran, C., Byrappa, N. & McBride, A., 2004. Stimulant psychosis: systematic review. *The British journal of psychiatry : the journal of mental science*, 185(3), pp.196–204.

Cussac, D. *et al.*, 2002. Stimulation by antipsychotic agents of mitogen-activated protein kinase (MAPK) coupled to cloned, human (h)serotonin (5-HT)(1A) receptors. *Psychopharmacology*, 162(2), pp.168–77.

Cuthbert, B.N. & Insel, T.R., 2010. Toward new approaches to psychotic disorders: the NIMH Research Domain Criteria project. *Schizophrenia bulletin*, 36(6), pp.1061–2.

Cuthbert, B.N. & Insel, T.R., 2013. Toward the future of psychiatric diagnosis: the seven pillars of RDoC. *BMC medicine*, 11(1), p.126.

Dai, H. & Carey, R.J., 1994. The NMDA antagonist MK-801 can impair attention to exteroceptive stimuli. Behavioural brain research, 62(2), pp.149–56.

Dandash, O. *et al.*, 2014. Altered striatal functional connectivity in subjects with an at-risk mental state for psychosis. *Schizophrenia bulletin*, 40(4), pp.904–13.

Davis, L.K. *et al.*, 2013. Partitioning the heritability of Tourette syndrome and obsessive compulsive disorder reveals differences in genetic architecture. M. C. Keller, ed. *PLoS genetics*, 9(10), p.e1003864.

Dawson, N. *et al.*, 2008. Novel analysis for improved validity in semi-quantitative 2-deoxyglucose autoradiographic imaging. Journal of neuroscience methods, 175(1), pp.25–35.

Dawson, N., Morris, B.J. & Pratt, J. a, 2011. Subanaesthetic ketamine treatment alters prefrontal cortex connectivity with thalamus and ascending subcortical systems. *Schizophrenia bulletin*, 39(2), pp.366–77.

Dawson, N. *et al.*, 2012. Modafinil reverses phencyclidine-induced deficits in cognitive flexibility, cerebral metabolism, and functional brain connectivity. Schizophrenia bulletin, 38(3), pp.457–74.

Dawson, N., Morris, B.J. & Pratt, J.A., 2013. Subanaesthetic ketamine treatment alters prefrontal cortex connectivity with thalamus and ascending subcortical systems. *Schizophrenia bulletin*, 39(2), pp.366–77.

Dawson, N. *et al.*, 2014. Subanesthetic ketamine treatment promotes abnormal interactions between neural subsystems and alters the properties of functional brain networks. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 39(7), pp.1786–98.

Degenhardt, F. *et al.*, 2012. Association between copy number variants in 16p11.2 and major depressive disorder in a German case-control sample. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, 159B(3), pp.263–73.

Dere, E., Pause, B.M. & Pietrowsky, R., 2010. Emotion and episodic memory in neuropsychiatric disorders. *Behavioural brain research*, 215(2), pp.162–71.

Dhanasekaran, D.N. & Reddy, E.P., 2008. JNK signaling in apoptosis. *Oncogene*, 27(48), pp.6245–51.

Dramsdahl, M. *et al.*, 2012. Adults with attention-deficit/hyperactivity disorder - a diffusion-tensor imaging study of the corpus callosum. *Psychiatry research*, 201(2), pp.168–73.

Dudchenko, P.A., Wood, E.R. & Eichenbaum, H., 2000. Neurotoxic hippocampal lesions have no effect on odor span and little effect on odor recognition memory but produce significant impairments on spatial span, recognition, and alternation. The Journal of neuroscience : the official journal of the Society for Neuroscience, 20(8), pp.2964–77.

Duncan, G.E. *et al.*, 1999. Comparison of brain metabolic activity patterns induced by ketamine, MK-801 and amphetamine in rats: support for NMDA receptor involvement in responses to subanesthetic dose of ketamine. *Brain research*, 843(1-2), pp.171–83.

Eckersley, R., 2006. Is modern Western culture a health hazard? *International journal of epidemiology*, 35(2), pp.252–8.

European College of Neuropsychopharmacology (ECNP) (2011) The Size and Burden of Mental Disorders and Other Disorders of the Brain in Europe – It's worse than we thought.

Egerton, A. *et al.*, 2005. Impairment in perceptual attentional set-shifting following PCP administration: a rodent model of set-shifting deficits in schizophrenia. *Psychopharmacology*, 179(1), pp.77–84.

Ehrlichman, R.S. *et al.*, 2008. Deviance-elicited changes in event-related potentials are attenuated by ketamine in mice. *Journal of cognitive neuroscience*, 20(8), pp.1403–14.

Eichele, T. *et al.*, 2008. Prediction of human errors by maladaptive changes in event-related brain networks. *Proceedings of the National Academy of Sciences of the United States of America*, 105(16), pp.6173–8.

el-Mallakh, R.S. & Wyatt, R.J., 1995. The Na,K-ATPase hypothesis for bipolar illness. *Biological psychiatry*, 37(4), pp.235–44.

Elvevåg, B. & Goldberg, T.E., 2000. Cognitive impairment in schizophrenia is the core of the disorder. *Critical reviews in neurobiology*, 14(1), pp.1–21.

Emsell, L. *et al.*, 2013. Limbic and callosal white matter changes in euthymic bipolar I disorder: an advanced diffusion magnetic resonance imaging tractography study. *Biological psychiatry*, 73(2), pp.194–201.

Enright, S.J. & Beech, A.R., 1990. Obsessional states: anxiety disorders or schizotypes? An information processing and personality assessment. *Psychological medicine*, 20(3), pp.621–7.

Van Ewijk, H. *et al.*, 2012. Diffusion tensor imaging in attention deficit/hyperactivity disorder: a systematic review and meta-analysis. *Neuroscience and biobehavioral reviews*, 36(4), pp.1093–106.

Fearon, P. & Morgan, C., 2006. Environmental factors in schizophrenia: the role of migrant studies. *Schizophrenia bulletin*, 32(3), pp.405–8.

Ferrari, A.J. et al., 2013. Global variation in the prevalence and incidence of major depressive disorder: a systematic review of the epidemiological literature. Psychological medicine, 43(3), pp.471–81.

Ferrarelli, F. & Tononi, G., 2011. The thalamic reticular nucleus and schizophrenia. *Schizophrenia bulletin*, 37(2), pp.306–15.

Ferreira, M.A.R. *et al.*, 2008. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nature genetics*, 40(9), pp.1056–8.

Filippi, M. *et al.*, 2014. Patterns of brain structural changes in first-contact, antipsychotic drug-naive patients with schizophrenia. *AJNR. American journal of neuroradiology*, 35(1), pp.30–7.

Fineberg, N. a *et al.*, 2013. The size, burden and cost of disorders of the brain in the UK. *Journal of psychopharmacology (Oxford, England)*, 27(9), pp.761–70.

Fitzsimmons, J., Kubicki, M. & Shenton, M.E., 2013. Review of functional and anatomical brain connectivity findings in schizophrenia. *Current opinion in psychiatry*, 26(2), pp.172–87.

Fone, K.C.F. & Porkess, M.V., 2008. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. *Neuroscience and biobehavioral reviews*, 32(6), pp.1087–102.

Fornito, A. *et al.*, 2013. Functional dysconnectivity of corticostriatal circuitry as a risk phenotype for psychosis. *JAMA psychiatry*, 70(11), pp.1143–51.

Fornito, A. *et al.*, 2012. Schizophrenia, neuroimaging and connectomics. *NeuroImage*, 62(4), pp.2296–314.

Foussias, G. & Remington, G., 2010. Negative symptoms in schizophrenia: avolition and Occam's razor. Schizophrenia bulletin, 36(2), pp.359–69.

Fox, M.D. *et al.*, 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences of the United States of America*, 102(27), pp.9673–8.

Franklin, K.B.J & Paxinos, G. 1997. The mouse in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press

Freedman, R. *et al.*, 2003. The genetics of sensory gating deficits in schizophrenia. *Current psychiatry reports*, 5(2), pp.155–61.

Friedman, J.I. *et al.*, 2008. Diffusion tensor imaging findings in first-episode and chronic schizophrenia patients. *The American journal of psychiatry*, 165(8), pp.1024–32.

Friston, K.J. & Frith, C.D., 1995. Schizophrenia: a disconnection syndrome? *Clinical neuroscience (New York, N.Y.)*, 3(2), pp.89–97.

Frohlich, J. & Van Horn, J.D., 2014. Reviewing the ketamine model for schizophrenia. *Journal of psychopharmacology (Oxford, England)*, 28(4), pp.287–302.

Frohlich, J. & Van Horn, J.D., 2001. Reviewing the ketamine model for schizophrenia. *Journal of psychopharmacology (Oxford, England)*, 28(4), pp.287–302.

Fromer, M. *et al.*, 2014. De novo mutations in schizophrenia implicate synaptic networks. *Nature*, 506(7487), pp.179–84.

Funk, A.J. *et al.*, 2012. Abnormal activity of the MAPK- and cAMP-associated signaling pathways in frontal cortical areas in postmortem brain in schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 37(4), pp.896–905.

Gaigg, S.B., Bowler, D.M. & Gardiner, J.M., 2014. Episodic but not semantic order memory difficulties in autism spectrum disorder: Evidence from the Historical Figures Task. *Memory*, 22(6), pp.669–678.

Galaverna, F.S., Morra, C.A. & Bueno, A.M., 2012. Attention in patients with chronic schizophrenia: Deficit in inhibitory control and positive symptoms. *The European Journal of Psychiatry*, 26(3), pp.185–195.

Galehdari, H. *et al.*, 2009. Association between the G1001C polymorphism in the GRIN1 gene promoter and schizophrenia in the Iranian population. *Journal of molecular neuroscience : MN*, 38(2), pp.178–81.

Gallagher, P. *et al.*, 2014. Neurocognitive functioning in bipolar depression: a component structure analysis. *Psychological medicine*, 44(5), pp.961–74.

Gardner, R.J. *et al.*, 2014. Neural oscillations during non-rapid eye movement sleep as biomarkers of circuit dysfunction in schizophrenia. *The European journal of neuroscience*, 39(7), pp.1091–106.

Ge, Y. et al., 2010. Hippocampal long-term depression is required for the consolidation of spatial memory. *Proceedings of the National Academy of Sciences of the United States of America*, 107(38), pp.16697–702.

Geering, K., 2006. FXYD proteins: new regulators of Na-K-ATPase. *American journal of physiology. Renal physiology*, 290(2), pp.F241–50.

Geering, K. *et al.*, 2003. FXYD proteins: new tissue- and isoform-specific regulators of Na,K-ATPase. *Annals of the New York Academy of Sciences*, 986, pp.388–94.

Green, M.F., Kern, R.S. & Heaton, R.K., 2004. Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. Schizophrenia research, 72(1), pp.41–51.

Georgi, A. *et al.*, 2007. Possible association between genetic variants at the GRIN1 gene and schizophrenia with lifetime history of depressive symptoms in a German sample. *Psychiatric genetics*, 17(5), pp.308–10.

Geyer, M.A. *et al.*, 2001. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology*, 156(2-3), pp.117–54.

Goff, D.C. & Coyle, J.T., 2001. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *The American journal of psychiatry*, 158(9), pp.1367–77.

Goh, S. & Peterson, B.S., 2012. Imaging evidence for disturbances in multiple learning and memory systems in persons with autism spectrum disorders. *Developmental medicine and child neurology*, 54(3), pp.208–13.

Goldman-Rakic, P.S., 1994. Working memory dysfunction in schizophrenia. *The Journal of neuropsychiatry and clinical neurosciences*, 6(4), pp.348–57.

Goldstein, Sam, Naglieri, Jack A. 2014 – The Handbook of Executive Functioning (Eds.)2014, XIX, 567 p. 43 illus.

Gonzalez-Burgos, G., Fish, K.N. & Lewis, D. a, 2011. GABA neuron alterations, cortical circuit dysfunction and cognitive deficits in schizophrenia. *Neural plasticity*, 2011, p.723184.

Gordeev, S.A., 2008. Cognitive functions and the state of nonspecific brain systems in panic disorders. *Neuroscience and behavioral physiology*, 38(7), pp.707–14.

Gotlib, I.H. & Joormann, J., 2010. Cognition and depression: current status and future directions. *Annual review of clinical psychology*, 6, pp.285–312.

Grandy, D.K. *et al.*, 1989. The human dopamine D2 receptor gene is located on chromosome 11 at q22-q23 and identifies a TaqI RFLP. *American journal of human genetics*, 45(5), pp.778–85.

Gratten, J. *et al.*, 2014. Large-scale genomics unveils the genetic architecture of psychiatric disorders. *Nature Publishing Group*, 17(6), pp.782–790.

Greden, J. F., & Tandon, R,. 1991. Negative schizophrenic symptoms:Pathophysiology and clinical implications. *Progress in psychiatry, No. 28*.

Green, E.K. *et al.*, 2010. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. *Molecular psychiatry*, 15(10), pp.1016–22.

Greicius, M.D. *et al.*, 2003. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, 100(1), pp.253–8.

Greicius, M.D. *et al.*, 2009. Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cerebral cortex (New York, N.Y. : 1991)*, 19(1), pp.72–8.

Group, C. & Consortium, P.G., 2013. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet*, pp.1371–1379.

Guilfoyle, D.N. *et al.*, 2013. Functional connectivity fMRI in mouse brain at 7T using isoflurane. *Journal of neuroscience methods*, 214(2), pp.144–8.

Guo, W. *et al.*, 2014. Abnormal default-mode network homogeneity in first-episode, drug-naive schizophrenia at rest. *Progress in neuro-psychopharmacology & biological psychiatry*, 49, pp.16–20.

Gusnard, D.A. *et al.*, 2001. Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America*, 98(7), pp.4259–64.

Hamilton, J.P., Chen, M.C. & Gotlib, I.H., 2013. Neural systems approaches to understanding major depressive disorder: an intrinsic functional organization perspective. *Neurobiology of disease*, 52, pp.4–11.

Hampson, R.E., Hedberg, T. & Deadwyler, S.A., 2000. Differential information processing by hippocampal and subicular neurons. Annals of the New York Academy of Sciences, 911, pp.151–65.

Harrison, B.J. *et al.*, 2013. Brain corticostriatal systems and the major clinical symptom dimensions of obsessive-compulsive disorder. *Biological psychiatry*, 73(4), pp.321–8.

Harvey, P.D. *et al.*, 2005. Treatment of cognitive impairment in early psychosis: a comparison of risperidone and haloperidol in a large long-term trial. *The American journal of psychiatry*, 162(10), pp.1888–95.

Hatcher, J.P. *et al*., 2001. Development of SHIRPA to characterise the phenotype of gene-targeted mice. Behavioural brain research, 125(1-2), pp.43–7.

Hauber, W. & Sommer, S., 2009. Prefrontostriatal circuitry regulates effort-related decision making. *Cerebral cortex (New York, N.Y. : 1991)*, 19(10), pp.2240–7.

Hayashi-Takagi, A., Barker, P.B. & Sawa, A., 2011. Readdressing synaptic pruning theory for schizophrenia: Combination of brain imaging and cell biology. *Communicative & integrative biology*, 4(2), pp.211–2.

Van den Heuvel, M.P. & Fornito, A., 2014. Brain networks in schizophrenia. *Neuropsychology review*, 24(1), pp.32–48.

Hill, E.L., 2004. Executive dysfunction in autism. *Trends in Cognitive Sciences*, 8(1), pp.26–32.

Hill, E.L. & Frith, U., 2003. Understanding autism: insights from mind and brain. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 358(1430), pp.281–9.

Hill, K. *et al.*, 2004. Hypofrontality in schizophrenia: a meta-analysis of functional imaging studies. *Acta psychiatrica Scandinavica*, 110(4), pp.243–56.

Hill, S.K. *et al.*, 2010. Effect of second-generation antipsychotics on cognition: current issues and future challenges. *Expert review of neurotherapeutics*, 10(1), pp.43–57.

Hobaiter, C. & Byrne, R.W., 2011. The gestural repertoire of the wild chimpanzee. *Animal cognition*, 14(5), pp.745–67.

Hoffman, R.E. *et al.*, 2011. Elevated functional connectivity along a corticostriatal loop and the mechanism of auditory/verbal hallucinations in patients with schizophrenia. *Biological psychiatry*, 69(5), pp.407–14.

Hoffman, R.E., Stopek, S. & Andreasen, N.C., 1986. A comparative study of manic vs schizophrenic speech disorganization. *Archives of general psychiatry*, 43(9), pp.831–8.

Hong, S.-B. *et al.*, 2014. Connectomic Disturbances in Attention-Deficit/Hyperactivity Disorder: A Whole-Brain Tractography Analysis. *Biological psychiatry*.

Houenou, J. *et al.*, 2007. Increased white matter connectivity in euthymic bipolar patients: diffusion tensor tractography between the subgenual cingulate and the amygdalo-hippocampal complex. *Molecular psychiatry*, 12(11), pp.1001–10.

Howes, O.D. & Kapur, S., 2009. The dopamine hypothesis of schizophrenia: version III--the final common pathway. *Schizophrenia bulletin*, 35(3), pp.549–62.

Hughes, K.C. & Shin, L.M., 2011. Functional neuroimaging studies of post-traumatic stress disorder. *Expert review of neurotherapeutics*, 11(2), pp.275–85.

Hurd, Y.L., Suzuki, M. & Sedvall, G.C., 2001. D1 and D2 dopamine receptor mRNA expression in whole hemisphere sections of the human brain. *Journal of chemical neuroanatomy*, 22(1-2), pp.127–37.

Ihara, H., Berrios, G.E. & McKenna, P.J., 2000. Dysexecutive syndrome in schizophrenia: A cross-cultural comparison between Japanese and British patients. *Behavioural neurology*, 12(4), pp.209–220.

Illarionava, N.B. *et al.*, 2014. Role of Na,K-ATPase α1 and α2 isoforms in the support of astrocyte glutamate uptake. PloS one, 9(6), p.e98469.

Ino, Y. *et al.*, 2002. Dysadherin, a cancer-associated cell membrane glycoprotein, down-regulates E-cadherin and promotes metastasis. *Proceedings of the National Academy of Sciences of the United States of America*, 99(1), pp.365–70.

Iwata, Y. *et al.*, 2010. Failure to confirm genetic association of the FXYD6 gene with schizophrenia: the Japanese population and meta-analysis. American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics, 153B(6), pp.1221–7.

Jeong, H. *et al.*, 2001. Lethality and centrality in protein networks. *Nature*, 411(6833), pp.41–2.

Jonckers, E. *et al.*, 2011. Functional connectivity fMRI of the rodent brain: comparison of functional connectivity networks in rat and mouse. *PloS one*, 6(4), p.e18876.

Just, M.A. *et al.*, 2004. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain : a journal of neurology*, 127(Pt 8), pp.1811–21.

Kadowaki, K. *et al.*, 2004. Phosphohippolin expression in the rat central nervous system. *Brain research. Molecular brain research*, 125(1-2), pp.105–12.

Kalkstein, S., Hurford, I. & Gur, R.C., 2010. Neurocognition in schizophrenia. *Current topics in behavioral neurosciences*, 4, pp.373–90.

Kanaan, R.A.A. *et al.*, 2005. Diffusion tensor imaging in schizophrenia. *Biological psychiatry*, 58(12), pp.921–9.

Kasparek, T. *et al.*, 2013. Brain functional connectivity of male patients in remission after the first episode of schizophrenia. *Human brain mapping*, 34(3), pp.726–37.

Kay, S. R., Fiszbein, A., & Opler, L,. A. 1987. The Positive and Negative Syndrome Scale (PANSS) for Schizophrenia. Schizophrenia Bulletin, 13(2),261–276.

Keener, M.T. & Phillips, M.L., 2007. Neuroimaging in bipolar disorder: a critical review of current findings. *Current psychiatry reports*, 9(6), pp.512–20.

Keller, T.A., Kana, R.K. & Just, M.A., 2007. A developmental study of the structural integrity of white matter in autism. *Neuroreport*, 18(1), pp.23–7.

Kennedy, N.J. et al., 2007. Requirement of JIP scaffold proteins for NMDA-mediated signal transduction. *Genes & development*, 21(18), pp.2336–46.

Keown, C.L. *et al.*, 2013. Local functional overconnectivity in posterior brain regions is associated with symptom severity in autism spectrum disorders. *Cell reports*, 5(3), pp.567–72.

Kim, M.J. et al., 2007. Synaptic accumulation of PSD-95 and synaptic function regulated by phosphorylation of serine-295 of PSD-95. *Neuron*, 56(3), pp.488–502.

Kirov, G. *et al.*, 2009. A genome-wide association study in 574 schizophrenia trios using DNA pooling. Molecular psychiatry, 14(8), pp.796–803.

Kirov, G. *et al.*, 2012. De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. *Molecular psychiatry*, 17(2), pp.142–53.

Kjelgaard, M.M. & Tager-Flusberg, H., 2001. An Investigation of Language Impairment in Autism: Implications for Genetic Subgroups. *Language and cognitive processes*, 16(2-3), pp.287–308.

Kleinman, L. et al., 2003. Costs of bipolar disorder. PharmacoEconomics, 21(9), pp.601–22.

Kochunov, P. & Hong, L.E., 2014. Neurodevelopmental and neurodegenerative models of schizophrenia: white matter at the center stage. *Schizophrenia bulletin*, 40(4), pp.721–8.

Kong, A. *et al.*, 2012. Rate of de novo mutations and the importance of father's age to disease risk. *Nature*, 488(7412), pp.471–5.

De Kovel, C.G.F. *et al.*, 2010. Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies. *Brain : a journal of neurology*, 133(Pt 1), pp.23–32.

Krain, A.L. & Castellanos, F.X., 2006. Brain development and ADHD. *Clinical psychology review*, 26(4), pp.433–44.

Krause, M., Hoffmann, W.E. & Hajós, M., 2003. Auditory sensory gating in hippocampus and reticular thalamic neurons in anesthetized rats. *Biological psychiatry*, 53(3), pp.244–53.

Kumar, R.A. *et al.*, 2008. Recurrent 16p11.2 microdeletions in autism. *Human molecular genetics*, 17(4), pp.628–38.

Kurtz, M.M. & Gerraty, R.T., 2009. A meta-analytic investigation of neurocognitive deficits in bipolar illness: profile and effects of clinical state. *Neuropsychology*, 23(5), pp.551–62.

De La Fuente, A. *et al.*, 2013. A review of attention-deficit/hyperactivity disorder from the perspective of brain networks. *Frontiers in human neuroscience*, 7, p.192.

Lahti, A.C. *et al.*, 1995. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 13(1), pp.9–19.

Lahti, A.C. *et al.*, 2001. Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 25(4), pp.455–67.

Laruelle, M., 2014. Schizophrenia: from dopaminergic to glutamatergic interventions. *Current opinion in pharmacology*, 14, pp.97–102.

Lauer, A.M. *et al.*, 2009. Analysis of environmental sound levels in modern rodent housing rooms. *Lab animal*, 38(5), pp.154–60.

Laviola, G. *et al.*, 2009. Gene-environment interaction during early development in the heterozygous reeler mouse: clues for modelling of major neurobehavioral syndromes. *Neuroscience and biobehavioral reviews*, 33(4), pp.560–72.

Lee, I. & Kesner, R.P., 2003a. Differential roles of dorsal hippocampal subregions in spatial working memory with short versus intermediate delay. Behavioral neuroscience, 117(5), pp.1044–53.

Lee, I. & Kesner, R.P., 2003b. Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. The Journal of neuroscience : the official journal of the Society for Neuroscience, 23(4), pp.1517–23.

Lee, R.S.C. *et al.*, 2012. A meta-analysis of cognitive deficits in first-episode Major Depressive Disorder. *Journal of affective disorders*, 140(2), pp.113–24.

Legon, W. *et al.*, 2014. Transcranial focused ultrasound modulates the activity of primary somatosensory cortex in humans. *Nature neuroscience*, 17(2), pp.322–9.

Lewis, C.M. *et al.*, 2009. Learning sculpts the spontaneous activity of the resting human brain. *Proceedings of the National Academy of Sciences of the United States of America*, 106(41), pp.17558–63.

Lewis, D.A. & Levitt, P., 2002. Schizophrenia as a disorder of neurodevelopment. *Annual review of neuroscience*, 25, pp.409–32.

Li, M. *et al.*, 2012. Meta-analysis and brain imaging data support the involvement of VRK2 (rs2312147) in schizophrenia susceptibility. Schizophrenia research, 142(1-3), pp.200–5.

Lin, F. *et al.*, 2011. Abnormal frontal cortex white matter connections in bipolar disorder: a DTI tractography study. *Journal of affective disorders*, 131(1-3), pp.299–306.

Li, X.-M. et al., 2007. JNK1 contributes to metabotropic glutamate receptor-dependent long-term depression and short-term synaptic plasticity in the mice area hippocampal CA1. *The European journal of neuroscience*, 25(2), pp.391–6

Linnman, C. *et al.*, 2011. An fMRI study of unconditioned responses in post-traumatic stress disorder. *Biology of mood & anxiety disorders*, 1(1), p.8.

Lionel, A.C. *et al.*, 2011. Rare copy number variation discovery and cross-disorder comparisons identify risk genes for ADHD. *Science translational medicine*, 3(95), p.95ra75.

Lipska, B.K. & Weinberger, D.R., 2000. To model a psychiatric disorder in animals: schizophrenia as a reality test. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 23(3), pp.223–39.

Liu, F. *et al.*, 2012. Classification of different therapeutic responses of major depressive disorder with multivariate pattern analysis method based on structural MR scans. *PloS one*, 7(7), p.e40968.

Liu, J. & Lin, A., 2005. Role of JNK activation in apoptosis: a double-edged sword. *Cell research*, 15(1), pp.36–42.

Lodge, D.J. & Grace, A.A., 2008. Hippocampal dysfunction and disruption of dopamine system regulation in an animal model of schizophrenia. *Neurotoxicity research*, 14(2-3), pp.97–104.

Loi, F., Vaidya, J.G. & Paradiso, S., 2013. Recognition of emotion from body language among patients with unipolar depression. *Psychiatry research*, 209(1), pp.40–9.

Lord, C., Risi, S. & Pickles, A., 2004 Trajectory of Language Development in Autistic Spectrum Disorders. Developmental language disorders: From phenotypes to etiologies. , (pp. 7-29).

Lubarski, I. *et al.*, 2005. Interaction with the Na,K-ATPase and tissue distribution of FXYD5 (related to ion channel). *The Journal of biological chemistry*, 280(45), pp.37717–24.

Luck, S.J. & Gold, J.M., 2008. The construct of attention in schizophrenia. *Biological psychiatry*, 64(1), pp.34–9.

Lui, S. *et al.*, 2014. Resting-state brain function in schizophrenia and psychotic bipolar probands and their first-degree relatives. *Psychological medicine*, pp.1–12.

Lüscher, C. & Malenka, R.C., 2012. NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harbor perspectives in biology*, 4(6).

Magioncalda, P. *et al.*, 2014. Functional connectivity and neuronal variability of resting state activity in bipolar disorder-reduction and decoupling in anterior cortical midline structures. *Human brain mapping*.

Mahurin, R.K. *et al.*, 2006. Trail making test errors and executive function in schizophrenia and depression. *The Clinical neuropsychologist*, 20(2), pp.271–88.

Makris, N. *et al.*, 2008. Attention and executive systems abnormalities in adults with childhood ADHD: A DT-MRI study of connections. *Cerebral cortex (New York, N.Y. : 1991)*, 18(5), pp.1210–20.

Makris, N. *et al.*, 2009. Towards conceptualizing a neural systems-based anatomy of attention-deficit/hyperactivity disorder. *Developmental neuroscience*, 31(1-2), pp.36–49.

Malhotra, A.K. *et al.*, 1997. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 17(3), pp.141–50.

Mamah, D., Barch, D.M. & Repovš, G., 2013. Resting state functional connectivity of five neural networks in bipolar disorder and schizophrenia. *Journal of affective disorders*, 150(2), pp.601–9.

Manoach, D.S., 2003. Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. *Schizophrenia research*, 60(2-3), pp.285–98.

Manoliu, A. *et al.*, 2014. Aberrant dependence of default mode/central executive network interactions on anterior insular salience network activity in schizophrenia. *Schizophrenia bulletin*, 40(2), pp.428–37.

Manoliu, A. *et al.*, 2013. Insular dysfunction within the salience network is associated with severity of symptoms and aberrant inter-network connectivity in major depressive disorder. *Frontiers in human neuroscience*, 7, p.930.

Marighetto, a *et al.*, 2008. Comparative effects of the dopaminergic agonists piribedil and bromocriptine in three different memory paradigms in rodents. *Journal of psychopharmacology (Oxford, England)*, 22(5), pp.511–21.

Marino, L. *et al.*, 2007. Cetaceans have complex brains for complex cognition. *PLoS biology*, 5(5), p.e139.

Di Martino, A. *et al.*, 2011. Aberrant striatal functional connectivity in children with autism. *Biological psychiatry*, 69(9), pp.847–56.

Martino, D.J. *et al.*, 2014. Toward the identification of neurocognitive subtypes in euthymic patients with bipolar disorder. *Journal of affective disorders*, 167, pp.118–24.

Martinussen, R. *et al.*, 2005. A meta-analysis of working memory impairments in children with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, 44(4), pp.377–84.

McAlonan, K., Cavanaugh, J. & Wurtz, R.H., 2006. Attentional modulation of thalamic reticular neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience, 26(16), pp.4444–50.

McAlonan, K., Cavanaugh, J. & Wurtz, R.H., 2008. Guarding the gateway to cortex with attention in visual thalamus. Nature, 456(7220), pp.391–4.

Mccarroll, S.A., Feng, G. & Hyman, S.E., 2014. Genome-scale neurogenetics : methodology and meaning. *Nature Publishing Group*, 17(6), pp.756–763.

McCarthy, S.E. *et al.*, 2009. Microduplications of 16p11.2 are associated with schizophrenia. *Nature genetics*, 41(11), pp.1223–7.

McGrath, J.J. *et al.*, 2014. A comprehensive assessment of parental age and psychiatric disorders. *JAMA psychiatry*, 71(3), pp.301–9.

McGrath, L.M. *et al.*, 2014. Copy number variation in obsessive-compulsive disorder and tourette syndrome: a cross-disorder study. *Journal of the American Academy of Child and Adolescent Psychiatry*, 53(8), pp.910–9.

McIntosh, A.M. *et al.*, 2008. White matter tractography in bipolar disorder and schizophrenia. *Biological psychiatry*, 64(12), pp.1088–92.

McKinney, W.T. & Bunney, W.E., 1969. Animal model of depression. I. Review of evidence: implications for research. *Archives of general psychiatry*, 21(2), pp.240–8.

Menon, V., 2011. Large-scale brain networks and psychopathology: a unifying triple network model. *Trends in cognitive sciences*, 15(10), pp.483–506.

Menon, V. & Uddin, L.Q., 2010. Saliency, switching, attention and control: a network model of insula function. *Brain structure & function*, 214(5-6), pp.655–67.

Merikangas, K.R. et al., 2011. Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. Archives of general psychiatry, 68(3), pp.241–51.

Millan, M.J. *et al.*, 2012. Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. *Nature reviews. Drug discovery*, 11(2), pp.141–68.

Millan, M.J., 2006. Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacology & therapeutics*, 110(2), pp.135–370.

Millan, M.J., 2002. N-methyl-D-aspartate receptor-coupled glycineB receptors in the pathogenesis and treatment of schizophrenia: a critical review. *Current drug targets. CNS and neurological disorders*, 1(2), pp.191–213.

Millar, J.K. *et al.*, 2000. Disruption of two novel genes by a translocation co-segregating with schizophrenia. Human molecular genetics, 9(9), pp.1415–23.

Minzenberg, M.J. *et al.*, 2009. Meta-analysis of 41 functional neuroimaging studies of executive function in schizophrenia. *Archives of general psychiatry*, 66(8), pp.811–22.

Miyamoto, S. *et al.*, 2000. Effects of ketamine, MK-801, and amphetamine on regional brain 2-deoxyglucose uptake in freely moving mice. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 22(4), pp.400–12.

Molina, V. *et al.*, 2005. Hypofrontality in men with first-episode psychosis. *The British journal of psychiatry : the journal of mental science*, 186, pp.203–8.

Moore, S.A., 2009. Cognitive abnormalities in posttraumatic stress disorder. *Current opinion in psychiatry*, 22(1), pp.19–24.

Moorhead, T.W.J. *et al.*, 2007. Progressive gray matter loss in patients with bipolar disorder. *Biological psychiatry*, 62(8), pp.894–900.

Moran, L. V *et al.*, 2013. Disruption of anterior insula modulation of large-scale brain networks in schizophrenia. *Biological psychiatry*, 74(6), pp.467–74.

Morris, B.J., Cochran, S.M. & Pratt, J.A., 2005. PCP: from pharmacology to modelling schizophrenia. *Current opinion in pharmacology*, 5(1), pp.101–6.

Morrison, S.C., Brown, L.A. & Cohen, A.S., 2013. A multidimensional assessment of social cognition in psychometrically defined schizotypy. *Psychiatry research*, 210(3), pp.1014–9.

Morris, B.J. & Pratt, J.A., 2014. Novel treatment strategies for schizophrenia from improved understanding of genetic risk. Clinical genetics, 86(5), pp.401–11.

Mortensen, P.B. *et al.*, 1999. Effects of family history and place and season of birth on the risk of schizophrenia. *The New England journal of medicine*, 340(8), pp.603–8.

Mottron, L. *et al.*, 2013. Veridical mapping in the development of exceptional autistic abilities. *Neuroscience and biobehavioral reviews*, 37(2), pp.209–28.

Mueser, K. T., & McGurk, S. R., 2004. Schizophrenia. *Lancet*, 363(9426), 2063–72.

Mukherjee, P.K. et al., 1999. Glutamate receptor signaling interplay modulates stress-sensitive mitogen-activated protein kinases and neuronal cell death. *The Journal of biological chemistry*, 274(10), pp.6493–8.

Murray, J.B., 2002. Phencyclidine (PCP): a dangerous drug, but useful in schizophrenia research. *The Journal of psychology*, 136(3), pp.319–27.

Myers, A.K. et al., 2014. Cortical interneurons require Jnk1 to enter and navigate the developing cerebral cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 34(23), pp.7787–801.

Nakao, T. *et al.*, 2011. Gray matter volume abnormalities in ADHD: voxel-based meta-analysis exploring the effects of age and stimulant medication. *The American journal of psychiatry*, 168(11), pp.1154–63.

Nakao, T. *et al.*, 2009. Working memory dysfunction in obsessive-compulsive disorder: a neuropsychological and functional MRI study. *Journal of psychiatric research*, 43(8), pp.784–91.

Nakao, K. et al., 2002. Hippocampal long-term depression as an index of spatial working memory. *The European journal of neuroscience*, 16(5), pp.970–4.

Nazeri, A. *et al.*, 2013. Alterations of superficial white matter in schizophrenia and relationship to cognitive performance. *Neuropsychopharmacology : official*

publication of the American College of Neuropsychopharmacology, 38(10), pp.1954–62.

Neale, B.M. *et al.*, 2010. Case-control genome-wide association study of attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, 49(9), pp.906–20.

Nee, D.E., Jonides, J. & Berman, M.G., 2007. Neural mechanisms of proactive interference-resolution. *NeuroImage*, 38(4), pp.740–51.

Need, A.C. *et al.*, 2009. A genome-wide investigation of SNPs and CNVs in schizophrenia. PLoS genetics, 5(2), p.e1000373.

Neill, J.C. *et al.*, 2010. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. *Pharmacology & therapeutics*, 128(3), pp.419–32.

Nekovarova, T. *et al.*, 2014. Bridging disparate symptoms of schizophrenia: a triple network dysfunction theory. *Frontiers in behavioral neuroscience*, 8(May), p.171.

Newmark, R.E. *et al.*, 2013. Contributions of the hippocampal subfields and entorhinal cortex to disambiguation during working memory. Hippocampus, 23(6), pp.467–75.

Olff, M. *et al.*, 2014. Executive function in posttraumatic stress disorder (PTSD) and the influence of comorbid depression. *Neurobiology of learning and memory*, 112, pp.114–21.

Olton, D.S., 1987. The radial arm maze as a tool in behavioral pharmacology. *Physiology & Behavior*, 40(6), pp.793–797.

O'Mara, S.M. *et al.*, 2001. The subiculum: a review of form, physiology and function. Progress in neurobiology, 64(2), pp.129–55.

Orellana, G. & Slachevsky, A., 2013. Executive functioning in schizophrenia. *Frontiers in psychiatry*, 4, p.35.

Orliac, F. *et al.*, 2013. Links among resting-state default-mode network, salience network, and symptomatology in schizophrenia. *Schizophrenia research*, 148(1-3), pp.74–80.

Van Os, J., Kenis, G. & Rutten, B.P.F., 2010. The environment and schizophrenia. *Nature*, 468(7321), pp.203–12.

Palaniyappan, L. *et al.*, 2013. Neural primacy of the salience processing system in schizophrenia. *Neuron*, 79(4), pp.814–28.

Pasternak, O. *et al.*, 2012. Excessive extracellular volume reveals a neurodegenerative pattern in schizophrenia onset. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 32(48), pp.17365–72.

Paulus, M.P. & Stein, M.B., 2006. An insular view of anxiety. *Biological psychiatry*, 60(4), pp.383–7.

Paylor, R. & Lindsay, E., 2006. Mouse models of 22q11 deletion syndrome. *Biological psychiatry*, 59(12), pp.1172–9.

Pedersen, C.B. & Mortensen, P.B., 2006. Are the cause(s) responsible for urban-rural differences in schizophrenia risk rooted in families or in individuals? *American journal of epidemiology*, 163(11), pp.971–8.

Pelphrey, K.A. *et al.*, 2002. Visual scanning of faces in autism. *Journal of autism and developmental disorders*, 32(4), pp.249–61.

Pessoa, L., 2008. On the relationship between emotion and cognition. *Nature reviews. Neuroscience*, 9(2), pp.148–58.

Phillips, M.L., Ladouceur, C.D. & Drevets, W.C., 2008. A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. *Molecular psychiatry*, 13(9), pp.829, 833–57.

Pratt, J. *et al.*, 2012. Advancing schizophrenia drug discovery: optimizing rodent models to bridge the translational gap. *Nature reviews. Drug discovery*, 11(7), pp.560–79.

Proal, E. *et al.*, 2011. Brain gray matter deficits at 33-year follow-up in adults with attention-deficit/hyperactivity disorder established in childhood. *Archives of general psychiatry*, 68(11), pp.1122–34.

Radanovic, M. *et al.*, 2013. Formal Thought Disorder and language impairment in schizophrenia. *Arquivos de neuro-psiquiatria*, 71(1), pp.55–60.

Raichle, M.E. *et al.*, 2001. A default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America*, 98(2), pp.676–82.

Redrobe, J.P., Bull, S. & Plath, N., 2010. Translational Aspects of the Novel Object Recognition Task in Rats Abstinent Following Sub-Chronic Treatment with Phencyclidine (PCP): Effects of Modafinil and Relevance to Cognitive Deficits in Schizophrenia. *Frontiers in psychiatry*, 1, p.146.

Renkawek, K. *et al.*, 1992. Neonatal status convulsivus, spongiform encephalopathy, and low activity of Na+/K(+)-ATPase in the brain. *Epilepsia*, 33(1), pp.58–64.

Repovs, G., Csernansky, J.G. & Barch, D.M., 2011. Brain network connectivity in individuals with schizophrenia and their siblings. *Biological psychiatry*, 69(10), pp.967–73.

Richard Wilkinson., 2010. The Spirit Level: Why Equality is Better for Everyone. Penguin.

Ripke, S., Wray, N.R., *et al.*, 2013. A mega-analysis of genome-wide association studies for major depressive disorder. *Molecular psychiatry*, 18(4), pp.497–511.

Ripke, S. *et al.*, 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511, 421–427

Ripke, S., Dushlaine, C.O., *et al.*, 2013. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nature Publishing Group*, 45(10), pp.1150–1159

Rippon, G. *et al.*, 2007. Disordered connectivity in the autistic brain: challenges for the “new psychophysiology”. *International journal of psychophysiology : official journal of the International Organization of Psychophysiology*, 63(2), pp.164–72.

Risold, P.Y., Thompson, R.H. & Swanson, L.W., 1997. The structural organization of connections between hypothalamus and cerebral cortex. Brain research. Brain research reviews, 24(2-3), pp.197–254.

Rizig, M.A. *et al.*, 2012. A gene expression and systems pathway analysis of the effects of clozapine compared to haloperidol in the mouse brain implicates susceptibility genes for schizophrenia. *Journal of psychopharmacology (Oxford, England)*, 26(9), pp.1218–30.

Robinson, L.J. *et al.*, 2006. A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. *Journal of affective disorders*, 93(1-3), pp.105–15.

Rogers, D.C. et al., 2001. SHIRPA, a protocol for behavioral assessment: validation for longitudinal study of neurological dysfunction in mice. Neuroscience Letters, 306(1-2), pp.89–92.

Rose, E.M. *et al.*, 2009. Glutamate transporter coupling to Na,K-ATPase. The Journal of neuroscience : the official journal of the Society for Neuroscience, 29(25), pp.8143–55.

Roth, R.M. *et al.*, 2004. Procedural and declarative memory in obsessive-compulsive disorder. *Journal of the International Neuropsychological Society : JINS*, 10(5), pp.647–54.

Rybakowski, J.K. & Lehmann, W., 1994. Decreased activity of erythrocyte membrane ATPases in depression and schizophrenia. *Neuropsychobiology*, 30(1), pp.11–4.

Samamé, C., 2013. Social cognition throughout the three phases of bipolar disorder: a state-of-the-art overview. *Psychiatry research*, 210(3), pp.1275–86.

Santos, J.L. *et al.*, 2014. A five-year follow-up study of neurocognitive functioning in bipolar disorder. *Bipolar Disord. 2014 Nov;16(7):722-31*

Sarrazin, S. *et al.*, 2014. A multicenter tractography study of deep white matter tracts in bipolar I disorder: psychotic features and interhemispheric disconnectivity. *JAMA psychiatry*, 71(4), pp.388–96.

Sato, J.R. *et al.*, 2012. Abnormal brain connectivity patterns in adults with ADHD: a coherence study. Y. Fan, ed. *PloS one*, 7(9), p.e45671.

Savla, G.N. *et al.*, 2013. Deficits in domains of social cognition in schizophrenia: a meta-analysis of the empirical evidence. *Schizophrenia bulletin*, 39(5), pp.979–92.

Sayin, A. *et al.*, 2010. Theory of mind in obsessive-compulsive disorder: Comparison with healthy controls. *European psychiatry : the journal of the Association of European Psychiatrists*, 25(2), pp.116–22.

Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium 2011. Genome-wide association study identifies five new schizophrenia loci. *Nature genetics*, 43(10), pp.969–76.

Schizophrenia Commission 2012. The Abandoned Illness. A Report by the Schizophrenia Commission.

Schönenberg, M. & Abdelrahman, T., 2013. In the face of danger: exploring the attentional blink to emotional facial expressions in PTSD. *Psychiatry research*, 209(2), pp.180–5.

Schreiter, S., Pijnenborg, G.H.M. & Aan Het Rot, M., 2013. Empathy in adults with clinical or subclinical depressive symptoms. *Journal of affective disorders*, 150(1), pp.1–16.

Seeley, W.W. *et al.*, 2007. Dissociable intrinsic connectivity networks for salience processing and executive control. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(9), pp.2349–56.

Seeman, P. *et al.*, 1984. Bimodal distribution of dopamine receptor densities in brains of schizophrenics. *Science*, 225(4663), pp.728–731.

Seeman, P. & Lee, T., 1975. Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. *Science (New York, N.Y.)*, 188(4194), pp.1217–9.

Selvaraj, S. *et al.*, 2012. Grey matter differences in bipolar disorder: a meta-analysis of voxel-based morphometry studies. *Bipolar disorders*, 14(2), pp.135–45.

Sforazzini, F. *et al.*, 2014. Distributed BOLD and CBV-weighted resting-state networks in the mouse brain. *NeuroImage*, 87, pp.403–15.

Shang, C.Y. *et al.*, 2013. Disturbed microstructural integrity of the frontostriatal fiber pathways and executive dysfunction in children with attention deficit hyperactivity disorder. *Psychological medicine*, 43(5), pp.1093–107.

Shaw, P. *et al.*, 2007. Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proceedings of the National Academy of Sciences of the United States of America*, 104(49), pp.19649–54.

Shin, N.Y. *et al.*, 2014. Cognitive functioning in obsessive-compulsive disorder: a meta-analysis. *Psychological medicine*, 44(6), pp.1121–30.

Singh, K.D. & Fawcett, I.P., 2008. Transient and linearly graded deactivation of the human default-mode network by a visual detection task. *NeuroImage*, 41(1), pp.100–12.

Skudlarski, P. *et al.*, 2010. Brain connectivity is not only lower but different in schizophrenia: a combined anatomical and functional approach. *Biological psychiatry*, 68(1), pp.61–9.

Snyder, H.R. *et al.*, 2014. Obsessive-Compulsive Disorder Is Associated With Broad Impairments in Executive Function: A Meta-Analysis. *Clinical Psychological Science*, p.2167702614534210.

Sokoloff, L., 1981. Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, 1(1), pp.7–36.

Sonuga-Barke, E.J.S. & Castellanos, F.X., 2007. Spontaneous attentional fluctuations in impaired states and pathological conditions: a neurobiological hypothesis. *Neuroscience and biobehavioral reviews*, 31(7), pp.977–86.

Sorge, R.E. *et al.*, 2014. Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nature methods*, 11(6), pp.629–32.

Sporns, O., 2014. Towards network substrates of brain disorders. *Brain : a journal of neurology*, 137(Pt 8), pp.2117–8.

Sprengelmeyer, R. *et al.*, 2011. The insular cortex and the neuroanatomy of major depression. *Journal of affective disorders*, 133(1-2), pp.120–7.

Sridharan, D., Levitin, D.J. & Menon, V., 2008. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proceedings of the National Academy of Sciences of the United States of America*, 105(34), pp.12569–74.

Sripada, C. *et al.*, 2014. Disrupted network architecture of the resting brain in attention-deficit/hyperactivity disorder. *Human brain mapping*, 35(9), pp.4693–705.

Sripada, C.S., Kessler, D. & Angstadt, M., 2014. Lag in maturation of the brain's intrinsic functional architecture in attention-deficit/hyperactivity disorder. *Proceedings of the National Academy of Sciences*, 111(39), pp.14259–14264.

Sripada, R.K. *et al.*, Neural dysregulation in posttraumatic stress disorder: evidence for disrupted equilibrium between salience and default mode brain networks. *Psychosomatic medicine*, 74(9), pp.904–11.

St Clair, D. *et al.*, 1990. Association within a family of a balanced autosomal translocation with major mental illness. Lancet, 336(8706), pp.13–6.

Stam, AJ; *et al.*,. 2013. The genetic overlap of attention deficit hyperactivity disorder and autistic spectrum disorder. *The Application of Clinical Genetics*, 2, p.7.

Stergiakouli, E. *et al.*, 2012. Investigating the contribution of common genetic variants to the risk and pathogenesis of ADHD. *The American journal of psychiatry*, 169(2), pp.186–94.

Stern, E.R. *et al.*, 2012. Resting-state functional connectivity between fronto-parietal and default mode networks in obsessive-compulsive disorder. C. Soriano-Mas, ed. *PloS one*, 7(5), p.e36356.

Still, A.W., 1969. Proactive interference and spontaneous alternation in rats. *Quarterly Journal of Experimental Psychology*, 21(4), pp.339–345.

Stordal, K.I. *et al.*, 2004. Impairment across executive functions in recurrent major depression. *Nordic journal of psychiatry*, 58(1), pp.41–7.

Strakowski, S.M. *et al.*, 2012. The functional neuroanatomy of bipolar disorder: a consensus model. *Bipolar disorders*, 14(4), pp.313–25.

Substance Abuse and Mental Health Services Administration (SAMHSA) (2010). Results from the 2009 National Survey on Drug Use and Health: Mental Health Findings (Office of Applied Studies, NSDUH Series H-39, HHS Publication No. SMA 10-4609). Rockville, MD.

Sullivan, P.F., Daly, M.J. & Donovan, M.O., 2012. Genetic architectures of psychiatric disorders : the emerging picture and its implications. *Nature Publishing Group*, 13(8), pp.537–551.

Sumathipala, A., 2004. Culture-bound syndromes: the story of dhat syndrome. *The British Journal of Psychiatry*, 184(3), pp.200–209.

Sun, L. *et al.*, 2012. Abnormal functional connectivity between the anterior cingulate and the default mode network in drug-naïve boys with attention deficit hyperactivity disorder. *Psychiatry research*, 201(2), pp.120–7.

Supekar, K. *et al.*, 2013. Brain hyperconnectivity in children with autism and its links to social deficits. *Cell reports*, 5(3), pp.738–47.

Sweadner, K.J. & Rael, E., 2000. The FXYD gene family of small ion transport regulators or channels: cDNA sequence, protein signature sequence, and expression. *Genomics*, 68(1), pp.41–56.

Tamm, L., Barnea-Goraly, N. & Reiss, A.L., 2012. Diffusion tensor imaging reveals white matter abnormalities in Attention-Deficit/Hyperactivity Disorder. *Psychiatry research*, 202(2), pp.150–4.

Tamminga, C.A. *et al.*, 1992. Limbic system abnormalities identified in schizophrenia using positron emission tomography with fluorodeoxyglucose and neocortical alterations with deficit syndrome. *Archives of general psychiatry*, 49(7), pp.522–30.

Therien, A.G. *et al.*, 1997. Tissue-specific Distribution and Modulatory Role of the Subunit of the Na,K-ATPase. *Journal of Biological Chemistry*, 272(51), pp.32628–32634.

Therien, A.G., Karlish, S.J.D. & Blostein, R., 1999. Expression and Functional Role of the Subunit of the Na,K-ATPase in Mammalian Cells. *Journal of Biological Chemistry*, 274(18), pp.12252–12256.

Thomas Gualtieri, C., 2014. Autism and Schizophrenia Are Disorders of Evolvability. *Open Journal of Medical Psychology*, 03(02), pp.161–183.

Thomas, G.M. et al., 2008. Rapid and bi-directional regulation of AMPA receptor phosphorylation and trafficking by JNK. *The EMBO journal*, 27(2), pp.361–72.

Tian, L. *et al.*, 2006. Altered resting-state functional connectivity patterns of anterior cingulate cortex in adolescents with attention deficit hyperactivity disorder. *Neuroscience letters*, 400(1-2), pp.39–43.

Tim Kasser, The High Price of Materialism 2002, MIT Press, Cambridge, MA.

Tolin, D.F. *et al.*, 2001. Memory and memory confidence in obsessive-compulsive disorder. *Behaviour research and therapy*, 39(8), pp.913–27.

Torgerson, C.M. *et al.*, 2013. DTI tractography and white matter fiber tract characteristics in euthymic bipolar I patients and healthy control subjects. *Brain imaging and behavior*, 7(2), pp.129–39.

Torres, I.J., Boudreau, V.G. & Yatham, L.N., 2007. Neuropsychological functioning in euthymic bipolar disorder: a meta-analysis. *Acta psychiatrica Scandinavica. Supplementum*, (434), pp.17–26.

Tseng, K.Y., Chambers, R.A. & Lipska, B.K., 2009. The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. Behavioural brain research, 204(2), pp.295–305.

Tyler, W.J. *et al.*, 2008. Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. *PloS one*, 3(10), p.e3511.

Uddin, L.Q. *et al.*, 2014. Brain State Differentiation and Behavioral Inflexibility in Autism†. *Cerebral cortex (New York, N.Y. : 1991)*.

Uddin, L.Q. *et al.*, 2013. Salience network-based classification and prediction of symptom severity in children with autism. *JAMA psychiatry*, 70(8), pp.869–79.

Uekermann, J. *et al.*, 2010. Social cognition in attention-deficit hyperactivity disorder (ADHD). *Neuroscience and biobehavioral reviews*, 34(5), pp.734–43.

Ullmann, R. *et al.*, 2007. Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. *Human mutation*, 28(7), pp.674–82.

Umbricht, D. *et al.*, 2014. Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: a randomized, double-blind, proof-of-concept study. *JAMA psychiatry*, 71(6), pp.637–46.

Ussher, J.M. & Perz, J., 2013. PMS as a process of negotiation: women's experience and management of premenstrual distress. *Psychology & health*, 28(8), pp.909–27.

Vargas, C., López-Jaramillo, C. & Vieta, E., 2013. A systematic literature review of resting state network--functional MRI in bipolar disorder. *Journal of affective disorders*, 150(3), pp.727–35.

Veling, W. *et al.*, 2008. Ethnic density of neighborhoods and incidence of psychotic disorders among immigrants. *The American journal of psychiatry*, 165(1), pp.66–73.

Vorhees, C. V & Williams, M.T., 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature protocols*, 1(2), pp.848–58.

Vyazovskiy, V. V *et al.*, 2008. Cortical metabolic rates as measured by 2-deoxyglucose-uptake are increased after waking and decreased after sleep in mice. Brain research bulletin, 75(5), pp.591–7.

Wang, J. & Xia, Y., 2012. Assessing developmental roles of MKK4 and MKK7 in vitro. *Communicative & integrative biology*, 5(4), pp.319–24.

Wessa, M., Kanske, P. & Linke, J., 2014. Bipolar disorder: a neural network perspective on a disorder of emotion and motivation. *Restorative neurology and neuroscience*, 32(1), pp.51–62.

Wheeler, A.L. & Voineskos, A.N., 2014. A review of structural neuroimaging in schizophrenia: from connectivity to connectomics. *Frontiers in Human Neuroscience*, 8, p.653.

White, T. *et al.*, 2011. Global white matter abnormalities in schizophrenia: a multisite diffusion tensor imaging study. *Schizophrenia bulletin*, 37(1), pp.222–32.

White, T.P. *et al.*, 2010. Aberrant salience network (bilateral insula and anterior cingulate cortex) connectivity during information processing in schizophrenia. *Schizophrenia research*, 123(2-3), pp.105–15.

Witter, M.P. & Groenewegen, H.J., 1990. The subiculum: cytoarchitectonically a simple structure, but hodologically complex. Progress in brain research, 83, pp.47–58.

WHO Regional Committee for Europe (2013) WHO Fact Sheet: Mental Health

World Health Organization. (2008) The Global Burden of Disease: 2004 Update (WHO Press, 2008).

Williams, A.D. & Moulds, M.L., 2008. Negative appraisals and cognitive avoidance of intrusive memories in depression: a replication and extension. *Depression and anxiety*, 25(7), pp.E26–33.

Williams, J.M.G. *et al.*, 2007. Autobiographical memory specificity and emotional disorder. *Psychological bulletin*, 133(1), pp.122–48.

Williams, N.M. *et al.*, 2010. Rare chromosomal deletions and duplications in attention-deficit hyperactivity disorder: a genome-wide analysis. *Lancet*, 376(9750), pp.1401–8.

Winchester, C.L. *et al.*, 2012. Converging evidence that sequence variations in the novel candidate gene MAP2K7 (MKK7) are functionally associated with schizophrenia. *Human molecular genetics*, 21(22), pp.4910–21.

Winchester, C.L., Pratt, J.A. & Morris, B.J., 2014. Risk genes for schizophrenia: translational opportunities for drug discovery. *Pharmacology & therapeutics*, 143(1), pp.34–50.

Wong, D. *et al.*, 1986. Positron emission tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. *Science*, 234(4783), pp.1558–1563.

Wray, N.R. *et al.*, 2012. Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. *Molecular psychiatry*, 17(1), pp.36–48.

Wu, Y.-H. *et al.*, 2014. White matter tract integrity of frontostriatal circuit in attention deficit hyperactivity disorder: association with attention performance and symptoms. *Human brain mapping*, 35(1), pp.199–212.

Xia, S. *et al.*, 2012. Thalamic shape and connectivity abnormalities in children with attention-deficit/hyperactivity disorder. *Psychiatry research*, 204(2-3), pp.161–7.

Yamasaki, T. *et al.*, 2011. Stress-activated protein kinase MKK7 regulates axon elongation in the developing cerebral cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(46), pp.16872–83.

Yang, H. et al., 2011. Hippocampal long-term depression is enhanced, depotentiation is inhibited and long-term potentiation is unaffected by the application of a selective c-Jun N-terminal kinase inhibitor to freely behaving rats. *The European journal of neuroscience*, 33(9), pp.1647–55.

Yao, L. *et al.*, 2013. White matter deficits in first episode schizophrenia: an activation likelihood estimation meta-analysis. *Progress in neuro-psychopharmacology & biological psychiatry*, 45, pp.100–6.

Young, E.C. *et al.*, 2005. The use of two language tests to identify pragmatic language problems in children with autism spectrum disorders. *Language, speech, and hearing services in schools*, 36(1), pp.62–72.

Young, J.W. *et al.*, 2007. The odour span task: a novel paradigm for assessing working memory in mice. Neuropharmacology, 52(2), pp.634–45.

Young, J.W. *et al.*, 2009. Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. Pharmacology & therapeutics, 122(2), pp.150–202.

Yu, H. *et al.*, 2014. Protein-interaction-network-based analysis for genome-wide association analysis of schizophrenia in Han Chinese population. *Journal of Psychiatric Research*, 50, pp.73–78.

Zalesky, A. *et al.*, 2011. Disrupted axonal fiber connectivity in schizophrenia. *Biological psychiatry*, 69(1), pp.80–9.

Zandi, T. *et al.*, 2008. The need for culture sensitive diagnostic procedures: a study among psychotic patients in Morocco. *Social psychiatry and psychiatric epidemiology*, 43(3), pp.244–50.

Zhang, J. *et al.*, 2010. No association between the FXYD6 gene and schizophrenia in the Chinese Han population. *Journal of psychiatric research*, 44(6), pp.409–12.

Zheng, F. *et al.*, 2014. Further evidence for genetic association of CACNA1C and schizophrenia : New risk loci in a Han Chinese population and a meta-analysis. , 152, pp.105–110.

Zhong, N. *et al.*, 2011. A novel replicated association between FXYD6 gene and schizophrenia. *Biochemical and biophysical research communications*, 405(1), pp.118–21.

Zou, L. *et al.*, 2008. Diffusion tensor imaging study of the anterior limb of internal capsules in neuroleptic-naive schizophrenia. *Academic radiology*, 15(3), pp.285