Title:

Functional Brain Connectivity Phenotypes for Schizophrenia Drug Discovery

Authors and Affiliations:

Neil Dawson[1], Brian J Morris[2,4], Judith A Pratt[3,4]

1. Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, LA1 4YQ, UK
2. Institute of Neuroscience and Psychology, School of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK
3. Strathclyde Institute of Pharmacy and Biomedical Science, Taylor St, University of Strathclyde, Glasgow, G4 0RE, UK
4. Psychiatric Research Institute of Neuroscience in Glasgow (PsyRING), University of Glasgow, Glasgow, G12 8QQ, UK

Corresponding Author:

Dr Neil Dawson, Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, LA1 4YQ, UK. Email: n.dawson1@lancaster.ac.uk.

Tel: +44(0)1524 594 896.

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Abstract

While our knowledge of the pathophysiology of schizophrenia has increased dramatically this has not translated into the development of new and improved drugs to treat this disorder. Human brain imaging and electrophysiological studies have provided dramatic new insight into the mechanisms of brain dysfunction in the disease, with a swathe of recent studies highlighting the differences in functional brain network and neural system connectivity present in the disorder. Only recently has the value of applying these approaches in preclinical rodent models relevant to the disorder started to be recognised. Here we highlight recent findings of altered functional brain connectivity in preclinical rodent models and consider their relevance to those alterations seen in the brains of schizophrenia patients. Furthermore, we highlight the potential translational value of using the paradigm of functional brain connectivity phenotypes in the context of preclinical schizophrenia drug discovery, as a means both to understand the mechanisms of brain dysfunction in the disorder and to reduce the current high attrition rate in schizophrenia drug discovery.

Introduction

Schizophrenia is a common, severely debilitating form of mental illness that affects around 1% of the global population. Patients typically exhibit a combination of *positive symptoms*, that include hallucinations, disorganised speech, and delusions, *negative symptoms*, such as affective flattening and reduced motivation (avolition), and pronounced *cognitive deficits*, that include deficits in working memory, cognitive flexibility and attention. While the existing medications, the antipsychotics, relieve the positive symptoms of the disorder in the majority of patients, they fail to treat the negative or cognitive symptoms of the disorder adequately. Moreover, the drugs do not cure the disease and have many serious side effects, including dyskinesia and weight gain. Therefore, there is an urgent need to develop new therapies to treat the disorder.

The development of new drug therapies for schizophrenia relies upon their validation in preclinical rodent models. In the past many of these preclinical models have been "blunt instruments" with questionable construct validity; that is to say that these models have not been based on aetiologically established risk factors for schizophrenia, or on the known neurobiology of disorder (see Pratt *et al.*, 2012 for review). The use of these preclinical models has undoubtedly hindered the development of new therapeutics for the disorder. However, as our understanding of the contribution of a range of genetic and environmental risk factors for schizophrenia, and of the complex neurobiology of the disorder, has increased, so too has the now widespread recognition in the field that utilising preclinical models based on these aetiologically established risk factors and established neurobiology offers the greatest opportunity for success in schizophrenia drug discovery. In addition, the increased recognition that there has been an over reliance on the use of behavioural

phenotypes, in behavioural measures that also often have questionable validity, to test the potential of novel therapeutic compounds in these preclinical models has also revolutionised the thinking in this field, as has the recognition that we have a limited understanding of the neural circuitry underling these behaviours and the distinct symptom domains of schizophrenia. This thinking has driven changes in the field in two ways:

(1) The recent development of a range of preclinical behavioural tests that are more firmly based on deficits seen in schizophrenia patients. For example the attentional set shifting task in rodents (ASST, Birrell *et al.*, 2000) that is based on the Intradimensional-Extradimensional (ID-ED) Set Shifting deficit seen in schizophrenic patients in the Cambridge Neuropsychological Test Automated Battery, (CANTAB, Jazbec *et al.*, 2007) and the Wisconsin Card Sorting Task (WCST, Nieuwenstein *et al.*, 2001). In addition, other tests assessing translational aspects of attention (Bari *et al.*, 2008, Thomson *et al.*, 2011) and working memory in rodents (Marighetto *et al.*, 2008) have also been developed. The recent technological advance of utilising touchscreen technology for cognitive testing in rodents may further accelerate the development of new translational paradigms for schizophrenia drug discovery (Bussey *et al.*, 2012).

(2) A recognition of the potential of utilising endophenotypes, broadly defined as internal phenotypes that lie on the pathway between the underlying genetics and the symptoms of the disease (Walters *et al.*, 2007), in the schizophrenia drug discovery process. Intermediate phenotypes, internal phenotypes that are not necessarily genetically determined but relate to both known disease aetiology or neurobiology and disease symptoms, are also useful in this respect. A prime example of this is the utilisation of functional brain imaging and electrophysiological measures of brain activity that have been applied both in schizophrenia

patients and in translational preclinical models relevant to the disorder. However, while measures to characterise brain connectivity have been widely applied in the clinical context their application in preclinical models relevant to schizophrenia has been much more limited. Here we consider how the characterisation of brain connectivity phenotypes, both as endophenotypes or intermediate phenotypes in preclinical models, will not only provide new insight into the neurobiology of schizophrenia but also a valuable new translational framework that offers new opportunities for schizophrenia drug discovery.

Altered brain functional connectivity in schizophrenia patients and at risk human populations

The idea that schizophrenia is caused, not by focal abnormalities in the brain, but from pathological alterations in the interactions between distinct brain regions and neural subsystems has long been a central tenant of schizophrenia research. In 1906 Wernike, in his sejunction (the action of disjoining) hypothesis, was the first to postulate that psychosis arises from anatomical disconnectivity in the brain (for example through the disruption of white matter tracts), a notion supported today both by indirect measures of structural brain connectivity (based on covariations in regional brain volume [Bassett *et al.*, 2008] and the morphometric analysis of white matter tracts, [Sigmundsson *et al*., 2001]) and recent *in vivo* diffusion tensor imaging (DTI) studies of white matter tract integrity (Yao *et al*., 2013; Ellison-Wright & Bullmore*,* 2009). In 1988 Volkow *et al*., completed one of the first studies, using positron emission tomography (PET), characterising how functional interactions between brain regions are altered in schizophrenia (Volkow *et al*., 1988). While altered anatomical connectivity undoubtedly contributes to the functional dysconnectivity seen between brain regions in schizophrenia a central role for altered synaptic functioning is also

supported. In particular, altered N-methyl-D-aspartate receptor (NMDA-R) dependent synaptic plasticity, mediated either directly at the level of the NDMA-R or through the influence of altered activity in other modulatory neurotransmitter systems, is thought to contribute to the functional dysconnectivity seen in the disorder (Stephan *et al*., 2006; 2009). These mechanisms are particularly important from a drug development perspective, as these deficits in synaptic functioning, rather than alterations in anatomical connectivity mediated through altered connectivity in long-range white matter tracts, are likely to be more amenable to pharmacological intervention.

The most commonly applied methodologies that have been utilized to characterise altered functional connectivity in schizophrenia patients are the hemodynamics-based neuroimaging techniques, such as blood-oxygen dependent (BOLD) functional magnetic resonance imaging (fMRI), and electrophysiological methods, such as magnetoencephalography (MEG) and electroencephalography (EEG). While the neuroimaging techniques offer greater spatial resolution the electrophysiological methods offer greater temporal granularity. Using these methodologies brain connectivity in schizophrenia has been analysed both under resting state conditions and when patients engage in a range of cognitive tasks that commonly assess cognitive constructs known to be dysfunctional in schizophrenia such as working memory (Forbes *et al*., 2009) or attention (Nieuwenstein *et al.*, 2001). In general, two broad analytical frameworks have been utilised in characterising brain connectivity from these data:

(1) Methods focused on characterising the connectivity of a specific regional interactions or defined seed brain regions, such as through dynamic causal modelling (DCM, Benetti *et al.*,

2009; Deserno *et al*., 2012, Guller *et al*., 2012; Roiser *et al*., 2013; Smidt *et al*., 2013a) and structural equation modelling (SEM, Schlosser *et al.*, 2003) approaches.

(2) Data-driven methods that characterise brain connectivity in a more holistic manner, such as at the level of complex brain networks using the algorithms of graph theory (Bullmore *et al.*, 2009, Fornito *et al.*, 2011; 2012; van den Heuvel & Fornito, 2014) or independent components analysis (ICA, Camchong *et al.*, 2011).

An important distinction between these two analytical approaches is the concept of effective versus functional connectivity. Procedures like DCM and SEM (1) are often informed by established anatomical connectivity, and parameterise distributed neuronal networks in terms of directed effective connectivity (allowing the determination of the directed causal interactions in brain connectivity), while the more global approaches based upon functional connectivity (2) describe undirected correlated activity between brain regions.

Overall, the data from these studies support compromised functional brain connectivity in patients with chronic schizophrenia, whether that be defined at the scale of whole brain networks (Micheloyannis *et al.*, 2006, Liu *et al.*, 2008, Lynall *et al.*, 2010; van den Heuvel & Fornito, 2014), or through the characterisation of specific neural subsystem interactions (Schlosser *et al.*, 2003, Benetti *et al.*, 2009; Deserno *et al*., 2012). In chronic schizophrenia, this appears to be centred around reduced frontal cortex (van den Heuvel *et al.*, 2010, Camchong *et al.*, 2011, Fornito *et al.*, 2011; Pettersson-Yeo *et al*., 2011), thalamic (Welsh *et al.*, 2010, Tomasi *et al.*, 2014) and hippocampal-frontal cortex functional connectivity (Meyer-Lindenberg *et al.*, 2005, Zhou *et al.*, 2008, Godsil *et al.*, 2013). Reduced frontal cortex, thalamic and hippocampal-frontal cortex connectivity is also seen in first episode

schizophrenia patients (Zhou *et al.*, 2007, Benetti *et al.*, 2009; Pettersson-Yeo *et al*., 2011; Schmidt *et al*., 2013a), and in at risk individuals (Dauvermann *et al*., 2013; Diwadkar *et al*., 2012; Pettersson-Yeo *et al*., 2011; Schmidt *et al*., 2014), suggesting that this effect is not the result of prolonged antipsychotic treatment. Of course, other important functional interactions have also been identified as being compromised in the brains of schizophrenia patients, including altered cerebellar (Barch, 2014) and prefrontal-temporal cortex connectivity (Ford *et al.*, 2002, Lawrie *et al.*, 2002; Roiser *et al*., 2013), and these are particularly evident when more holistic approaches to characterising brain network connectivity have been used (Fornito *et al.*, 2011). In addition, abnormally increased region-specific alterations in functional connectivity have also been identified (Schlosser *et al.*, 2003, Whitfield-Gabrieli *et al.*, 2009). The relative importance of these specific alterations in functional connectivity in the disorder, and their relevance to each of the specific symptom domains of schizophrenia, certainly requires further detailed consideration.

In general, the data currently available in the field support the contention that the altered brain connectivity seen in schizophrenic patients is present, often to a lesser degree, in at risk individuals (Benetti *et al.*, 2009; Pettersson-Yeo *et al*., 2011; Schmidt *et al*., 2014), unaffected family members (Liu *et al.*, 2008, Repovs *et al.*, 2011, Khadka *et al.*, 2013, Collin *et al.*, 2014; Diwadkar *et al*., 2012; Dauvermann *et al*., 2013) or individuals with mutations in candidate risk genes for the disorder (Esslinger *et al.*, 2009, Callicott *et al.*, 2013), suggesting that disrupted connectivity may represent a valid biomarker and endophenotype for the disorder. However, it should also be noted that many of the alterations seen in functional brain connectivity in chronic schizophrenia patients are not seen in first episode patients, this is particularly evident in terms of some measures that consider network connectivity on

a global scale, including measures of network clustering, small-worldness and efficiency for information transfer (Fornito *et al.*, 2011), suggesting either that the temporal progression of the disease is accurately reflected in evolving alterations in brain network connectivity, at least when functional connectivity is considered on a global scale, or that the prolonged antipsychotic treatment experienced by chronic schizophrenia patients modifies the global properties of functional brain networks. This certainly warrants further systematic investigation with dedicated longitudinal studies and studies focused on elucidating the impact of prolonged antipsychotic treatment on brain functional connectivity.

Many connectivity studies assess brain activity and connectivity during cognitive task performance, typically employing tasks that engage the prefrontal cortex (PFC), where patients commonly show functional impairment (Hill *et al.*, 2004). However, it has become clear relatively recently that patients also show altered connectivity at the resting state – in the so-called default mode network (DMN, Buckner *et al.*, 2008, Rotarska-Jagiela *et al.*, 2010, Woodward *et al.*, 2011) – in addition to the cognitive control networks (Whitfield-Gabrieli *et al.*, 2012). The DMN includes areas such as the dorsolateral prefrontal cortex (DLPFC) and temporal cortex that are frequently identified as dysfunctional in schizophrenia patients (Ford *et al.*, 2002). Activity in the DMN, which decreases during task performance, has been related to thought processes and attention to emotional state. The general picture in schizophrenia has been that patients show hyperactivity of the DMN along with an impaired ability to suppress the DMN during cognitive task performance. These abnormalities are present in first-episode and chronically ill patients as well as in those at high risk of developing psychosis (Fryer *et al*., 2013; Shim *et al*., 2010; Wotruba *et al*., 2014). Interestingly, it has recently been proposed that rodents exhibit a DMN-like pattern of

connectivity at rest, involving orbitofrontal, prelimbic and temporal cortices (Lu *et al.*, 2012). It will be very interesting to determine whether this DMN-like activity is similarly perturbed in translational models relevant to schizophrenia.

Altered brain connectivity following NMDA-R antagonist administration in humans

NMDA-R antagonists such as phencyclidine (PCP) and ketamine are well-known for their ability to produce schizophrenia-like symptoms when administered acutely in both healthy individuals (Krystal *et al.*, 1994) or in remitted schizophrenia patients (Lahti *et al.*, 1995), and when administered chronically (Morris *et al.*, 2005). Assessing the impact of these antagonists on functional connectivity is also particularly pertinent given the suggested central role for altered NMDA-R mediated synaptic plasticity in the functional dysconnectivity seen in schizophrenia (Stephan *et al*., 2006; 2009).

An overt increase in metabolic activity is observed in prefrontal areas following acute ketamine treatment in healthy volunteers (Vollenweider *et al.*, 1997, Langsjo *et al.*, 2004). This is in contrast to the hypometabolism observed in the prefrontal cortex in patients with schizophrenia (Hill *et al.*, 2004). The effects of ketamine on brain connectivity might show greater correspondence with the disease, but these are less well studied, particularly in humans. For example, the ability of ketamine to reduce the auditory mismatch negative (MMN) signal, in a similar way to that seen in schizophrenia patients (Umbrict & Krljes, 2005), may result from altered synaptic plasticity and connectivity in the projection between the auditory and superior temporal gyrus (Schmidt *et al.*, 2013b), two brain regions strongly implicated in the auditory hallucinations experienced by schizophrenia patients (Lawrie *et al*., 2002). Furthermore, acute ketamine treatment has been shown to reduce prefrontal connectivity, in association with impaired working memory performance (Anticevic *et al.*,

2012, Driesen *et al.*, 2013a), while reportedly increasing connectivity at rest (Driesen *et al.*, 2013b), suggesting that the ability of NMDA-R antagonists to modulate functional connectivity may be different under baseline and in task-activated situations. Additionally, timing also seems to be important as while acute subanaethetic ketamine treatment increases connectivity at rest (Driesen *et al.*, 2013b) this appears to be decreased 24 hours after ketamine treatment (Scheidegger *et al.*, 2012). The temporal relationship between these alterations in connectivity and the symptoms exhibited needs to be more clearly defined. For example, the acute effects of ketamine on brain connectivity may relate more directly to its ability to induce schizophrenia-like symptoms, whereas the connectivity alterations at 24 hours post-ketamine treatment may relate more to its antidepressant effects (Scheidegger *et al.*, 2012, Driesen *et al.*, 2013a ; 2013b). Furthermore, the relationship between NMDA-R antagonist induced alterations in functional connectivity and the stage of the disease needs to be carefully considered, as evidenced by recent data suggesting that the effects of acute ketamine treatment on prefrontal cortex connectivity most closely resemble those seen at early stages of the disease (Anticevic *et al*., 2014).

Altered brain connectivity in translational rodent models relevant to Schizophrenia

Only recently have the connectivity measures employed in human brain imaging and electrophysiology studies been utilised in preclinical translational models relevant to the schizophrenia and these generally support the concept that translationally-relevant alterations in brain connectivity exist in these models. This includes data generated from studies using aetiologically relevant genetic (Sigurdsson *et al.*, 2010) and environmental (Dickerson *et al.*, 2010) risk factor models, and pharmacological models based on known neurobiology of the disorder, including those based on dopamine system hyperfunction

(Schwarz *et al.*, 2007, Schwarz *et al.*, 2009) and NMDA-R hypofunction (Dawson *et al.*, 2013; 2014a; 2014b]). Moreover, the presence of overlapping brain connectivity phenotypes shared by these various models suggest that common patterns of altered brain connectivity exist in these models that may represent common pathways directly linking them to the altered patterns of brain connectivity seen in schizophrenia. Here we briefly review some of the brain connectivity alterations seen in various translational animal models. It is important to note that as this field of enquiry is in its infancy, both in terms of the preclinical models to which these measures have been applied and the analytical methods adopted. Future studies will undoubtedly expand our knowledge of the connectivity alterations that are present in these models.

Altered Hippocampal-Prefrontal Connectivity

Altered hippocampal-prefrontal cortex connectivity has been most widely characterised in preclinical rodent models relevant to schizophrenia using electrophysiological methods, in part due to the fact that these neural systems are relatively accessible to characterisation using these methodologies, and due to the observation that dysconnectivity between these systems has been robustly demonstrated in a range of psychiatric disorders (Godsil *et al.*, 2013). Using electrophysiological methods reduced hippocampal-prefrontal coupling (synchrony) has been reported in various genetic, environmental and pharmacological models relevant to the disorder (summarised in Table 1). For example, in a seminal piece of recent work, functional synchrony between the hippocampus and prefrontal cortex was found to be reduced in Df(16)$A^{+/-}$ mice, a translational model of a deletion in human chromosome 22 (22q11.2) that dramatically increases the risk of developing schizophrenia (Karayiorgou *et al.*, 2004), during a working memory task (Sigurdsson *et al.*, 2010). This

parallels the reduced connectivity seen between these neural systems during working memory tasks in schizophrenia patients. In addition, reduced hippocampal-prefrontal synchrony has been shown as a result of maternal immune activation (MIA, Dickerson *et al.*, 2010), a translational rodent model based on epidemiological evidence of an increased risk of developing schizophrenia in adulthood following prenatal exposure to infection (Brown *et al.*, 2010). Furthermore, reduced hippocampal-prefrontal synchrony has also been shown in the widely utilised methylazoxymethanol acetate (MAM) neurodevelopmental model (Dickerson *et al.*, 2010, Phillips *et al.*, 2012, Belujon *et al.*, 2013).

Altered hippocampal-prefrontal connectivity has also been shown in preclinical animal models when brain imaging methods have been utilised. For example, reduced hippocampal-prefrontal cortex connectivity has been shown in NMDA-R hypofunction models relevant to schizophrenia. This includes reduced functional connectivity between these neural systems following subchronic memantine treatment, assessed using fMRI (Sekar *et al.*, 2013), and following subchronic PCP treatment, assessed using 2-deoxyglucose functional brain imaging (Dawson *et al.*, 2012, Dawson *et al.*, 2014a). Furthermore, when brain network connectivity is considered at the global level subchronic PCP treatment induces a reduced connectivity in brain networks which parallels that reported in schizophrenia (Dawson *et al.*, 2014a). By contrast, acute treatment with subanaesthetic doses of the NMDA-R antagonist ketamine has been shown to increase connectivity in brain networks when considered on a global scale, increases the connectivity of prefrontal cortex (Dawson *et al.*, 2014b) and increases hippocampal-prefrontal connectivity (Gass *et al.*, 2014). These alterations directly contrast with those reported in the brains of patients with schizophrenia (Micheloyannis *et al.*, 2006, Liu *et al.*, 2008, van den Heuvel *et al.*, 2010).

Overall, these data suggest that prolonged rather than acute administration of NMDA-R antagonists induces alterations in brain connectivity that are most relevant to those seen in chronic schizophrenia, a contention further supported by the observation that while acute ketamine administration increases PFC connectivity (Gass *et al.*, 2014) this is reduced when connectivity is characterised 24 hours after ketamine treatment (Scheidegger *et al.*, 2012).

Overall, the clinical and preclinical data, utilising diverse preclinical models relevant to the disorder, suggest that altered hippocampal-prefrontal connectivity may be a primary mechanism contributing to brain dysfunction in schizophrenia. This may be particularly relevant to the working memory (Sigurdsson *et al.*, 2010), prepulse inhibition (Dickerson *et al.*, 2010) or sleep (Phillips *et al.*, 2012) deficits seen in these models and in the disorder, but further detailed consideration of the relationship between these altered patterns of brain connectivity and the other specific symptom domains of schizophrenia are required. Furthermore, whether a deficit in hippocampal-prefrontal connectivity is both sufficient and necessary to induce these specific deficits remains to be determined. While this may represent a potential endophenotype or intermediate phenotype against which the potential validity of novel drugs can be assessed, the relative importance of this specific neural functional interaction in preclinical models must be interpreted with some caution, as the relevance of other neural subsystem interactions using electrophysiological methods remains largely unexplored. However, the recent observation that this interaction is disrupted in preclinical models when dysfunction is considered at the level of large scale complex brain networks (Dawson *et al.*, 2014a), further supports the contention that the interaction between these neural systems is highly relevant in these translational models.

Altered Thalamic Connectivity

The importance of altered thalamic connectivity to brain dysfunction in schizophrenia has become increasingly recognized, in part due to the greater anatomical resolution of the functional brain imaging methods that are now available. While precise delineation of the different nuclei within the thalamus is still not possible with fMRI techniques, there is strong evidence to support dysfunction of mediodorsal and anteroventral nuclei in schizophrenia (Welsh *et al.*, 2010), which matches the areas showing abnormalities in post-mortem tissue (Pakkenberg *et al.*, 2009, Cronenwett *et al.*, 2010). Many recent studies have reported reduced thalamo-prefrontal cortical connectivity at resting state in patients with schizophrenia (Woodward *et al.*, 2012, Anticevic *et al.*, 2013, Klingner *et al.*, 2013).

While there is widespread evidence to support thalamic dysfunction in a range of preclinical models relevant to the disorder, the functional connectivity of the thalamic nuclei has only been characterised in a selection of these models. For example, in the subchronic PCP model there is evidence to support reduced thalamic (Dawson *et al.*, 2014a) and reduced thalamic-prefrontal connectivity (Dawson *et al.*, 2012). This includes reduced reticular thalamus connectivity in animals treated subchronically with PCP. By contrast, reticular thalamus connectivity to the prefrontal cortex is enhanced after acute ketamine treatment (Dawson *et al.*, 2014b). More studies need to be dedicated to elucidating how the functional connectivity of the thalamic nuclei are altered in a broader range of preclinical models relevant to schizophrenia, as well as in the disease itself. In particular the specific role of distinct thalamic nuclei, such as the reticular thalamic nucleus which may have a prominent role in the disorder (Ferrarelli and Tononi, 2011; Pratt and Morris in this issue), needs to be more rigorously defined. As the reticular thalamus is a key regulator of information flow

between the thalamus and cortex, and an important hub brain region in complex brain networks (Dawson *et al.*, 2014a), it has a central role in regulating connectivity between distributed neural subsystems. Therefore, it's contribution to the disorder in particular should be more thoroughly considered.

Challenges and Future Directions

The application of connectivity phenotypes in preclinical models relevant to psychiatric disorders is in its infancy. Luckily, a range of paradigms have been widely applied in the clinical literature that can be reverse translated into the preclinical context. Elucidating connectivity phenotypes in preclinical models relevant to schizophrenia provides not only added insight into the nature of brain dysfunction in the disorder, and its contributory mechanisms, but also provide a translational biomarker against which the efficacy of novel therapeutics can be tested. Of course, their greatest utility in this respect will be in combination with the use of other, translationally-relevant behavioural, neurophysiological and neurochemical measures. The value of taking this type of approach is highlighted in our recent work where we identified the alterations in regional connectivity that underlie the ability of the putative procognitive drug modafinil to reverse the deficits in cognitive flexibility seen in the subchronic PCP model of schizophrenia (Dawson *et al.*, 2012). This allowed us to not only further to elucidate the neural systems contributing to the deficit in cognitive flexibility in this model, but also provided important new insight into the mechanisms through which modafinil achieves it's procognitive effects. Using a systems-level, holistic approach to characterising the alterations in brain connectivity, such as through the application of graph theory algorithms to preclinical brain imaging data (Bullmore *et al.*, 2009, Dawson *et al.*, 2014a; 2014b), that allows for the characterisation of

brain connectivity across a range of scales, from the global to the regional, rather than focusing in on pre-specified neural subsystem interactions, may offer the greatest advantage and the most robust approach when characterising brain connectivity in preclinical models. This may also allow for the closer alignment of the connectivity alterations seen in preclinical models to those identified in schizophrenia, and may also offer the advantage of integrating connectivity as defined through diverse modalities, including measures of both functional and structural connectivity. A primary advantage in this regard, is that this data-driven approach will likely identify those alterations in brain connectivity that are most central to the phenotype seen in the animals model, and will also identify novel functional interactions of interest. While analysing functional connectivity phenotypes in the context of preclinical schizophrenia drug discovery is in its infancy, the potential utilisation of effective connectivity phenotypes in this context has not yet been recognised. This is particularly surprising given that analytical paradigms for characterising effective connectivity in the brains of rodents, such as through the application of structural equation modelling (SEM), already exist (McIntosh & Gonzalez-Lima, 1994). Determining endophenotypes based upon effective connectivity in this way may have even greater predictive and diagnostic validity than those based on functional connectivity. Indeed, much current work is being dedicated to developing paradigms and biophysically constrained models of effective connectivity to further understand brain connectivity in psychiatric disorder and the pharmacological modulation of this connectivity. The utilisation of both the functional and effective connectivity paradigms will undoubtedly offer the greatest opportunity for valid translation in preclinical schizophrenia drug discovery.

Despite the potential promise of utilising functional connectivity phenotypes for schizophrenia drug discovery the field faces many challenges that must be overcome before the true translational value of this approach can be fully realised. Many of these challenges will only be overcome by further systematic study, both in patients and in preclinical models, and a greater understanding of the various mechanisms, and their dysfunction in psychiatric diseases, regulating functional connectivity in the brain. At this point in time there are many open questions regarding the utility of functional connectivity endophenotypes in the context of drug discovery. In terms of the functional connectivity alterations seen in schizophrenia patients some of these questions include;

(1) How do alterations in functional connectivity relate to the specific symptoms domains of the disorder, and which of these are specific to schizophrenia as compared to other psychiatric disorders?

(2) Which alterations in functional connectivity are amenable to treatment with medication, and which most accurately predict treatment response?

(3) How do we differentiate between the impact of chronic antipsychotic treatment and the biology of the disease itself on functional connectivity in schizophrenia patients?

(4) How do alterations in functional connectivity develop as the disease progresses, including from the prodrome to first episode and then into the chronic phase of the disease? Which of these are disease-stage specific and which are amenable to treatment? Do these offer predictive biomarkers for modifying disease progression?

(5) Are trait alterations in functional connectivity amenable to medication, and are these useful predictors of symptom resolution?

(6) Do state-dependent or non-state dependent alterations in functional connectivity offer the best opportunity for drug discovery?

Some groups are already working towards addressing some of these challenges. For example, recent studies have characterised the alterations in functional brain connectivity seen in at risk individuals who subsequently develop psychosis as compared to at risk individuals who do not (Allen *et al*., 2012; Lord *et al*., 2012). This work not only suggests that different stages of the disease are reflected by specific alterations in functional connectivity, but also highlights the alterations in functional connectivity contributing to the psychotic symptoms of the disorder. While many studies have been committed to understanding the alterations in functional connectivity underlying distinct cognitive deficits in the disorder (Fornito *et al*., 2011; Hensler *et al*., 2010; Meyer-Lindenberg *et al*., 2001) little research has been committed to elucidating those deficits that contribute to the negative symptoms of schizophrenia. Thus the relationship between alterations in functional connectivity and the specific symptom domains of the disorders is only now beginning to be elucidated. Furthermore, a major limitation in this field of research as it currently stands is the general lack of robust longditudinal studies characterising alterations in functional connectivity as the disease progresses in schizophrenic patients and a lack of studies characterising the reversal of these deficits with antipsychotic treatment, along with their relationship to psychopathology. In addition to studies conducted in patients, preclinical studies conducted in rodents will be able to help to address some of these important issues, such as gaining clearer information of the biological and biochemical basis of specific alterations in functional connectivity in the brain. This includes elucidating the alterations in functional connectivity mediated by mutations in candidate risk genes for the disorder (Sigurdsson *et*

al., 2010) and disease relevant alterations in neurotransmitter system functioning (Dawson *et al*., 2013; 2014a; 2014b).

In addition to these high-level challenges many technical challenges also exist that need to be more systematically addressed. These include challenges around the variability and stability of functional connectivity measures gained from brain imaging data, addressing the poor noise-to-signal ratio of some state-induced alterations in functional connectivity and, for analysis under resting state conditions, establishing protocols that reduce the variability of the measures by ensuring participants are engaged in similar mental processes "at rest".

Overall, although in their infancy and despite the many challenges faced in this developing field of research, methods allowing the characterisation of functional brain connectivity phenotypes in preclinical models offer an important translational paradigm that should be effectively integrated, and developed iteratively, with other translational approaches to reduce the current high attrition rate in the schizophrenia drug discovery process and to develop new effective drugs to treat this disorder.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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Reduced Hippocampal – PFC Connectivity			
Preclinical Model	**Reference**	**Modality**	**Summary**
Df(16)A$^{+/-}$ mutant mice (genetic risk model)	Siggurdsson *et al*., 2010	EEG	Reduced synchrony during working memory task
MIA (environmental risk model)	Dickerson *et al*., 2010	EEG	Reduced coherence in freely moving animals
MAM model (developmental model)	Phillips *et al*., 2012	EEG	Impaired phase locking in sleeping animals
	Belujon *et al*., 2013	Electrophysiology	Reduced mPFC-evoked synaptic plasticity induced by high-frequency stimulation of the fimbra
NMDA-R antagonist models (glutamate hypofunction models)	Sekar *et al*., 2013	rs-fMRI	Reduced functional connectivity (correlation based) following subchronic memantine treatment
	Dawson *et al*., 2012	2-DG functional imaging	Reduced connectivity (PLSR) following subchronic PCP treatment
	Dawson *et al*., 2014a	2-DG functional imaging	Reduced connectivity (Graph Theory Measures) following subchronic PCP treatment
Reduced Thalamocortical Connectivity			
NMDA-R antagonist models (glutamate hypofunction model)	Dawson *et al*., 2012	2-DG functional imaging	Reduced thalamic-mPFC connectivity (PLSR) following subchronic PCP treatment
	Dawson *et al*., 2013	2-DG functional imaging	Reduced PFC-AV/MD thalamus connectivity (PLSR) following subanaesthetic ketamine treatment
	Dawson *et al*., 2014a	2-DG functional imaging	Reduced thalamic connectivity (Graph Theory Measures) following subchronic PCP treatment

Table 1: Alterations in hippocampal-PFC and thalamic functional connectivity reported in preclinical rodent models relevant to schizophrenia. 2-DG: 2-deoxyglucose; AV: anteroventral; MD: mediodorsal; EEG: electroencephalogram (electrodes) ; MIA: maternal immune activation; MAM: methylazoxymethanol acetate; rs-fMRI; resting state fMRI; PCP: phencyclidine; PLSR: partial least squares regression analysis; PFC: prefrontal cortex