# Deconstructing schizophrenia: Advances in preclinical models for biomarker identification

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**Abstract**

Schizophrenia is considered to develop as a consequence of genetic and environmental factors impacting on brain neural systems and circuits during vulnerable neurodevelopmental periods, thereby resulting in symptoms in early adulthood. Understanding of the impact of schizophrenia risk factors on brain biology and behavior can help in identifying biologically relevant pathways that are attractive for informing clinical studies and biomarker development. In this chapter, we emphasise the importance of adopting a reciprocal forward and reverse translation approach that is iteratively updated when additional new information is gained, either preclinically or clinically, for offering the greatest opportunity for discovering panels of biomarkers for the diagnosis, prognosis and treatment of schizophrenia. Importantly, biomarkers for identifying those at risk may inform early intervention strategies prior to the development of schizophrenia.

Given the emerging nature of this approach in the field, this review will highlight recent research of preclinical biomarkers in schizophrenia that show the most promise for informing clinical needs with an emphasis on relevant imaging, electrophysiological, cognitive behavioural and biochemical modalities. The implementation of this reciprocal translational approach is exemplified firstly by the production and characterisation of preclinical models based on the glutamate hypofunction hypothesis, genetic and environmental risk factors for schizophrenia (reverse translation), and then the recent clinical recognition of the thalamic reticular thalamus (TRN) as an important locus of brain dysfunction in schizophrenia as informed by preclinical findings (forward translation).

## 1. Introduction

Contemporary thinking on the causes of schizophrenia is that a combination of genetic and environmental risk factors interact during neurodevelopment to disrupt neural processes which then give rise to a diverse range of symptoms that typically emerge in late adolescence. Despite recent advances in the identification of these risk factors, we are now only at the beginning of the journey to translate this information into identified biomarkers with utility that can be used to predict clinical outcome, disease progression, therapeutic responsiveness and to inform drug discovery. Currently, diagnosis (via DSM 5 and ICD10) is based upon a descriptive collection of behaviours, that lack disorder specificity and show high heterogeneity, and treatments are based upon the dopamine hypothesis of schizophrenia developed over 60 years ago. Not surprisingly there is extremely limited scope at present for a personalised medicine approach in schizophrenia and in psychiatry in general. The identification of symptom domain relevant biomarkers would dramatically impact on our ability to diagnose and treat psychiatric disorders. For schizophrenia however, no biomarkers are currently adopted in clinical practice. Prata et al (2014) have reviewed the diverse literature on potential biomarkers for psychosis, noting that only one of hundreds of outcome prediction biomarkers demonstrated clinical utility; namely a pharmacogenetic biomarker that predicts the side –effects of clozapine (Prata, Mechelli and Kapur, 2014).

Nonetheless, with recent advances in genetic, genomic, neural imaging, immunological, electrophysiological and cognitive neuroscience approaches, the path to developing biomarkers for schizophrenia continues, and is becoming increasingly refined. (See chapters by ; Lyndon-Staley and Bassett; Hunter and Lawrie; Herron, Nerurkar and Cavanagh this volume). Indeed, a reliable diagnostic biomarker now exists for psychosis resulting from autoimmune limbic encephalitis which can occur as a result of autoantibodies to NMDA receptors. This diagnostic biomarker is in the form of a serum assay to detect anti-NMDA antibodies. Whilst patients experiencing this form of psychosis represents a small percentage of those experiencing schizophrenia overall, the identification of this patient subgroup is important as the disorder is treatable particularly if identified early (see Herron, Nerurkar and Cavanagh this volume).

Key remaining questions include (a) how can preclinical research impact on the development of clinically relevant biomarkers? (b) how can clinically identified biomarkers be effectively 'reverse translated' in preclinical models? and (c) how can these biomarkers be effectively utilized in preclinical research to improve the drug discovery process?

First it is important to define biomarkers and highlight their clinical utility. Biomarkers are objective biological measures that can broadly be divided into several categories: a) diagnostic - where they can aid in predicting risk and diagnosis b) prognostic - where they can provide a signpost for clinical course and c) predictive of drug response/therapeutic intervention - whether beneficial or adverse, and thereby provide a potential means of patient stratification.

There is of course, the theoretical potential for biomarkers to overlap in clinical utility; a diagnostic biomarker might not only be a marker for a particular symptom of the disorder but may also be a marker for treatment response. Moreover, given the overlapping biological basis and symptom profile of different psychiatric disorders it is likely that some biomarkers may be relevant across the traditional diagnostic boundaries. Given this, and the diverse range of symptoms present in schizophrenia, it is likely that a select panel of biomarkers will be required for effective diagnostic, prognostic and therapeutic intervention.

In this chapter we provide a brief overview of the current status of biomarkers in preclinical schizophrenia research. We then focus on the importance of forward and reverse translation approaches for biomarker development with specific recent advances in the areas of brain imaging, brain network connectivity, oscillations, behavioural analysis and biochemical research that are increasing our understanding of disease risk and aetiology and how they could be used for identifying novel effective treatments.

## 2. Current status

In order for preclinical biomarkers to translate to a clinically relevant outcome they should be measured in preclinical models of translational value and high validity. The preclinical model should encompass a relevant feature of the disorder (e.g. a genetic variant) and the measurement taken (the biomarker in this case) should be of clinical relevance.

Historically, preclinical models relevant to schizophrenia have been based upon whether they fall into the category of construct, face and predictive validity. Given that the causes of schizophrenia are multifactorial and not fully established, full construct validity is not yet, and is arguably unlikely to ever be fully, achievable in a single specified animal model. Nevertheless, recent advances in the understanding of the genetics of schizophrenia have enabled models of higher construct validity to be generated, with the caveat that relatively few of the genetic hits are simple single gene loss of function variants. Furthermore as our knowledge of the multifactorial basis of the disease increases, so does our ability to integrate multiple aetiologically relevant mechanisms into one preclinical model, as exemplified by recent studies combining genetic and environmental risk factors ((Nagai, Ibi and Yamada, 2011; Ayhan, McFarland and Pletnikov, 2016; Moran *et al.*, 2016). Hence, the construct validity of the preclinical models used in the field is increasing.

Models with face validity and predictive validity have typically been most widely employed for drug discovery. Face validity applies to many behavioural tasks including sensorimotor gating as measured by prepulse inhibition (PPI), where similar phenomenon can be observed in humans and rodents. Indeed, reversal of rodent PPI deficits by antipsychotic drugs also supports the potential predictive validity of this behavioural model (Swerdlow and Geyer, 1998; Martinez *et al.*, 2000; Leng *et al.*, 2003; Clapcote *et al.*, 2007) in that these antipsychotic drugs can ameliorate the positive symptoms of schizophrenia. However, the evidence for antipsychotic drugs actually reversing PPI deficits in humans is less substantiated (Mackeprang, Kristiansen and Glenthoj, 2002; Duncan *et al.*, 2003). This raises the question of whether the phenomenon of PPI is measuring similar neurobiological processes in rodents and humans. Arguably, a key element of face validity is to establish readouts that index the relevant neural circuitry across species. In addition, PPI deficits are not specific to schizophrenia, being present in a broad range of other brain disorders including Alzheimer's, Parkinson's and Huntington's disease (Swerdlow *et al.*, 1995; Perriol

*et al.*, 2005) and there is a great degree of overlap in performance between individuals in clinical and healthy samples, limiting the diagnostic potential of PPI as a biomarker for schizophrenia.

In terms of drug discovery, existing models have been of value in predicting antipsychotic drugs that ameliorate the hallucinations and delusions that form the positive symptoms of schizophrenia and for predicting extrapyramidal motor side-effects. However, these models have tended to identify drugs with the same mechanism e.g D2 receptor blockade. From a clinical perspective, there is considerable room for improvement as antipsychotic drugs are not always effective, do not cure the disease and have many side effects. In the past 20 years, it has become apparent that the cognitive deficits and negative symptoms of schizophrenia are major factors in determining patient functionality/outcome and quality of life (Green *et al.*, 2000; Green, Kern and Heaton, 2004; Nuechterlein *et al.*, 2011; Fervaha *et al.*, 2014). Despite substantial investment by the pharmaceutical industry, no effective treatments have emerged for cognitive deficits and negative symptoms (Dunlop and Brandon, 2015).

Arguably this largely results from the limited construct validity of existing models combined with the use of 'biomarker' measures in animal models that do not translate to the clinic or which only replicate previous mechanisms (Pratt *et al.*, 2012). As a result of this translational bottleneck, new approaches to improve translation are underway.

## 3. Forward and Reverse Translation

The lack of biologically informed diagnosis for schizophrenia presents a significant challenge for developing preclinical biomarkers. It has been argued that the diagnostic frameworks of DSM and ICD provide information on syndromes making it unlikely that any biomarker will associate with these descriptors (Scarr *et al.*, 2015). Instead, it is more likely that biomarkers will align with particular symptoms present in a subpopulation of individuals and which may cross current diagnostic boundaries (Scarr *et al.*, 2015; Clementz *et al.*, 2016). This ethos of striving for stratification forms the basis of a recent classification scheme proposed by the US National Institute of Mental Health (NIMH) termed Research Domain

Criteria (RDoC). A 'new way of classifying mental disorders based on dimensions of observable behavior and neurobiological measures' (http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml).
From a preclinical perspective, the RDoC approach of seeking to identify genes, molecules, brain circuits and physiological measures associated with specific behavioural constructs offers opportunities to more effectively align translation in the search for biomarkers.

Another initiative, CNTRICS (Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia) has the goal of ''developing measurement approaches from cognitive, social and affective neuroscience so that they may be implemented in efforts to develop treatments for impaired cognition in schizophrenia''(http://cntrics.ucdavis.edu/).

CNTRICS was developed in order to apply advances in cognitive neuroscience to the clinic, moving beyond the previously used standardized tests to those that could investigate specific cognitive constructs. An important outcome of CNTRICS has been the identification of cognitive tasks that can be implemented in parallel in animal models (Dudchenko *et al.*, 2013a; Lustig *et al.*, 2013a; Moore *et al.*, 2013). Moreover, integrating this information with neural imaging findings is a key goal.

Together RDoC and CNTRICS form a feasible clinical approach to inform the development of preclinical models and the identification of biomarkers. For example, working memory deficits have been related to dysfunctional prefrontal cortex activity (Perlstein *et al.*, 2001; Manoach, 2003; Potkin *et al.*, 2009; Kauppi *et al.*, 2014; Senkowski and Gallinat, 2015; Van Snellenberg *et al.*, 2016) in schizophrenia and other psychiatric disorders. This not only paves the way for developing clinical biomarkers but importantly offers the opportunity for effective '*Reverse translation*' in the sense that preclinical models could adopt similar imaging and behavioural measures. Moreover, preclinical research can help to identify the genetic, cellular and environmental mechanisms that contribute to these deficits using identified cognitive tasks and imaging modalities, thereby providing 'biomarker' tools to discover new treatments and predict which treatments might prove most effective in a subset of patients. Notably this strategy would be helpful for identifying treatments to improve a particular cognitive construct rather than the disorder as a whole and may of course cross existing diagnostic boundaries. A further benefit of preclinical research is that

it can directly inform clinical research through the more classically accepted paradigm of *'Forward translation.'* For example, current clinical imaging techniques provide less anatomical resolution and cellular specificity than preclinical imaging. Hence novel brain regions, cells and circuits identified preclinically, can be taken forward to investigate potential clinical biomarkers in patients. Of course, the most broadly recognized paradigm of '*Forward translation'* is that of preclinical drug validation prior to clinical testing. A historic lack of '*Reverse translation'* in the context of schizophrenia preclinical drug discovery has been a major limiting factor in effective '*Forward translation'* in this regard. Adopting a reciprocal forward and reverse translation approach, that is iteratively updated when additional new information is gained offers the greatest opportunity for discovering panels of biomarkers for the diagnosis, prognosis and treatment of schizophrenia.

In this chapter, we review recent research on preclinical biomarkers that show most promise for informing clinical need. In this context we focus on relevant imaging, electrophysiological, cognitive and biochemical modalities in preclinical models based upon the glutamate hypothesis and genetic and environmental risk factors (reverse translation). We then discuss one example of productive forward and reverse translation in the use of acute ketamine in human volunteers and in rodents. Finally, we provide an example of the importance of forward translation, which has revealed the thalamic reticular nucleus (TRN) as an important locus of brain dysfunction in schizophrenia.

*Insert Fig 1 here: 'Framework for biomarker development through the adoption of forward and reverse translation approaches'.*

## 4. Reverse Translation: Imaging Biomarkers

### 4.1 Glutamate and hypofrontality

The glutamate hypothesis of schizophrenia is based upon the observations that NMDA receptor antagonists such as phencyclidine (PCP) and ketamine can induce the positive and negative symptoms of schizophrenia together with cognitive deficits in normal volunteers which are exacerbated in schizophrenia ((Javitt and Zukin, 1991; Krystal *et al.*, 1994). In

addition, individuals who chronically abuse PCP show cognitive deficits that are similar to those seen in the disorder (Cosgrove and Newell, 1991). The search for the underlying biological mechanisms has revealed changes in expression of glutamatergic synaptic markers in post mortem brain (Meador-Woodruff *et al.*, 2003) as well as corresponding genetic evidence (e.g. GRIN1, GRIN2A-D association) (Demontis *et al.*, 2011; Ripke *et al.*, 2014; Harrison, 2015; Hu *et al.*, 2015). Importantly both common and rare genetic risk factors for schizophrenia, including copy number variants, exert a biological impact on the glutamatergic synapse ; including the postsynaptic density associated with the NMDA receptor complex (Pratt *et al.*, 2012; Morris and Pratt, 2014; Pocklington, O'Donovan and Owen, 2014; Hall et al., 2015; Harrison, 2015; Reddaway et al this volume ).

Although, there is accumulating evidence for an impact of schizophrenia genetic risk upon the glutamatergic synapse, the huge investment in NMDA receptor anatagonist models, particularly for drug discovery, began ~30 years years ago. This was largely based upon the observational findings that NMDA receptor antagonists, can induce symptoms resembling schizophrenia in normal subjects, which are exacerbated in schizophrenia ((Jentsch and Roth, 1999; Morris, Cochran and Pratt, 2005; Large, 2007; Jones, Watson and Fone, 2011; Pratt *et al.*, 2012)). The behavioural, neurochemical and brain imaging deficits identified in these models provide translational biomarkers for discovering new treatments. It is important to recognize that no single NMDA receptor model exists. Acute, subchronic and chronic drug treatment regimens have been utilized and a diverse range of standard of care and novel drugs have been evaluated for their ability to restore a range of rodent behaviours. As an existing example of forward translation in schizophrenia, studies performed in these models were key in the assessment of mGlu2/3 receptor agonists for the disorder, but unfortunately these drugs did not show a clear clinical benefit in recent trials(Patil St *et al.*, 2007; Dunlop and Brandon, 2015). Several factors may have explained this disappointing finding including trial design, the outcome measures taken and patient selection. From a preclinical perspective, the lack of predictive validity could be in part be explained by the use of behavioural measures that do not align with CNTRICS, coupled with the NMDA receptor model itself (with variables such as drug dose, acute treatment, chronic treatment, treatment with washout, assessments in the presence or absence of drug contributing to the inconsistent results). Interestingly, an investigation of a range of NMDA

receptor antagonists in rodent cognitive tests has shown inconsistent results across tasks with no common deficits produced by all drugs investigated. (Smith *et al.*, 2011). Furthermore, it has been argued that there does not seem to be an NMDA receptor antagonist regime that engages NMDA receptors equivalently in humans and animals that reliably produces the same cognitive deficits in each species (Gilmour *et al.*, 2012). In addition, we do not yet understand how and when abnormal glutamate transmission develops in schizophrenia, hence acute and repeated NMDA receptor antagonist treatment models in rodents are likely to reflect different stages of the disorder.

With these caveats in mind, an alternative approach is to explore 'intermediate' phenotypes, lying between a disease-relevant mechanism and the behavioural outcome, and 'endophenotypes', lying between an established genetic risk factor for the disease and the behavioural outcome. As these intermediate and endophenotypes lie on the path between the basic biology of the disorder and its symptoms they could potentially be more robust and useful, in comparison to behavioural outcomes, in aiding the discovery of schizophrenia biomarkers. Imaging biomarkers fall into this category and we have therefore focused on developing a model based upon the clear evidence that prefrontal cortex activity is disrupted in schizophrenia (Lewis *et al.*, 1999; Hill *et al.*, 2004; Potkin *et al.*, 2009). Using a low dose repeated PCP treatment regime with a washout period, we demonstrated hypofrontality ( Cochran *et al.*, 2003; Dawson *et al.*, 2012) mirroring that observed in schizophrenia. This imaging deficit was accompanied by deficits in markers of GABAergic function, namely reduced parvalbumin and Kv3.1 mRNA expression and importantly deficits in cognitive flexibility as measured by the CNTRICS recommended translationally relevant attention-set shifting task (Cochran *et al.*, 2002; Cochran *et al.*, 2003; Egerton *et al.*, 2008; Pratt *et al.*, 2008).

Hence imaging 'hypofrontality' could be an important preclinical biomarker for discovering drugs to ameliorate cognitive deficits, particularly in relation to the executive cognitive deficits, present in schizophrenia. Current antipsychotic drugs have minimal impact on restoring cognitive deficits in patients and predictably clozapine and haloperidol did not restore hypofrontality in this model. Similarly, PCP-induced deficits in cognitive flexibility are not reversed by haloperidol or risperidone in a rodent repeated PCP model (Goetghebeur and Dias, 2009). This further supports the mechanistic and translational

validity of this model; avoiding the generation of false-positive drug effects in preclinical models is central to ensuring their translational validity.

### 4.2 Glutamate and functional connectivity

Non-invasive imaging technologies have enabled the identification of brain regions, such as dysfunctional activity of the prefrontal cortex (PFC) in schizophrenia. Nevertheless, it is clear that the PFC does not act in isolation and exhibits multiple complex neural interactions within and beyond the PFC. Recent advances in the field of network science are enabling this complexity to be defined. Hence, in the clinical literature there has been huge interest in understanding the alterations in structural (anatomical) and functional brain network connectivity that are present in patients with schizophrenia, and how these relate to specific symptom domains in the disorder. These studies provide valuable new insight into how interactions between brain regions and neural subsystems are disturbed in the disorder and have the potential to be developed as imaging biomarkers (See Lydon-Stanley and Bassett this volume) . When utilized in combination with specific cognitive tasks these approaches can also give valuable insight into how these disturbed interactions contribute to the cognitive deficits present in schizophrenia (Nieuwenstein, Aleman and De Haan, 2001; Forbes *et al.*, 2009). Overall, these studies are generally supportive of reduced functional brain network connectivity on the global scale (Micheloyannis *et al.*, 2006; Liu *et al.*, 2008; Lynall *et al.*, 2010; van den Heuvel *et al.*, 2010; A Fornito *et al.*, 2011), and between defined neural systems (Schlösser *et al.*, 2003; Benetti *et al.*, 2009; Deserno *et al.*, 2012) in patients with chronic schizophrenia. The most consistently defined alterations in neural system connectivity include reduced frontal cortex connectivity (van den Heuvel *et al.*, 2010; Alex Fornito *et al.*, 2011; Camchong *et al.*, 2011; Pettersson-Yeo *et al.*, 2011; Roiser *et al.*, 2013) with a prominent reduction in hippocampal-frontal cortex functional connectivity (Meyer-Lindenberg *et al.*, 2005; Zhou *et al.*, 2007; Godsil *et al.*, 2013), and compromised thalamic connectivity (Welsh, Chen and Taylor, 2010; Tomasi and Volkow, 2014). Many of these alterations appear to be conserved in patients with first episode patients (Zhou *et al.*, 2007; Benetti *et al.*, 2009; Schmidt and Borgwardt, 2013) and in at risk individuals (Dauvermann *et al.*, 2013; Schmidt *et al.*, 2014) suggesting that they are not merely the consequence of

chronic antipsychotic treatment, which has also been shown to independently impact on brain functional connectivity (Cole *et al.*, 2013).

From a drug discovery perspective, it is hoped that the alterations in brain network connectivity seen in patients represent an intermediate phenotype/endophenotype, and thus a useful biomarker, that will be both responsive to pharmacological intervention and whose correction/restoration would closely align with the improvement of specific disease symptoms. Of course, there is also great interest in using brain network functional connectivity as an intermediate phenotype in the ***preclinical*** drug validation process (Dawson, Morris and Pratt, 2015) for further review). Therefore, an essential first step in the process has been to reverse translate some of the analytical approaches used in the clinical literature to quantitatively define brain network connectivity and to apply these to brain imaging data gained in preclinical models relevant to the disorder. If found to be successfully conserved in translationally valid preclinical models these alterations in brain network connectivity should represent effective biomarkers against which the efficacy of novel drugs can be tested, in combination with other experimental approaches including relevant behavioural assessment and biochemical analysis. Moreover, if assessed in combination with translationally relevant behavioural paradigms (recommended by CNTRICS) these functional connectivity biomarkers could potentially be used to stratify patients and predict treatment response in clinical populations. Importantly, many of the functional connectivity alterations present in patients also appear to be conserved in individuals with mutations in specific candidate risk genes or CNV's for the disorder (Esslinger *et al.*, 2009; Callicott *et al.*, 2013; Padula *et al.*, 2017), suggesting that these alterations in brain network connectivity represent a useful endophenotypic biomarker in the disorder. Given that preclinical models based on established genetic risk factors for schizophrenia represent a key translational approach, with high construct validity, assessing the alignment of alterations in brain network connectivity present in these models and in humans with relevant risk gene mutations is likely to be central in establishing the utility of this approach. It is important to note that this area of research is in its infancy, but the emerging data support its potential validity. Intriguingly, the alterations in connectivity currently reported appear to be both conserved across species (ie. between humans and

rodents) and across imaging modalities (ie. 2-DG, fMRI, EEG, electrophysiology, MEG, (Dauvermann, Lee and Dawson, 2017; Pratt *et al.*, 2017)

A leading exemplar of reverse translation in this context is the characterization of functional brain network connectivity in preclinical models based on glutamate system hypofunction. For example, characterization of altered functional brain network connectivity in the subchronic PCP model supports reduced connectivity at the global scale along with a reduction in hippocampal-prefrontal connectivity, reduced thalamo-prefrontal and reduced thalamic nuclei connectivity overall (N. Dawson *et al.*, 2012, 2014). These results were gained by analyzing $^{14}$C-2-deoxyglucose autoradiographic functional brain imaging data using graph theory algorithms and other analytical approaches (namely the partial least squares regression (PLSR) algorithm) that have previously been applied to functional brain imaging data gained in humans (McIntosh and Lobaugh, 2004; Micheloyannis *et al.*, 2006; Liu *et al.*, 2008). The validity of this approach is further supported by the observation that subchronic treatment with the NMDA receptor antagonist memantine also reduces hippocampal-PFC connectivity in rodents, as assessed using fMRI (Sekar *et al.*, 2013). Emphasising the translational relevance of the alterations in functional connectivity seen in the subchronic PCP model, the global network alterations seen in the model are consistent with the altered neural systems connectivity, including reduced hippocampal-PFC and thalamic connectivity, seen in patients with schizophrenia (Micheloyannis *et al.*, 2006; Liu *et al.*, 2008; Godsil *et al.*, 2013; Anticevic *et al.*, 2014). Moreover, the potential translational relevance and the utility of these alterations as biomarkers in the drug validation process is supported by evidence that these alterations in functional connectivity, including hippocampal-PFC and thalamo-PFC connectivity, are amenable to pharmacological correction by a drug, modafinil, known to have procognitive effects in both the subchronic PCP model and patients with schizophrenia (Goetghebeur and Dias, 2009; Dawson *et al.*, 2012; Scoriels, Jones and Sahakian, 2013). In addition, modafinil both increases PFC cerebral metabolism in schizophrenia patients and reverses the hypofrontality seen in the PCP model (Spence *et al.*, 2005; Dawson *et al.*, 2012), supporting PFC hypometabolism as a biomarker for the cognitive flexibility deficit seen in the disorder.

### 4.3 Emerging data in genetic, environmental and neurodevelopmental models relevant to schizophrenia

More recently the approach of characterizing brain network connectivity in preclinical models relevant to schizophrenia has been extended. To date, this includes the characterization of connectivity alterations in genetic models relevant to the disorder, including the Df(16)$A^{+/-}$ mutant mouse model of the 22q11.2 microdeletion, which increases the risk of developing schizophrenia by ~20 fold , and mouse models based on the psychiatric risk gene *Disrupted-in-Schizophrenia-1* (*DISC1*), which increases the risk of developing a major mental illness by ~ 50 fold; (Brandon *et al.*, 2009). In both of these models reduced hippocampal-PFC connectivity is supported (Sigurdsson *et al.*, 2010; Dawson, Morris and Pratt, 2015). While the findings from the Df(16)$A^{+/-}$ model are limited to the electrophysiological characterization of hippocampal-PFC connectivity, the analysis of brain network connectivity in the *Disc1* mouse models from $^{14}$C-2-DG brain imaging data has allowed the more extensive systems-level analysis of alterations in functional brain network connectivity, that also identified altered thalamo-PFC connectivity as a consequence of *Disc1* truncation (Dawson *et al.*, 2015). Importantly, the reduced hippocampal-PFC connectivity identified through the analysis of functional brain imaging $^{14}$C-2-DG data has been confirmed using electrophysiology, proving the validity of this approach. In addition to these genetic risk factor models, reduced hippocampal-PFC connectivity is also evident in a model of environmental risk (maternal immune activation; MIA (Lisman and Jensen, 2013)) (Dickerson, Wolff and Bilkey, 2010) and a model of impaired neurodevelopment (using the mitotic toxin methylazoxymethanol acetate; MAM) ( Phillips *et al.*, 2012; Belujon, Patton and Grace, 2013). To date, the analysis of connectivity in these animals has been limited to electrophysiological characterisation of the hippocampus-PFC. Future systems-levels analysis of the functional connectivity alterations present in the brains of these animals, gained for example through the analysis of brain imaging data, could be used to identify how global network properties are altered in these models, other relevant neural system interactions that are compromised in these models, and their translational relevance to schizophrenia.

## 5. Reverse Translation: Oscillations

The co-ordination of cortical and subcortical neuronal networks is achieved by neural oscillations. Such oscillations can be detected by electroencephalography (EEG) and magnetoencephalography (MEG) and represent synchronous activity of a population of neurons. These neuronal oscillations serve to integrate sensory processing with cognitive and motor outcomes and operate across many spatial and temporal scales (Uhlhaas and Singer, 2010; Buzsáki and Watson, 2012; Lisman and Jensen, 2013). Oscillatory activity occurs over a range of frequency bands from slow delta (0-4Hz) through to fast gamma (30-80Hz). In schizophrenia, there are alterations in delta, theta and gamma power and impaired cross-frequency coupling between gamma and the slower frequency bands (theta and alpha). The disruption in gamma oscillations has been a particular research focus with evidence for an involvement in dysfunctional neurocognition and perceptual disturbances in schizophrenia (see (Gandal *et al.*, 2012; Gonzalez-Burgos, Cho and Lewis, 2015; Pittman-Polletta *et al.*, 2015; Uhlhaas and Singer, 2015)

Hence gamma oscillatory activity may represent a potential biomarker for studying pathophysiological processes, illness progression and therapeutic interventions. To this end, deficits in gamma oscillatory activity are reported at first episode pyschosis, in unmedicated patients and to some degree in unaffected relatives. Taken together this suggests that abnormal gamma synchrony is a heritable feature of schizophrenia and represent a neural endophenotype (Gandal *et al.*, 2012). It is important to note that gamma oscillations are found in the majority of mammalian brain structures and appear to be phylogenetically conserved in their sensory co-ordinating role. This enables both forward and reverse translational research.

Informed by clinical research, preclinical researchers are probing the neural mechanisms underpinning oscillations. Inhibitory GABA interneurons, which vary in structure, function and location in cortical layers and circuits are key to the generation of oscillations. Of particular interest for the production of gamma oscillations are the fast spiking parvalbumin (PV) containing GABA interneurons. PV interneurons of the basket cell subtype synapse on the cell body and proximal dendrites of pyramidal cells, whereas the chandelier cell subtype synapse at the initial axon segment of the pyramidal cells, thereby influencing pyramidal cell

activity (Gonzalez-Burgos, Cho and Lewis, 2015). A range of pyramidal cell glutamatergic receptors (AMPA, NMDA and mGlu) are involved in regulating oscillations, along with NMDA receptors present on PV containing interneurons. Whittington's group have probed the mechanisms of cortical gamma rhythms *in vitro* and shown that the generation of rhythms exists in three distinct forms. In all three cases the gamma rhythm is an emergent property of a local neuronal network. The differences depend on the interneurons recruited (basket vs chandelier), pyramidal cell involvement and fundamental dynamic properties (Whittington *et al.*, 2000, 2011). Arguably, the Pyramidal Interneuron Network Gamma (PING) model is likely to be particularly relevant to schizophrenia. In this model, a strong inhibitory input from PV containing basket cells would transiently silence the activity of a local population of asynchronously firing pyramidal neurons. Once the inhibitory effect has subsided the pyramidal cells fire in synchrony. Whittington et al (Whittington *et al.*, 2000, 2011) have shown that if this GABAergic inhibition is rhythmic at gamma frequency then the pyramidal cell activity also becomes rhythmic and this leads to synchronous gamma oscillations in the network. One hypothesis is that the reduced gamma power observed in schizophrenia could result from two aberrant processes 1) excitatory inputs to pyramidal cells are normal but feedback inhibition from PV interneurons is weak 2) excitatory drive to pyramidal cells is low because of a reduced number of dendritic spines. This in turn leading to a compensatory reduction in strength of feedback inhibition from PV basket cells (Gonzalez-Burgos, Cho and Lewis, 2015).

There is a growing body of evidence that gamma oscillations are disrupted in preclinical models of relevance to schizophrenia. These include models based upon neurotransmitter system dysfunction and established genetic risk factors. The disruption of GABAergic circuitry could be a common pathway leading to gamma oscillation disturbances seen in these models. In models based upon the glutamate hypothesis of schizophrenia, acute NMDA receptor blockade (PCP, ketamine) leads to increased gamma power (Phillips *et al.*, 2012) arguably through reduced GABA release onto pyramidal neurons following increased pyramidal cell activity after NMDA receptor blockade on GABAergic interneurons (Homayoun and Moghaddam, 2007). Furthermore, selective deletion of the NR1 subunit from PV positive neurons increased gamma power and resulted in deficits in spatial and working memory (Korotkova *et al.*, 2010).

Models that reflect chronic disruption of NMDA receptor activity include repeated PCP administration and NMDA-NR1 neo-/- (NR1-/-) mice, which express less than 10% of obligatory NMDAR1 subunit. Repeated NMDA receptor blockade with PCP, altered theta power but not gamma power whereas NR1-/- mice showed an increase in baseline gamma power (similar to acute PCP) and disrupted gamma-theta band cross frequency phase-coupling between hippocampus and prefrontal cortex (Dzirasa *et al.*, 2009). This suggests that the temporal dynamics of NMDA receptor blockade, or neuroplastic/developmental events related to a chronic reduction in NMDA receptor activity, have a profound influence on the emergent effects of NMDA receptor hypofunction on cortical oscillations.

In a genetic mouse model of the human 22q11.2 microdeletion, Df(16)A+/- mice showed reduced in vivo synchrony between the prefrontal cortex and hippocampus during a working memory task, although theta activity was the focus of this study, there was a trend for gamma activity be reduced (Sigurdsson *et al.*, 2010). Further support for gamma oscillation dysfunction in preclinical models relevant to schizophrenia is reviewed in (Gandal *et al.*, 2012). In summary, disruption of oscillatory activity in preclinical models of schizophrenia risk factors shows some support for altered gamma activity as a common deficit across models. Future work to characterize the neurobiology of these neural oscillations, and compare with findings in patients, is necessary. Potentially this translational research could lead to patient stratification and inform drug discovery strategies. For example, novel compounds that target specific GABA interneurone markers could be assessed for efficacy in patients with a particular gamma oscillation characteristic and genetic phenotype.

## 6. Reverse Translation: Behavioural biomarkers

Historically, schizophrenia has been deconstructed into positive symptoms, negative symptoms and cognitive deficits. As previously noted, despite the large impact on functional outcome, the negative symptoms and cognitive deficits are most resistant to

treatment and represent a large unmet clinical need (Green *et al.*, 2000; Green, Kern and Heaton, 2004; Nuechterlein *et al.*, 2011; Fervaha *et al.*, 2014).

Arguably one reason for the dearth of new compounds reaching the market to address this need is that preclinical behavioural studies do not adequately access similar behavioural domains that are underpinned by similar brain circuitry to that recruited by human tasks (Pratt *et al.*, 2012). In this section we focus on cognitive domains and briefly discuss positive and negative symptoms.

### 6.1 Positive symptoms

The delusions and typically auditory hallucinations of schizophrenia are those that are responsive (albeit not in all patients) to antipsychotic medication. Rodent models are considered a proxy marker at best of these symptoms. Based upon the dopamine hypothesis of schizophrenia and aberrant dopaminergic transmission, these models typical measure amphetamine –induced hyperactivity (Van Den Buuse, 2010). In preclinical studies, current antipsychotic drugs show an ability to reverse amphetamine-induced hyperactivity and as such show some predictive validity. However, one major challenge to the translational validity of this approach is the concept of receptor tautology – the antipsychotics acting primarily as D2 receptor antagonists are simply antagonizing the D2 activating effects of amphetamine administration, mediated through increased dopamine release. Thus, these studies prove the D2 receptor dependence of amphetamine-induced hyperlocomotion, rather than proving the therapeutic validity of these compounds in relation to the positive symptoms of schizophrenia. Moreover, there is still scope for improved treatments for the positive symptoms of the disorder, primarily based upon the incomplete efficacy and side effect profiles of existing drugs. For example, convincing arguments have been made that aberrant salience processes can explain the positive symptoms of schizophrenia (Kapur, Mizrahi and Li, 2005). Hence models that tackle this domain could prove useful. Furthermore, increased understanding of the neural circuitry underpinning hallucinations may inform new model development.

### 6.2 Negative symptoms

Negative symptoms are characterized by a range of sub-domains: affective flattening (reduced intensity and range of emotional expression), poverty of speech (alogia), anhedonia and motivational deficits. Sub-domains such as anhedonia and motivational deficits are readily accessible in rodent models and have been widely reviewed elsewhere (Millan *et al.*, 2014; Felice Reddy, Horan and Green, 2016). Notably, with respect to anhedonia, the 'liking' of an experience is less impaired than the looking forward to a reward/pleasurable experience in schizophrenia. Arguably, rodent tasks that tackle the ability of an animal to work for a reward, such as the progressive ratio task, show better translation than tasks such as the sucrose preference task which relate more to the 'pleasurable experience' (consummatory aspect). Additional tasks related to emotional processing (e.g. cognitive affective bias) also show great promise (See Emma Robinson this volume). Nevertheless, as with cognitive deficits, drugs that ameliorate negative symptoms in rodent models have yet to translate into meaningful improvements of negative symptoms in patients.

### 6.3 Cognition

As noted earlier an important outcome of CNTRICS has been the identification of construct specific elements of human cognition that can be implemented in parallel in animal models ((Young and Geyer, 2015)and MacQueen et al this volume). Table 1 summarises the recommendations made by CNTRICS for many of these cognitive domains.

It is notable that many cognitive domains can be broken down into various components, known as constructs. For example, executive function comprises planning, problem solving, organisation, cognitive flexibility and inhibition of inappropriate responses. CNTRICS have selected those components that are most affected in schizophrenia and which can be assessed in paradigms. In several cases, there are animal paradigms that can be considered as having good construct validity and which could be optimized further for development.

Another important advance has been the introduction of automated operant touchscreen platforms in rodents for the measurement of multiple cognitive domains in settings similar to touchscreen tasks used in human cognitive assessments (Bussey *et al.*, 2012; Oomen *et al.*, 2013).

*Insert Table 1 around here*

Whilst cognitive domains have been assessed in a range of models of risk factors for schizophrenia (Pratt *et al.*, 2012; Moran *et al.*, 2016) further work is required to establish the utility of genetic and genetic-environmental interaction models for schizophrenia drug discovery. Nevertheless, a leading example of the drug discovery potential in a NMDA receptor antagonist model, is the finding that modafinil reverses PCP-induced attentional set-shifting deficits in rats (Goetghebeur and Dias, 2009; Dawson *et al.*, 2012) , and improves attentional set-shifting in patients with schizophrenia (Turner *et al.*, 2004). To date, however compounds which ameliorate cognitive deficits in rodent models and which translate into meaningful improvements in a patients' quality of life are awaited.

In other domains, for example social cognition there are even larger challenges. Social cognition is a particularly complex and multidimensional domain that also impacts on positive and negative symptoms (Millan and Bales, 2013). It encompasses our ability to interpret social signals, understand beliefs, intentions and actions thereby enabling appropriate behaviours in a social context. Clearly translation to rodents is challenging, despite their sociable nature. To this end CNTRICS have selected the general construct of social and emotional recognition (and appropriate response selection) as being appropriate to translate across species. Tasks such as the social recognition/preference task are currently being widely used (Yang, Silverman and Crawley, 2011) although other tasks relevant to social behaviours warrant further investigation. Arguably, the concept of measuring social behaviours in a more naturalistic home cage setting offers improved translation. Indeed, such approaches are revealing differences in social behaviours in adolescent rodents treated with PCP and cage mates treated with saline (Mitchell et al 2017). Further work, to establish how genetic and environmental risk factors for schizophrenia impact on the development of social behaviours could conceivably show utility for drug discovery.

Perceptual disturbances also feature strongly in schizophrenia. CNTRICS has identified 'gain control and sensory integration' as relevant constructs for assessment across species. Rodent paradigms such as pre-pulse inhibition (PPI) and mismatch negativity (MMN) have

been proposed to align with the human construct (Siegel, Talpos and Geyer, 2013; Young and Geyer, 2015), but at present there is not a clear consensus as to whether the same cognitive construct is being measured and other paradigms are awaited. One candidate could be the recently developed mouse touchscreen task for global motion perception (Stirman, Townsend and Smith, 2016).

## 7. Reverse translation: Biochemical biomarkers

### 7.1 Imaging of neurotransmitter function

Traditionally, schizophrenia has been viewed as reflecting dysfunction primarily of dopaminergic and/or glutamatergic neurotransmission. A substantial literature, stretching back over many years, covers *in vivo* imaging of various aspects of dopaminergic function. Current theories emphasise increased dopaminergic activity in the striatum, alongside reduced dopaminergic activity in cortical and limbic regions (Kambeitz *et al.*, 2014; Slifstein *et al.*, 2015; Weinstein *et al.*, 2017). While these findings, derived from various different measures of dopaminergic function, appear to be robust, the effect sizes are small and insufficient for biomarker use. Nevertheless, future research into the causes of this divergent regulation of dopaminergic activity promises to provide important insight into the aetiology of the disease.

More recently, some consensus has emerged concerning glutamate abnormalities in schizophrenia. Prefrontal cortical glutamate levels, as monitored via proton magnetic resonance spectroscopy ($^{1}$H-MRS), appear to be decreased in patients with chronic schizophrenia although elevated anterior cingulate glutamatergic activity has been reported in several studies (Hugdahl *et al.*, 2015; Merritt *et al.*, 2016). Intriguing evidence suggests that the cingulate cortical changes, accompanied by reduced thalamic glutamate levels, may be present during the prodrome (Stone *et al.*, 2009; Allen *et al.*, 2015). While important mechanistically for understanding the disease process, again these effects have little imminent clinical biomarker potential.

Abnormalities in cortical glutamate transmission have however attracted attention as a translational biomarker at preclinical stages of drug development. A dramatic elevation of glutamate release in the prefrontal cortex, along with a surge in metabolic activity, is observed in adult rodents and humans following acute NMDA receptor blockade (Moghaddam *et al.*, 1997; Miyamoto *et al.*, 2001; Dawson, Morris and Pratt, 2013). This is suggested to result from disinhibition of prefrontal pyramidal neurons following blockade of tonically active NMDA receptors on the parvalbumin subtype of GABAergic interneurones (Homayoun and Moghaddam, 2007; Pratt *et al.*, 2008) although an action at NMDA receptors on parvalbumin neurons may vary according to the stage of neurodevelopment (Rotaru, Lewis and Gonzalez-Burgos, 2012). Simultaneously, human volunteers experience a range of schizophrenia-related symptoms when treated with the NMDA receptor antagonist ketamine (Krystal et al., 1994). The enhanced glutamate release is postulated to be linked to the disease-relevant effects in humans and to behavioural deficits, most notably impaired PPI and increased locomotor activity, in rodents. Hence there are rodent-human phenotypes apparently related to ketamine-induced cortical glutamate release which can be employed as biomarkers for drug development purposes (Javitt *et al.*, 2018).

### 7.2 Plasma biomarkers

Many studies have searched for peripheral markers related to “state” or “trait” in patients with schizophrenia. It is probably fair to say that, to date, little has emerged of clear utility, whether for monitoring disease progression or treatment response, or for patient stratification during clinical trials.

Patients with schizophrenia do tend to show altered levels of certain proteins in blood. Increased levels of C reactive protein (CRP) – generally indicative of immune system activation – are reliably detected in plasma of chronic patients (Miller, Culpepper and Rapaport, 2014; Fernandes *et al.*, 2016), although this may not be the case at early stages of the disease (Dickerson *et al.*, 2016). Meta-analyses consistently report elevations in plasma interleukin 1$\beta$, soluble interleukin 2 receptor, interleukin 6 and TNF$\alpha$ in patients (Potvin *et al.*, 2008; Miller *et al.*, 2011; Upthegrove, Manzanares-Teson and Barnes, 2014). Similarly, patient groups show reduced levels of the anti-inflammatory cytokine interleukin 10 (Zhang *et al.*, 2017). This is frequently interpreted as a “smoking gun” indication of an abnormal response to a developmental immune challenge. However, this pattern of change in

immune mediators could alternatively reflect the expression of genetic risk factors, acting not only on neuronal circuit development to cause schizophrenia, but also on the peripheral immune system, and even potentially the gut microbiome (Schirmer *et al.*, 2016), to perturb cytokine expression. In either case, the altered blood cytokine levels can be seen as a "trait" marker. The relatively subtle nature of the changes, and their obvious lack of specificity for schizophrenia, has precluded any exploitation as a biomarker. However, a blood-based multi-protein biomarker profile (including interleukin 10) has recently been proposed as a method for identifying at risk prodromal patients who are likely to transition to the full disease (Chan *et al.*, 2015) hence representing a possible biomarker for disease progression.

Arguably the most immediate prospects of exploiting plasma biomarkers may be in relation to patient stratification. There is some interest in anti-inflammatory agents as potential novel therapeutics in schizophrenia. In this regard, initial stratification of patient groups into those with and without a clearly elevated immune response profile might be useful. An immune-related biomarker for drug side-effect liability has also been suggested (Prata, Mechelli and Kapur, 2014)

### 7.3 Gene expression

Progress in development of novel drugs to treat Alzheimer's disease has accelerated with the arrival of tools for *in vivo* imaging of the pathology of the disease, where this previously could only be assessed post-mortem. These imaging tools provide a rapid and direct readout of effects on disease progression. The ultimate aspiration would be to identify some similar tools for use in schizophrenia research. This remains some way into the future, but we note, from evidence in post-mortem tissue, that decreased parvalbumin expression in the prefrontal cortex is a robust observation in patients with schizophrenia, with a substantial effect size, and possibly disease-specificity. A method for imaging parvalbumin neurones in vivo would undoubtedly represent a major advance in schizophrenia research.

For further discussion of potential biochemical and gene expression biomarkers see (Pickard, 2015)and Herron, Nerurkar and Cavanagh (this volume).

## 8. Panels of Biomarkers

In the future it is likely that, both preclinically and clinically, a multiscale and integrated high dimensional approach to biomarker identification and utilization will be required to effectively impact on schizophrenia diagnosis, prognosis and treatment. For example, valid preclinical models will need to replicate, across a range of scales –molecular, biochemical, cellular, complex brain network and behavioral levels – core aspects of neural impairment in schizophrenia, to generate a suite of disease relevant biomarkers that can be maximally useful and predictive. Preclinical models based on aetiologically established risk factors, such as models based on glutamate system hypofunction and genetic risk factors combined with environmental insults during neurodevelopment, offer the greatest hope in this regard. Clinically, it is likely that advanced analytical approaches, such as machine learning, will play a key role in identifying and integrating information across a broad panel of biomarkers in order to have clinically relevant impacts for patients. Early studies have recently proven the diagnostic potential of machine learning approaches in relation to the analysis of brain imaging data (Salvador et al., 2017; de Witt et al., 2017), whose value could be further increased and refined through the inclusion of a greater range of biomarkers, including cognitive behavioural markers and polygenic risk scores.

## 9. Forward Translation

There is a general scepticism about the feasibility of modelling a disease as subtle and complex as schizophrenia in rodents. Unsurprisingly, there are few examples of successful forward translation, where data from preclinical models has informed understanding of the clinical disease or illuminated new routes to therapeutic treatments. One example of productive forward and reverse translation is the use of acute ketamine in human volunteers and in rodents. The ability of NMDA receptor antagonists, such as ketamine or phencyclidine, to produce symptoms of schizophrenia on acute administration in humans has led to many acute administration studies in rodents (Morris, Cochran and Pratt, 2005). Once it became clear that these drugs cause a profound increase in glutamate release and metabolic activity in the rodent prefrontal cortex ((Tamminga *et al.*, 1987; Moghaddam *et al.*, 1997; Duncan *et al.*, 1999), then similar effects were confirmed in humans via imaging

studies (Lahti *et al.*, 1995; Breier *et al.*, 1997; Holcomb *et al.*, 2000; Stone *et al.*, 2012). The extent to which the elevated prefrontal glutamate release is responsible for the schizophrenia-like symptoms is still a matter of debate (Stone *et al.*, 2012; Hugdahl *et al.*, 2015). Nevertheless, as mentioned previously, the directly comparable phenomena in rodents and humans can facilitate translation from preclinical to clinical arenas, for drug classes active in this model (Javitt *et al.*, 2018).

There is a further instance of forward translation that has only been validated recently and which demonstrates that rodent models can provide new insight into the human condition. . As noted above, we found that rats receiving chronic, intermittent low-doses of the NMDA receptor antagonist phencyclidine ( Cochran *et al.*, 2003) develop metabolic hypofrontality and prefrontal cortex GABAergic interneuron deficits (reduced parvalbumin expression) that parallel those in the brains of patients with chronic schizophrenia. Importantly, chronic PCP administration to rats also reduced metabolic activity and parvalbumin in the thalamic reticular nucleus (TRN) ( Cochran *et al.*, 2003) and the changes in the TRN actually preceded those in the PFC (Cochran *et al.*, 2002) Indeed, since cortical parvalbumin expression is known to be regulated by the TRN (Alcantara, Soriano and Ferrer, 1996) we have proposed that the hypofrontality and PFC GABAergic deficits highly characteristic of schizophrenia (Hill *et al.*, 2004; Molina *et al.*, 2005; Lewis *et al.*, 1999)) may be a consequence of TRN dysfunction. More recently we have identified TRN hypofunction and dysconnectivity in not only the PCP model relevant to schizophrenia (Dawson *et al.*, 2012)but also following subanaesthetic ketamine administration (Dawson *et al.*, 2014) and as a result of mutation in the Disc1 gene (Dawson *et al.*, 2015) suggesting that TRN dysfunction may be a key mechanism in multiple preclinical models relevant to the disorder. In humans, the TRN is too narrow for resolution by current imaging techniques, and so any dysfunction in schizophrenia had not been detected. Remarkably, however, recent evidence, from analysis of post-mortem tissue, has now revealed that in schizophrenia there is a similar loss of parvalbumin expression in the TRN (Steullet *et al.*, 2017). Moreover, TRN dysfunction is also supported by observations in patients with schizophrenia of altered sleep spindle generation (Ferrarelli and Tononi, 2018). The fact that the rodent model was able to predict this pathological feature of the human disease clearly increases confidence in the translational value of other aspects of this and other relevant models model, be they cognitive, metabolic or neurochemical measures. Furthermore, the knowledge that

schizophrenia entails dysfunction of the TRN, and that this occurs at an early stage in the preclinical model, suggests new approaches to monitoring disease progression and treatment response clinically. Tracers for the selective imaging of the human TRN could be developed, based on existing knowledge of the neurochemistry of this region (Pratt and Morris, 2015).

**Summary**

Overall, in this chapter we have sought to highlight recent advances in the development of translational preclinical biomarkers for schizophrenia, with an emphasis of key work currently developing in this area. This includes leading examples of reverse and forward translation, the fruition of which is yet to be fully realized. In addition, we have highlighted key theoretical considerations at the forefront of current thinking in the field that focus on improving the translational value of preclinical models in this context, and the mechanisms of translation across the clinical-preclinical gap. Taking these approaches into consideration provides new hope for the advancement of biomarker identification in schizophrenia and the greater clinical impact of preclinical research in this area.

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| Cognitive domain | Construct selected of relevance to schizophrenia | Human | Rodent Tasks | References |
|---|---|---|---|---|
| Attention | Control of attention (esp input selection)<br><br>Ability to guide and refocus attention in accordance with internal goals and representations | Continuous performance tasks | 5-Choice serial reaction time task (5-CSRTT)<br><br>5-choice continuous performance test (5-C-CPT)<br><br>Distractor condition sustained attention task (dSAT) | (Luck and Gold, 2008; Lustig *et al.*, 2013b) |
| Executive function | Rule generation and control | ID/ED task | Reversal learning and Attentional Set shifting task | (Gilmour *et al.*, 2013; Young and Geyer, 2015) |
| Working memory | Goal maintenance and Interference control | Goal maintenance<br><br>Interference control | Operant delayed non-match to position task<br><br>N-back task | (Barch and Smith, 2008; Barch *et al.*, 2009; Dudchenko *et al.*, 2013b) |
| Long Term memory | Reinforcement learning<br><br>Declarative memory (Episodic memory) | Probabilistic learning tasks<br><br>Paired Associate learning (in CANTAB battery) | Probabilistic learning<br><br>No clear consensus<br><br>Novel/Spontaneous object recognition – not recommended<br><br>Touchscreen Task: object-location paired-associated learning<br><br>Further development | Ragland et al 2009<br><br>(Bussey, Barch and Baxter, 2013) |

| | | | | |
|---|---|---|---|---|
| | | | and validation recommended | |
| Social Cognition | Acquisition and recognition of affective (emotional) states coupled to social recognition | Tasks related to Identification of and response to emotional cues | Social recognition/preference<br><br>Recognition of multidimensional nature of social cognition and challenges of developing animal model.<br><br>Further development and improvements recommended | (Carter *et al.*, 2009; Millan and Bales, 2013) |

**Table 1**. Tasks proposed by CNTRICS as possessing high construct validity and potential for further development. The evidence for the translational capacity of the tasks is based upon the ability of the task to measure the construct of interest, that there is evidence that similar brain regions (and possibly brain networks) are recruited in the task and are impaired in schizophrenia. From a pragmatic perspective, important considerations for inclusion by CNTRICS were the ability to standardize the tasks across laboratories and task reliability. In some cases, further development and validation is highlighted

**Figure Legends:**

**Fig 1. Framework for biomarker development through the adoption of forward and reverse translation approaches**

**Fig Legend.** The importance of adopting a reciprocal forward and reverse translation approach that is iteratively updated when additional new information is gained, either preclinically or clinically, offers the the greatest opportunity for discovering panels of biomarkers for the diagnosis, prognosis and treatment of schizophrenia. Note that biomarkers are objective biological measures that can broadly be divided into several categories: i) diagnostic - where they can aid in predicting risk and diagnosis ii) prognostic - where they can provide a signpost for clinical course and iii) predictive of drug response/therapeutic intervention - whether beneficial or adverse, and thereby provide a potential means of patient stratification.