Current Topics in Behavioral Neurosciences 40

Judith Pratt · Jeremy Hall *Editors*

Biomarkers in Psychiatry

Springer

Current Topics in Behavioral Neurosciences

Volume 40

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Biomarkers in Psychiatry

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ISSN 1866-3370 ISSN 1866-3389 (electronic)
Current Topics in Behavioral Neurosciences
ISBN 978-3-319-99641-7 ISBN 978-3-319-99642-4 (eBook)
https://doi.org/10.1007/978-3-319-99642-4

Library of Congress Control Number: 2018954497

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

A focus of twenty-first-century medicine is to transform patient care through early interventions and personalized treatments. For conditions such as cancer and diabetes, the discovery and development of biomarkers has had a large impact on management and treatment. Unfortunately, the development of biomarkers for psychiatric disorders has lagged behind that of other areas of medicine and is a major barrier to improved care and the advancement of new therapies for these common and impactful conditions.

A key factor for the lack of biomarkers in routine use is in part attributable to the way mental health conditions are currently diagnosed. Diagnosis through DSM-5 and ICD10 relies on descriptions of symptoms rather than biological evidence. With around 1 in 4 of us suffering from a mental health condition each year and the fact that responses to interventions often do not exceed 50%, there is an urgent need to reappraise current therapeutic strategies.

In the past decade, new ways of conceptualizing psychiatric disorders have emerged. This together with the enormous technological advances in genetics, genomics, neuroscience, imaging, and behavioral research has accelerated our knowledge to the extent that "biomarkers" may be a tangible prospect in the twenty-first century. Worldwide multidisciplinary and interdisciplinary integrated translational research programs are carving a new debate in terms of utilizing diagnostic constructs that span current diagnostic categories to provide new insight into the causes of mental health conditions. This will inform "biomarkers" for diagnosis, patient stratification, and treatment. The ultimate aim is to develop new interventions that can prevent illness onset and treat these complex disorders more effectively.

This volume addresses the evidence for and potential to adopt "biomarkers" for prevention (early intervention), diagnosis, and treatment responses in mental health conditions. It will mesh together state-of-the-art research from internationally renowned preclinical and clinical scientists to illustrate how the fields of anxiety disorders, depression, schizophrenia, psychosis, and autism spectrum disorder have advanced in recent years.

The initial chapters inform how methodological advances are providing insights into the utility of biomarkers; ranging from stem cells, immunology, genetics and genomics, imaging, and network science to cognition. Thereafter, the chapters cohere around biomarker themes for the conditions with an emphasis on the importance of forward and reverse translation approaches. Hence these encompass evidence from preclinical neurobiology of disease models, clinical imaging, genetics, and behavior. Attention is given to how potential biomarkers can inform new interventions across current diagnostic categories.

The book will make a valuable contribution to the field of biological psychiatry and scholarship in this area. It will appeal to a broad readership, from psychiatrists and mental health practitioners to neuroscientists and academics. Moreover, it may be of particular interest to early career researchers and undergraduates in the fields of medicine, neuroscience, pharmacy, and pharmacology who are seeking an overview of the contemporary status of biomarkers in the field of psychiatry and mental health disorders.

Glasgow, UK — Judith Pratt
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Biomarkers in Neuropsychiatry: A Prospect for the Twenty-First Century?

Judith Pratt and Jeremy Hall

Contents

Abstract The search for biomarkers to aid in the diagnosis and prognosis of psychiatric conditions and predict response to treatment is a focus of twenty-first century medicine. The current lack of biomarkers in routine use is attributable in part to the existing way mental health conditions are diagnosed, being based upon descriptions of symptoms rather than causal biological evidence. New ways of conceptualizing mental health disorders together with the enormous advances in genetic, epidemiological, and neuroscience research are informing the brain circuits and physiological mechanisms underpinning behavioural constructs that cut across current diagnostic DSM-5 categories. Combining these advances with 'Big Data', analytical approaches offer new opportunities for biomarker development. Here we provide an introductory perspective to this volume, highlighting methodological strategies for biomarker identification; ranging from stem cells, immune mechanisms, genomics, imaging, network science to cognition. Thereafter we emphasize key points made by contributors on affective disorders, psychosis, schizophrenia, and autism spectrum disorder. An underlying theme is how preclinical and clinical research are informing biomarker development and the importance of forward and

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Curr Topics Behav Neurosci (2018) 40: 3–10
DOI 10.1007/7854_2018_58
Published Online: 30 June 2018

reverse translation approaches. In considering the exploitation of biomarkers we note that there is a timely opportunity to improve clinical trial design informed by patient 'biological' and 'psychological' phenotype. This has the potential to reinvigorate drug development and clinical trials in psychiatry. In conclusion, we are poised to move from the descriptive and discovery phase to one where biomarker panels can be evaluated in real-life cohorts. This will necessitate resources for large-scale collaborative efforts worldwide. Ultimately this will lead to new interventions and personalized medicines and transform our ability to prevent illness onset and treat complex psychiatric disorders more effectively.

Keywords Affective disorders · Autism spectrum disorder · Diagnostic biomarker · Early intervention · Forward and reverse translation · Genetics · Genomics · Immune · Inflammation · Patient stratification · Personalized medicines · Predictive biomarker · Prognosis biomarker · Psychosis · Schizophrenia · Stem cells

1 Introduction

A quest of contemporary medicine is to provide improved patient care, through early intervention and personalized medicines. For conditions such as cancer and diabetes, the discovery and development of biomarkers has had a large impact on management and treatment. However, the development of biomarkers for psychiatric conditions has lagged behind that of other areas of medicine, with no biomarkers currently in routine use for the major psychiatric disorders. This lack of relevant biomarkers has represented a major impediment to improved care and the development of new treatments for these common and impactful conditions.

Whilst there are multiple reasons for this, a key factor is the current way mental health conditions are diagnosed (see Prata et al. 2014; Kalia et al. 2015; Scarr et al. 2015). Current diagnosis of these conditions through DSM-5 and ICD10 relies on descriptions of symptoms rather than utilizing causal biological evidence. As a consequence, broad syndromes encompassing a range of symptoms result in disorders being overtly heterogeneous in nature. Furthermore, traditional diagnostic methods lack reliability and at present the ability to predict prognosis and response to treatment are limited. Hence the ability to align broad-based disease domains with biomarker discovery is particularly challenging.

The launch of the Research Domain Criteria (RDoC) project by the National Institute of Mental Health (http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml) some 10 years ago has led to new ways of conceptualizing mental health disorders out with DSM-5. In essence RDoC proposes that mapping the cognitive, circuit, and genetic aspects of mental disorders will yield new and better targets for treatment. This research framework provides opportunities to stratify patients for improved treatment options. Hence adopting an RDoC approach offers great opportunities for biomarker identification and development. Part of the ethos of RDoC is to align preclinical and clinical research, through forward and reverse translation. For example, it is envisaged that by assessing neural imaging and behavioural

'biomarkers' in preclinical models of disease risk (e.g. genetic risk variant) could assist in the discovery of new treatments and predict which treatments are likely to be most effective in a subset of patients.

In considering biomarkers, it is important to articulate their purpose; for Psychiatry, they can be considered broadly useful for

1. Diagnosis – including identification of those at high risk.
2. Prognosis – Assessment or prediction of the course of the disorder.
3. Prediction of treatment response (therapeutic or adverse).

Understanding the neurobiology of neuropsychiatric disorders is key to the process of identifying and developing biomarkers. Given the complexity of psychiatric disorders, this will require a multidisciplinary approach. In recent years, enormous strides have been made in understanding the causes of mental health disorders. This has been driven in part from advances in genetics, epigenetics, omics, neurochemistry, physiology, imaging and behavioural technologies that enable the probing of the human brain with greater precision from early in neurodevelopment through to adolescence and adulthood.

2 Methodological Strategies for Biomarker Identification and Development

The initial chapters inform how technological advances are providing insights into the utility of biomarkers from the genetic and cellular level through to imaging and behaviour.

Johnstone et al. (2018) discuss recent advances in human induced pluripotent stem cell (hiPSC) technology for understanding neurodevelopmental disorders such as schizophrenia and autism, and also affective disorders. They highlight the promises and pitfalls of reprogramming cell fate for investigation of these disorders and provide recommendations for future directions which may lead to biomarker identification.

Herron et al. (2018) review the diverse range of pro-inflammatory mediators implicated in psychiatric disorders. Although the mechanisms through which altered inflammatory signaling impact on brain, mood and behaviour is unclear, several possible mechanisms have been proposed. For example, the enhanced levels of cytokines (released from brain microglia or by accessing the brain from the periphery through traversing the BBB or via vagus neural feedback) are hypothesized to impact on brain serotonin, dopamine and glutamate systems together with modifying BDNF expression. It is notable that several cytokines (IL-6, TNF alpha, IL-1beta) are altered in depression, schizophrenia and bipolar disorder, raising questions about a common role of inflammatory mediators in psychiatric illness. Interestingly in depression markers of inflammatory response are reported to reduce in response to treatment. Herron et al. conclude that notwithstanding the challenges of the

heterogeneity of psychiatric disorders and the dynamic nature of the immune response, neuroimmune biomarkers hold the potential for stratification of illness, personalized treatments and may inform the development of immunologically targeted therapeutics.

Lydon-Staley and Bassett (2018) provide insight into how recent advances in the field of applied mathematics (specifically network science) provide a language to articulate how brain regions interact with each other at the structural and functional level. By analogy with, for example, social and transport networks, algorithms from network science enable the properties of a network to be interrogated. This work has enabled major advances in our understanding of brain connectivity during development and changes (dysconnectivity) arising from genetic and environmental factors that may lead to mental health conditions; particularly schizophrenia and depression. Lydon-Staley and Bassett note that the 'applications of network analysis have revealed organizational principles of healthy brains that allow for efficient, flexible, and robust information processing and how this may deviate in psychiatric disorders'. Importantly these recent advances have moved the brain imaging field from one where local changes in structure and function are visualized to one where the dynamic interactions of brain structures can be characterized, which will be of particular value for the development of biomarkers.

MacQueen et al. (2018) highlight the utility of RDoC constructs as potential cognitive biomarkers which cross existing diagnostic categories. In particular, they focus on the constructs of attention, cognitive control, working memory, declarative memory, perception and language along with relevant subdomains. Consideration is given to linking neural processes to these constructs and the techniques available to measure these in human and preclinical models. By utilizing forward and reverse translation techniques to assess cognitive constructs it is argued that animal models can provide insight in dysfunction relevant to cognitive disorders and that potential treatments for a particular cognitive dysfunction can be tested similarly first in preclinical models and then in patient populations.

3 Preclinical and Clinical Research to Inform Biomarkers for Affective Disorders, Psychosis, Schizophrenia and Autism Spectrum Disorder

In subsequent chapters, there is a focus on how preclinical and clinical research are informing biomarker development for affective disorders, psychosis and autism.

Slaney et al. (2018) discuss the challenges of developing preclinical models relevant to depression, which can aid in biomarker identification. They note the limitations of conventional animal models of depression such as the forced swim/tail suspension test and reward sensitivity tests such as the sucrose preference test. For the latter, it is notable that patients with depression do not show similar deficits when tested using techniques which assess 'in the moment' pleasure. Clearly a major limitation is the challenge of developing animal models for disorders currently

diagnosed by a description of symptoms. Slaney et al. provide persuasive evidence that neuropsychological deficits, specifically affective biases, (observed when emotional states affect cognitive function) evident in major depressive disorder, can inform the development of translational tasks in animals. In this regard, they review evidence that assessment of affective bias in rodents is feasible and has utility for predicting antidepressant efficacy as well as pro-depressant risk.

Battaglia and Khan (2018) focus on preclinical models for panic disorder and separation anxiety disorder involving early life interference with parental care. In common with the clinical conditions, CO2 hypersensitivity represents a common biomarker. In their review they discuss the possible mechanisms underpinning this pathophysiological readout, including gene–environment interactions and epigenetic changes and the implications for biomarker development.

Maron et al. (2018) emphasize the high prevelance rates of anxiety diosrders, their hetereogeneity and limitations in being treating successfully. In this review, they consider the large volume of data generated on clinical evidence from neuroimaging and genetic measurements as biomarkers in anxiety disorders, covering generalized anxiety disorder, panic disorder, social anxiety disorder, simple phobias as well as obsessive compulsive disorder and post-traumatic stress disorder, that are no longer part of the anxiety category in DSM-5. Although it is apparent that the amygdala is important in the expression of anxiety and the medial prefrontal cortex in restraining it, no biomarkers have yet to be associated with discrete anxiety disorders. Similarly genetic variants that are robustly associated with these particular disorders have yet to be confirmed. It is predicted that future research on gene-environmental factors combined with imaging and hormone biomarkers could lead to a panel of biomarkers for diagnosis and treatment management.

Notter (2018) provides a critical insight into immunological processes in schizophrenia pathology and their potential as biomarkers. Following an overview of immune system components, Notter reviews the results of immune dysfunctions along the clinical course of schizophrenia and discusses this in the context of their potential as predictive, trait or state biomarkers. On a negative note it appears that immune dysfunction in schizophrenia may be as heterogeneous as the condition itself. Nevertheless, this provides an opportunity for patient segregation based upon their immune profile. Clearly this type of stratification may help in the development and introduction of immune modulating drugs for specific groups of patients defined by their 'immune biomarker' profile. It is perhaps ironic that an association of schizophrenia with immune system dysfunction was postulated over a century ago, yet genetic evidence of genes that play an important role in immune functioning and neurodevelopment (e.g. complement component 4) was discovered very recently.

Reddaway et al. (2018) review advances in the genetic and genomic understanding of schizophrenia, and highlight the ways that this information may be used to better stratify patients to existing or new treatments. In addition they examine the way genetic information can be used to identify imaging markers associated with risk for disease through genomic imaging approaches. They particularly focus on studies examining the aggregated polygenic effect of common risk variants on brain structure and function, as well as the impact of rarer but more penetrant genetic

variants such as chromosomal micro-deletions at 22q11.2. Overall the chapter highlights the value of integrating genetic information with imaging and other biomarker studies in order to define biologically relevant markers.

Hunter and Lawrie (2018) review the current status of imaging and genetic biomarkers predicting transition to psychosis. The early detection and intervention of high risk groups has been proposed as the most effective way of improving outcomes in individuals who transition to psychosis. Important advances in the understanding of dynamic brain changes that occur during the transition from health to a psychotic disorder have been obtained from longitudinal follow-up studies. Imaging studies have shown grey matter abnormalities in frontal, cingulate and temporal cortices, hippocampal structures and the cerebellum as being potentially predictive of those patients who will develop a psychotic disorder. Similarly, the development of polygenic risk scores (a measure of the cumulative genetic risk generated through the combination of many SNPs) is showing increased predictive power. Hunter and Lawrie emphasize the power of combining genetic and imaging data as being more effective than either biomarker alone. They note that continued advances in the genetics field along with 'the application of machine learning techniques and imaging protocols in the context of every increasing computational power offer novel and exciting approaches to the development of tools that in their view likely, one day, offer clinicians a viable and acceptable method of assisting in complex diagnostic and treatment decisions'.

Pratt et al. (2018) discuss advancement in preclinical models for biomarker identification in schizophrenia. They note the importance of adopting a reciprocal forward and reverse translation approach as exemplified in RDoC for discovering panels of biomarkers for diagnosis, prognosis and treatment response. In particular, they emphasize that for preclinical biomarkers to translate to a clinically relevant outcome they should be measured in preclinical models of translational value and high validity. In this context, the review focusses on relevant imaging, electrophysiological, biochemical and cognitive behavioural modalities (informed by CNTRICS) in preclinical models based upon the glutamate hypofunction hypothesis, genetic and environmental risk factors for schizophrenia (reverse translation). Furthermore, the importance of forward translation is exemplified by the example that preclinical research has identified the thalamic reticular nucleus as a locus of brain dysfunction in schizophrenia which has recently been demonstrated in schizophrenia samples.

Andrews et al. (2018) provide an overview of the complex interactions between the many genetic and environmental factors implicated in autism spectrum disorder (ASD). They note that a major challenge in development of biomarkers for ASD is due to the aetiological and phenotypic complexity. In their review, they present evidence that new techniques, specifically combining neuroimaging with 'machine learning' based pattern classification methods may assist in making individual diagnostic predictions. Ultimately these 'brain imaging' biomarkers may not only assist in diagnosis but also help in the discovery and development of personalized treatments.

4 How Can Biomarkers Be Best Exploited?

Current research is shaping the way forward for the development of panels of biomarkers. This relies on the ever-increasing sophistication of 'Big Data' analytical approaches, whereby multifactorial, multidisciplinary findings can be integrated to reveal and test the sensitivity, specificity and reproducibility of biomarkers for early intervention, diagnosis and stratification.

The RDoC initiative, combined with fundamental advances in genetic, epidemiological and neuroscience research, is yielding new information on the circuits and physiological mechanisms underpinning particularly cognitive and behavioural constructs that cut across current diagnostic DSM-5 categories. Given that many disorders encompass a neurodevelopmental component and behavioural phenotypes (e.g. working memory deficits), that cross diagnostic categories, it is likely that 'panels of biomarkers' will be discovered that will enable the discovery and development of interventions suitable for treating particularly constructs (e.g. working memory) that cut across current diagnostic DSM-5 categories.

What are the prospects for Biomarkers in the twenty-first century Psychiatry? Notwithstanding, the existence of a small repertoire of biomarkers that already exist, namely a phamacogenetic biomarker for the side effects of clozapine along with a diagnostic biomarker to detect NMDA receptor antibodies in psychosis resulting from autoimmune limbic encephalitis, the closest biomarker for development is arguably an 'early intervention in psychosis' biomarker panel (incorporating genetic, imaging and behavioural biomarkers). The advanced status of research in this area supports 'early intervention biomarker' development as being ripe for translation into the clinic (see Hunter and Lawrie 2018). Ultimately, this would not only offer clinicians a method for assisting in complex diagnostic and treatment decisions, but importantly would have an enormous impact on patient care and society as a whole.

There is a timely opportunity to improve clinical trial design informed by patient phenotype. The availability of relevant biomarkers for psychiatric disorders, informed by advances in understanding of the underpinning biology, has the potential to reinvigorate drug development and clinical trials in psychiatry, which currently suffer from their reliance of "soft" endpoints. The assessment of therapeutic interventions is likely to yield richer data if patients are stratified according to their 'biological' and 'psychological' phenotypes. This would be the first step into the arena of personalized medicines.

From the reviews presented in this volume, it is clear that the journey towards the identification of biomarkers is poised to move from the descriptive and discovery phase to one where assessments of biomarker panels for diagnosis, patient stratification and treatment can be evaluated in real-life cohorts. This will require resources for large-scale collaborative efforts worldwide. In summary, we should view with optimism our capabilities to develop biomarkers that will ultimately lead to new interventions and transform our ability to prevent illness onset and treat complex psychiatric disorders more effectively.

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Stem Cells to Inform the Neurobiology of Mental Illness

Mandy Johnstone, Robert F. Hillary, and David St. Clair

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Abstract The inception of human-induced pluripotent stem cell (hiPSCs) technology has provided an exciting platform upon which the modelling and treatment of human neurodevelopmental and neuropsychiatric disorders may be expedited. Although the genetic architecture of these disorders is far more complex than previously imagined, many key loci have at last been identified. This has allowed in vivo and in vitro technologies to be refined to model specific high-penetrant genetic loci involved in both disorders. Animal models of neurodevelopmental disorders, such as schizophrenia and autism spectrum disorders, show limitations in recapitulating the full complexity and heterogeneity of human neurodevelopmental disease states. Indeed, patient-derived hiPSCs offer distinct advantages over classical animal models in the study of human neuropathologies. Here we have discussed the current, relative translational merit of hiPSCs in investigating human neurodevelopmental and neuropsychiatric disorders with a

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Curr Topics Behav Neurosci (2018) 40: 13–44
DOI 10.1007/7854_2018_57
Published Online: 7 June 2018

specific emphasis on the utility of such systems to aid in the identification of biomarkers. We have highlighted the promises and pitfalls of reprogramming cell fate for the study of these disorders and provide recommendations for future directions in this field in order to overcome current limitations. Ultimately, this will aid in the development of effective clinical strategies for diverse patient populations affected by these disorders with the aim of also leading to biomarker identification.

Keywords Affective disorders · Autism · Biomarkers · Cerebral organoids · hiPSCs · In vitro models · Schizophrenia

1 Introduction

The National Institutes of Health Biomarkers Definitions Working Group in 1998 defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Biomarkers Definitions Working Group 2001). Biomarkers have transformed modern medicine but remain largely elusive in psychiatry, due in part to the way we diagnose mental illness, and so ongoing efforts to rethink our approach to the diagnosis and treatment of mental illness will be necessary.

The relative inaccessibility of the human brain, the inherent problems associated with brain biopsies and post-mortem tissue studies, and the limited findings from longitudinal neuroimaging studies collectively have hampered our ability to make robust connections between underlying molecular mechanisms and the clinical course of neuropsychiatric disorders. Perhaps unsurprising then to date we have yet to identify a clinically translatable biomarker in psychiatry, and so we have lagged behind in comparison to other medical specialties. The idea of having biomarkers for a disorder such as schizophrenia, for example, is further complicated by the fact that it is a complex and heterogeneous disorder which can be further broken down into subtypes. It will be essential to correlate symptoms with findings from brain imaging and the molecular analysis of patient-derived cells, harnessing genomics, transcriptomics, and proteomics to develop a truly integrated approach (Fig. 1). With the advances made in identifying highly penetrant risk variants, it is now possible to translate the significance of these in terms of the underlying pathophysiology of disease. Such efforts in deep phenotyping and genotyping will allow us to link specific symptoms to dysfunction in related brain circuits with the goal of better methods of detection and treatment. The ideal scenario is the identification of biomarkers that are involved in aetiological pathways and are therefore likely to change in response to disease-modifying treatments and to change according to disease status. The development of biomodels to further our understanding of the mechanisms underlying and contributing to biomarkers in psychiatric disease is paramount towards these goals.

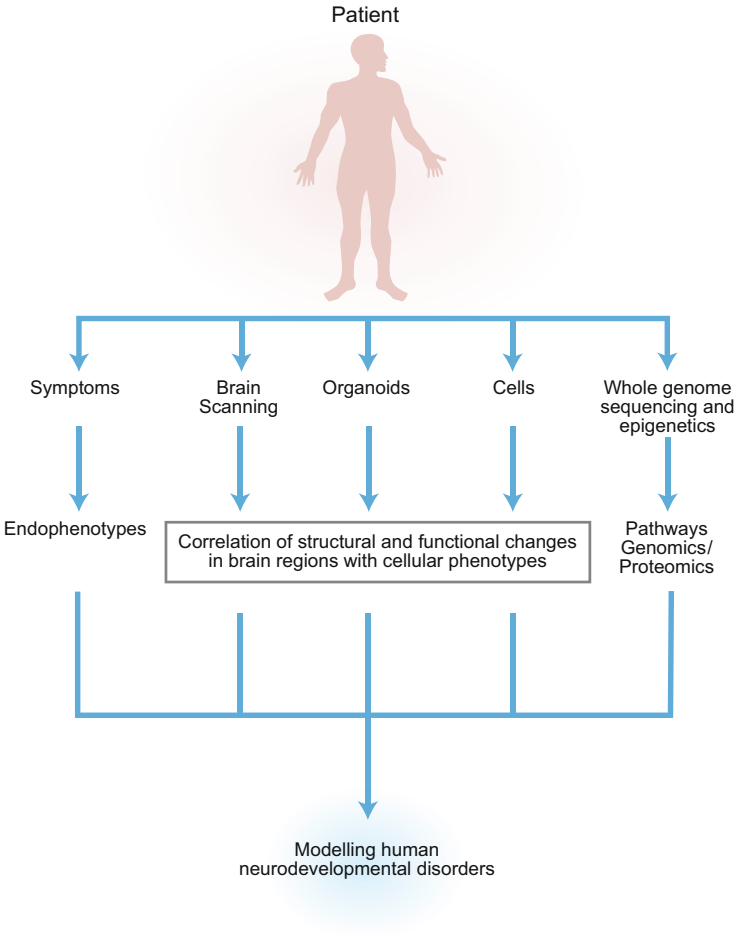

Fig. 1 "Concerted" research efforts will bring a truly integrated approach to the study of neuropsychiatric disorders. To develop clinically translatable biomarkers in psychiatry and improved disease-modifying treatments, it will be essential to correlate patient symptoms with findings from brain imaging and the molecular analysis of patient-derived cells, harnessing genomics, transcriptomics, and proteomics as well as 2D and 3D cellular modelling to develop a truly integrated strategy

2 Stem Cell Models Herald the Dawn of a New Era in Biological Psychiatry

The advent of human-induced pluripotent stem cell (hiPSC) technology has the potential to revolutionise modern biomedicine and has provided a new platform upon which the mechanisms underlying human pathologies may be interrogated. In 2006, Yamanaka and his colleagues reprogrammed murine fibroblasts to cells

exhibiting gene expression profiles and developmental potential akin to embryonic stem cells (ESCs) by using a cocktail of four transcription factors (Fig. 2a) (Takahashi and Yamanaka 2006). These cells were termed induced pluripotent stem cells (iPSCs), and the four factors – OCT4, SOX2, KLF4, and MYC – were immortalised as the "Yamanaka factors". Just 1 year following this discovery, two groups independently reported the generation of iPSCs from human fibroblasts, ushering in a new era of developmental biology and regenerative medicine (Takahashi et al. 2007; Yu et al. 2007). Although most iPSC studies have started with fibroblasts (Takahashi et al. 2007), it is also possible to reprogramme keratinocytes (Aasen et al. 2008), peripheral blood (Staerk et al. 2010), and hair follicles (Petit et al. 2012) which are often easier and more acceptable to patients. The inception of induced pluripotency has expedited the study of neurodevelopmental disorders, which previously relied on the utilisation of animal models and ESCs. Patient-derived iPSCs provide a novel means to overcome the biomedical and translational challenges associated with animal and ESC paradigms. Animal models do not recapitulate the full genomic complexity and heterogeneity of human neurodevelopmental disorders, and improved translational approaches are required (See MacQueen et al. 2018; Pratt et al. 2018). Moreover, the ethical concerns surrounding the derivation of human ESCs from diseased embryos diminish the value of these cells in the exploration of developmental stages that are otherwise inaccessible to research (Ardhanareeswaran et al. 2017). Indeed, studies pertinent to autism spectrum disorders (ASD), and fragile X syndrome, have been limited to aborted foetuses and adult *post-mortem* brain tissue (Mor-Shaked and Eiges 2016). Thus, hiPSCs provide a much needed alternative approach in neuroscientific research by permitting the examination of dynamic molecular, cellular, and anatomical correlates of neurodevelopmental and neuropsychiatric disease states.

3 Modelling Schizophrenia and Autism Using hiPSC Technologies

Schizophrenia (SCZ) and autism (ASD) are two of the most important neurodevelopmental disorders encountered in routine clinical psychiatric practice, and both are diagnosed on the basis of clinical history, symptoms, and behaviour. For schizophrenia these include positive symptoms, such as hallucinations, delusions, and thought disorder and negative symptoms such as social withdrawal, anhedonia, and poverty of thought: there are also a range of cognitive abnormalities especially of attention, memory, and executive function. Autism is characterised by abnormalities of social communication and interaction and repetitive patterns of interests and behaviour. Unfortunately, in spite of intensive efforts spanning several decades, there are still no objective tests (biomarkers) in routine clinical psychiatric practise to assist with diagnosis of psychiatric disorders (Kapur et al. 2012). Although ages of clinical presentation of schizophrenia and autism are normally

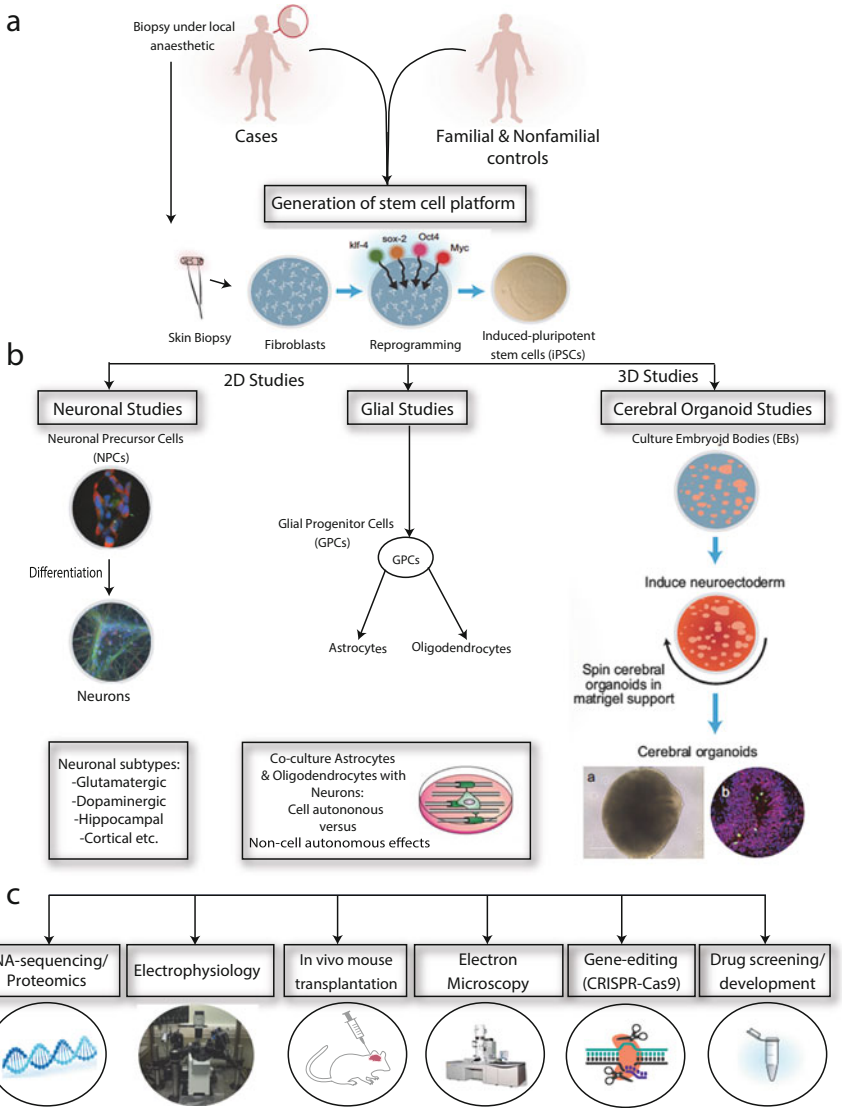

Fig. 2 Overview of human iPSC model systems to study psychiatric disorders. Human iPSC is generated by reprogramming fibroblasts from skin biopsies from volunteers with a variety of techniques, most commonly using standard Yamanaka factors, delivered in non-integrative episomal vectors. Other starting cellular materials can be used such as keratinocytes from hair or from peripheral blood mononuclear cells. Once generated and extensively tested, the hiPSCs can be used to either make neuronal precursor cells or glial precursor cells (e.g. oligodendrocyte/astrocyte precursors) or grown and lifted to make 3D organoids as shown in frame (**a**) phase-bright image of a cerebral organoid at 2 months of age, and frame (**b**) shows an organoid that has been sectioned and stained with antibodies to Pax6 and phospho-histone H3, clearly demonstrating a ventricular zone. (**c**) The cellular platforms generated can then be used for further downstream studies including electrophysiology, transcriptomic and proteomic studies, drug screening, as well as morphological studies and coculturing with other cell types. In addition the hiPSCs can be gene edited using CRISR-Cas9 to attempt to rescue phenotypes observed

early adult life and early infancy, respectively, both have at least in part neurodevelopmental origins; namely, antecedents affecting brain development and in turn predisposition to one or both disorders can occur at any point in the life cycle probably from conception onwards. There are also preconceptual intergenerational effects, the most studied being parental and grandparental ages. Antecedents may be environmental or genetic/epigenetic or the effects of gene-environment (G × E) interactions. Environmental risk factors are often discussed in the context of a "stress-vulnerability" aetiological model where early biological and psychological insults, occurring in both the pre- and postnatal periods, result in changes of gene and protein expression, changing the intracellular and extracellular milieu of the developing brain (Modabbernia et al. 2017; Opler et al. 2013). Essentially such environmental risk factors may be important in DNA methylation. However, perhaps the most intriguing finding to emerge from epidemiological studies is that schizophrenia and autism appear to share a remarkable number of environmental risk factors (Chisholm et al. 2015; Hamlyn et al. 2013) as well as overlapping genetic risk profiles as discussed below. Therefore, modelling methods in the future will ideally permit us to also recapitulate at least some of these environmental insults to study the dynamic G × E interactions.

3.1 The Genetic Architecture of Schizophrenia and Autism

Both schizophrenia and autism are strongly familial neuropsychiatric disorders. Our understanding of the genetic architecture of both neurodevelopmental disorders expanded enormously with the advent of methods for systematic interrogation of DNA across the whole genome, first through genome-wide association studies (GWAS) and more recently whole-exome and whole-genome sequencing. These advances allow us to detect association with rare (<1%) high-penetrant genetic lesions including copy number variants (CNVs) and association with common low-penetrant genetic risk factors identified using single nucleotide polymorphism (SNP) microarrays. These latter common low-penetrant risk factors have odds ratios around 1.0–1.2. Rare low-penetrant genetic lesions also exist, but sample sizes required for their detection with reasonable statistical supporting evidence are far beyond those currently available worldwide; a similar problem confounds potential genome-wide studies of gene/gene interactions/epistasis (Wei et al. 2014). It is also possible to examine nonstatistically significant common variants for association as a whole – so-called polygenic liability risk (Purcell et al. 2009). This latter approach does not however help with selection of individual gene targets for in vivo or in vitro modelling.

The evidence in schizophrenia comes from multiple twin family and adoption studies and points to a heritability of up to 80% with MZ concordance of 40–50% (Lichtenstein et al. 2009; Sullivan et al. 2003). There have been many fewer twin and family studies of autism and surprisingly no adoption studies. Earlier twin studies suggested heritability as high as 80–90% for ASD with little contribution from the

environment (Bailey et al. 1995). Newer studies of monozygotic twins have yielded concordance rates of <50%, with lower concordance for dizygotic twins, suggesting that both genes and environment play roles in the development of ASD (Ronald and Hoekstra 2011). The current consensus is that up to 40–50% of variance is determined by environmental factors (Modabbernia et al. 2017).

Early linkage and candidate gene mapping studies of schizophrenia and autism have not lived up to their promise in the way of findings that have stood the test of time. The most studied are *Disrupted in Schizophrenia 1* (*DISC1* gene) identified by cloning the breakpoints of a balanced 1:11 chromosomal rearrangement associated with multiple cases of mental illness including schizophrenia in a large Scottish pedigree (Millar et al. 2000), chromosome 22 deletion syndrome associated with a range of severe neurodevelopmental disorders (Schneider et al. 2014), fragile X syndrome (Richards and Sutherland 1992), Rett syndrome (Amir et al. 1999), and rare cases of autism with mutations of neuroligin genes (Jamain et al. 2003).

3.1.1 Rare Variants in Schizophrenia and Autism

Schizophrenia and autism share an enormous degree of genetic heterogeneity, but only a small proportion (5–10%) of the overall genetic risk results from rare highly penetrant genetic mutations such as CNVs. Many of these latter loci (causing deletions or duplications of stretches of DNA) show pleiotropy, i.e. they display a range of clinical phenotypic abnormalities that include autism, intellectual impairment, schizophrenia, and epilepsy (Conrad et al. 2010) resulting in a considerable overlap of high-penetrant loci between schizophrenia and autism. Many mutations especially in autism have arisen de novo and are not found in the parents of the affected proband (De Rubeis et al. 2014; Levy et al. 2011; Neale et al. 2012; Sanders et al. 2012). Although recurrent CNVs have high mutation rates due to non-allelic homologous recombination, they are eliminated fast by negative selection and seldom survive more than two or three generations (Stefansson et al. 2008). Around 800 rare loci are reported in autism (far fewer in schizophrenia), but the evidence to support their causal involvement varies enormously, and in only a few dozen including recurrent CNVs is there statistical evidence of genetic association (Sanders et al. 2012). Among these genes are *NGLN4X* (Jamain et al. 2003), *SHANK3* (Durand et al. 2007; Gauthier et al. 2009), *NRXN1* (Bucan et al. 2009; Kim et al. 2008), *SHANK2* (Berkel et al. 2010), *CNTN4* (Fernandez et al. 2004, 2008; Glessner et al. 2009), and *CNTNAP2* (Bakkaloglu et al. 2008; Strauss et al. 2006). The findings in schizophrenia are broadly similar (Harrison 2015; Jablensky 2015; Need and Goldstein 2014).

3.1.2 Common Variants in Schizophrenia and Autism

Initial genome-wide schizophrenia SNP association studies involving several thousand cases and controls yielded only two or three loci that meet statistical

significance ($p < 1 \times 10^{-7.5–8}$). The precise significance level depends upon the number of tests performed (Purcell et al. 2009; Stefansson et al. 2008). However with increased sample sizes to around 150,000 individuals, over 100 loci were reported to meet genome-wide significance (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014), and with even larger sizes up to 150 loci have now been identified (PGC unpublished). Although multiple common low-risk variants are reported associated with autism, to date, no loci for autism have consistently met criteria for genome-wide significant association; this is probably the result of inadequate sample sizes. There are a number of excellent articles discussing gene/gene interactions/epistasis (Wei et al. 2014), schizophrenia epigenetics (Hannon et al. 2016), and modelling of polygenic risk (Purcell et al. 2009, 2014). In particular, two earlier studies highlight the potential of being able to elucidate a better understanding of the effects of regulatory polymorphism on the expression of genes essential to mental health (Davidson et al. 2011; Hing et al. 2012). Furthermore, the identification of these regulatory determinants will in turn permit critical insights into the role of epigenetic factors such as DNA methylation that are known to influence gene expression.

3.1.3 Missing Heritability in Schizophrenia and Autism

The majority of genetic risk for both schizophrenia and autism is yet to be elucidated and is likely to involve many more rare high- and low-risk factors, common low-risk factors, epistasis, and epigenetic interactions – the so-called missing heritability. Their tiny effect sizes represent a formidable challenge: what sort of clinical or behavioural phenotype if any should one expect to find? A recent "omnigenic model" was proposed that gene regulatory networks are sufficiently interconnected such that all genes expressed in disease-relevant cells are liable to affect the functions of core disease-related genes and that most heritability can be explained by effects on genes outside core pathways (Boyle et al. 2017). The genes we have chosen to discuss here are likely to affect the function of core pathways and so are likely to shed insight into wider populations of patients with these disorders even though the majority of patients are not enriched for high-impact variants.

3.2 Loci Selection for Disease Modelling in Schizophrenia and Autism

In spite of their large numbers and widespread involvement in schizophrenia and autism, there have been few attempts to model individual common low-penetrant SNP-associated loci using either animal or human iPSC technologies. This is unsurprising. The vast majority of SNPs significantly associated in GWAS are located outside gene coding regions and in many instances often a considerable

distance from the nearest coding region. Most effort has therefore concentrated on attempting to fine map putative functional variants presumed to be in linkage disequibrium with the GWAS-associated SNPs. This is paralleled by in silico bioinformatic investigations using pathway analyses/gene ontology (GO) studies to try to obtain further corroboration of their functional significance. To date success has been very limited (Need and Goldstein 2014). Fortunately, successful studies designed to ascribe regulatory functionality to directly associated SNPs, or those in linkage disequilibrium (LD), using comparative genomics and CRISPR modified preclinical mouse models are well underway. These studies promise to develop a better understanding of the effects of regulatory polymorphism on the expression of genes essential to mental health. Furthermore, the identification of these regulatory determinants will permit critical insights into the role of epigenetic factors such as DNA methylation that are known to influence gene expression.

To date however there are very few instances where specific low-penetrance loci for autism or schizophrenia have been considered for modelling in animals or iPSCs. Their tiny effect sizes represent a formidable challenge: what sort of clinical/behavioural phenotype if any should one expect to find? An exception is the very strong allelic association of the MHC region of chromosome 6 to schizophrenia. This has prompted detailed exploration of the putative involvement of complex variation at the complement component 4 candidate gene being linked to excess synaptic pruning (Sekar et al. 2016), a dynamic process proposed to rid the brain during development of wasteful neural connections and strengthen others and proposed to be a reason why brains from patients with schizophrenia have fewer synaptic connections in multiple brain regions (Boksa 2012). Another noteworthy exception is the association of SNPs within *CACNA1C* to autism, bipolar disorder, and schizophrenia (Ripke et al. 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). The risk-associated genotypes appear to affect RNA abundance, but results are inconclusive. *CACNA1C* has many dozens of exons, with multiple transcripts and promoters. The locus however also harbours de novo Mendelian dominant exonic mutations responsible for Timothy syndrome (TS), a neurodevelopmental disorder which has features of autism. The most interesting findings have emerged from studying hiPSC cells from individuals with TS (Birey et al. 2017; Pasca et al. 2011). Building on previous work that showed in rodents that L-type calcium channel (LTCC) genes play a critical role in interneuron migration (Bortone and Polleux 2009), Birey et al. (2017) found that cortical interneurons derived from patients with TS display a cell-autonomous migration defect whereby they move more frequently but less efficiently (Birey et al. 2017). Furthermore the TS interneuron defect is rescued by pharmacologically manipulating LTCCs.

The selection of which highly penetrant genetic mutations to model using hiPSCs poses separate challenges from common low-risk SNP-associated loci as these mutations are often so rare that statistical evidence of association with the disorder is lacking. This is less of a problem in schizophrenia where linkage with the mutation in multiplex families is often available for additional corroboration. Also through PGC and other consortia, DNA from many thousands of cases is available for

interrogation to try to identify additional mutations at the locus of interest. In autism, where de novo mutation is more common, corroborating data from multiplex families is usually not available. It can be argued that de novo mutation itself may support a causative role in a disorder where the absence of familial cases is due to negative selection. A word of caution is merited. It must be borne in mind that each individual harbours around 60–100 de novo events (Gratten et al. 2013), and deciding which/if any are causative is not a trivial problem especially if it has implications for genetic counselling. Often therefore one of the main purposes of modelling is to try to demonstrate a causative mechanism that may result in the disorder under investigation. This especially applies where the gene is not an obvious candidate for the disorder under investigation, e.g. complement component 4 discussed above. In the case of rare variants, biology does have a role in both establishing a genetic association and later in understanding its role (Porteous et al. 2014). In some cases the statistical and/or circumstantial evidence for involvement of the locus with the disorder is sufficiently compelling that modelling in hiPSCs justifies the time and cost. Many such loci are currently being examined using hiPSC technologies as summarised below for DISC1 and CYF1P.

DISC1 is a major vulnerability factor for a wide range of chronic mental illnesses (Brandon and Sawa 2011) and was first isolated by cloning the breakpoints of a 1:11 balanced translocation co-segregating with major psychiatric disorders in a large Scottish pedigree (Millar et al. 2000; St Clair et al. 1990). Within this one family, the logarithm of the odds (LOD) score for schizophrenia alone met stringent genome-wide significance, whilst for schizophrenia plus bipolar disorder and major depressive disorder, it substantially exceeded genome-wide significance (MLOD = 7.1). A second wave of follow-up confirmed these original findings (Blackwood et al. 2001). Further evidence supporting the involvement of *DISC1* in mental disorders has been more recently debated (Guha et al. 2013; Porteous et al. 2014; Sullivan 2013).

DISC1 expression in the brain is particularly high in the hippocampus during neurogenesis and remains high in the adult dentate gyrus, olfactory bulb, and limbic regions (Duan et al. 2007; Meyer and Morris 2008), and it appears that DISC1 regulates important developmental processes such as neuronal migration, integration (Lipska et al. 2006), synapse formation, and neuronal stem cell maturation (Duan et al. 2007; Kamiya et al. 2005; Miyoshi et al. 2003; Ozeki et al. 2003). DISC1 is thus critical for neurodevelopment and normal adult neuronal function. In addition, transgenic or mutant mice with impaired DISC1 function show brain morphological changes, deficits in neural circuits, working memory impairment, and behavioural traits related to schizophrenia and also bipolar disorder (Tomoda et al. 2016). One of the more interesting of the mice transgenic models, denoted Disc1tr, expresses two copies of truncated Disc1 encoding the first eight exons generated using a bacterial artificial chromosome (BAC) (Shen et al. 2008). With this partial simulation of the human situation, they discovered a range of phenotypes including a series of novel features not previously reported. Disc1tr transgenic mice display enlarged lateral ventricles, reduced cerebral cortex, partial agenesis of the corpus callosum, and thinning of layers II and III with reduced neural proliferation at mid-neurogenesis (Shen et al. 2008). Parvalbumin (PV+) GABAergic neurons are reduced in the

hippocampus and medial prefrontal cortex and displaced in the dorsolateral frontal cortex. In culture, transgenic neurons grow fewer and shorter neurites. Behaviourally, these transgenic mice exhibit increased immobility and reduced vocalisation in depression-related tests and impairment in conditioning of latent inhibition. The BAC mouse model uses the full mouse genomic sequence and natural promoters. This may be responsible for the considerable schizophrenia reminiscent brain pathology observed in this study compared to other studies using more artificial constructs.

In terms of potential future biomarker selection, DISC1 requires far more validation and study and has not come out as a target from "GWAS" studies: it is still not clear the mode of action of the t(1:11) mutation and haploinsufficiency seems most likely. No truncated DISC1 protein has ever been identified, suggesting elimination of mutated RNA by nonsense-mediated decay. It has also been shown that transient knockdown of DISC1 by in utero electroporation in mouse, in the pre- and perinatal stages, specifically in a lineage of pyramidal neurons mainly in the prefrontal cortex, leads to selective abnormalities in postnatal mesocortical dopaminergic maturation and behavioural abnormalities associated with disturbed cortical neurocircuitry after puberty (Niwa et al. 2010). Nevertheless, a dominant negative mode of action from mutated DISC1 protein dimerising with the wild type cannot be ruled out. What is clear is that the mutations reported in *Disc1* do seem to alter the structural organisation of the DISC1 protein (Yerabham et al. 2017).

It has been possible to generate isogenic hiPSCs with an engineered disease-relevant disruption of *DISC1* which affects neural progenitor cell (NPC) proliferation, baseline WNT signalling, and expression of NPC fate markers such as FOXG1 and Tbr2 (Srikanth et al. 2015). Ming and Song's group have since generated hiPSCs from four members of an American family in which a frameshift mutation of *DISC1* co-segregated with major psychiatric disorders (Chiang et al. 2011) and furthermore produced different isogenic iPS cell lines via gene editing (Wen et al. 2014). In an elegant series of experiments, they showed that mutant DISC1 causes synaptic vesicle release deficits in hiPSC-derived forebrain neurons (Crabtree et al. 2017; Maher and LoTurco 2012; Tang et al. 2016). Mutant DISC1 depleted wild-type DISC1 protein and, furthermore, dysregulated expression of many genes related to synapses and psychiatric disorders in human forebrain neurons providing new insight into the molecular and synaptic etiopathology of psychiatric disorders (Wen et al. 2014). This group have also gone on to show that DISC1 regulates neurogenesis via modulating kinetochore attachment of Ndel1/Nde1 during mitosis in neural stem cells in mice and in human organoids derived from hiPSC from patients with schizophrenia who harbour a DISC1 mutation (Ye et al. 2017). Although similar studies have not yet been published from the Scottish DISC1 family, it will be interesting to see whether synaptic dysregulation is also evident in neurons derived from these family hiPSCs.

Chromosome 15q11.2 CNVs have emerged as prominent risk factors for various neuropsychiatric disorders, including schizophrenia, autistic spectrum disorder, and intellectual disability (Malhotra and Sebat 2012). 15q11.2 microdeletion (15q11.2 del) was identified as one of the most frequent CNVs associated with increased risk

for schizophrenia (Stefansson et al. 2008), a finding subsequently confirmed in additional cohorts (Kirov et al. 2009; Tam et al. 2010; Zhao et al. 2013). 15q CNVs are not as penetrant as other recurrent CNVs associated with neurodevelopmental disorders. They are however under negative selection (Stefansson et al. 2008), and even in normal subjects, 15q11.2 del is associated with cognitive variation and changes in structural measures on MRI scanning (Stefansson et al. 2014). 15q11.2 CNVs encompass four genes: nonimprinted in Prader/Willi Angelman 1 and 2 (*NIPA1* and *NIPA2*), *CYFIP1*, and *TUBGCP5*. Whilst little is known about functions of these genes in mammalian neural development, *CYFIP1* has been shown to interact with *Rac1* (Kobayashi et al. 1998), *FMRP* (Schenck et al. 2001), and *eIF4E* (Napoli et al. 2008). Biochemical studies have also identified CYFIP1 as a regulator of the WAVE complex, consisting of WAVE1, WAVE2, Nap1, and Abi1, a complex known to regulate Arp2/3-mediated actin polymerisation and membrane protrusion formation in non-neuronal cell lines (Kobayashi et al. 1998; Kunda et al. 2003; Steffen et al. 2004). The function of WAVE signalling in mammalian neurogenesis is not well understood. However, an elegant study has been published using stem cells from patients with 15q11.2 CNVs (Yoon et al. 2014). Yoon et al. (2014) took a multifaceted approach to investigate why 15q11.2 CNVs are prominent risk factors for schizophrenia and autism. Even in normal control subjects, carriers of the 15q11.2 deletion have cognitive deficits and structural changes on MRI scanning raising questions about how this genetic variant brings about these changes in the carriers. They showed that hiPSC-derived neural progenitor cells carrying 15q11.2 microdeletions exhibited deficits in adherens junctions and apical polarity resulting from haploinsufficiency of *CYFIP1* (Yoon et al. 2014). Furthermore, they showed that deficiency in CYFIP1 and WAVE in the developing mouse cortex affects radial glial cell migration causing ectopic localisation outside of the ventricular zone (Yoon et al. 2014). Targeted human genetic association analyses revealed an epistatic interaction between CYFIP1 and WAVE signalling mediator ACTR2 and risk for schizophrenia. Therefore, by integrating human neural stem cells, in vivo animal modelling, and targeted human genetic association studies, a mechanistic understanding of how 15q11.2 microdeletions affect neural development has been uncovered. In the future this signalling pathway may reveal important insights for the development of biomarkers.

Autism spectrum disorders affect approximately 1% of the population and are characterised by impairments in social interaction, stereotyped behaviours, and language deficits (Fombonne 2005). Certain disorders, including fragile X syndrome and Rett syndrome, have defined genetic bases; however, the majority of ASD cases lack a clear aetiology. Moreover, difficulties in recapitulating human brain development and the paucity of human neural tissue have precluded comprehensive understanding of ASD pathophysiology (Chaste and Leboyer 2012). Crucially, animal models fail to reflect the complexity of human behaviour. Mice deficient in receptors for the "social neuropeptides" oxytocin and vasopressin (both important for social attachment and affiliation and implicated in ASD aetiology) emit fewer ultrasonic vocalisations in various social challenges, including isolation. The degree to which

Table 1 Large-scale drug screening using iPSCs from patients with fragile X syndrome

Cell type derived from iPSCs	Readout	Number of compounds screened	Hit rate (%)	References
Patient neural progenitor cells	*FMR1* expression	50,000	4.2	Kaufmann et al. (2015)
Patient neural stem cells	*FMR1* expression	~5,000	0.12	Kumari et al. (2015)
Neural progenitor cells	*FMR1* expression	1,134	0.17	Li et al. (2017)

FMR1 fragile X mental retardation 1, *iPSC* induced pluripotent stem cell

these findings may be extrapolated to human social behaviour in syndromic and nonsyndromic ASD states remains challenging (Scattoni et al. 2008; Takayanagi et al. 2005). Additionally, the contribution of epigenetics in ASD pathophysiology, and species differences, confounds the clinical relevance of translational animal ASD models. IPSC technology allows for patient-specific disease modelling and therapeutic development and has provided novel neurobiological insights pertinent to ASD. For instance, iPSCs derived from patients with idiopathic ASD exhibit overproduction of GABAergic inhibitory neurons (Mariani et al. 2015). Moreover, the authors showed that the transcription factor FOXG1 contributes to such overproduction and FOXG1 expression correlates with clinical severity. In relation to pharmacotherapies, iPSCs have been utilised in large-scale drug screening for syndromic autism disorders such as fragile X syndrome which is characterised by epigenetic silencing of the *FMR1* gene (Table 1). High-throughput screening on iPSCs derived from patients with *SHANK3* haploinsufficiency, an autism risk gene which regulates synaptic organisation, illustrated that lithium pharmacotherapy rescued associated phenotypes in vitro (Darville et al. 2016). This was complemented by a trial in which lithium was administered to one patient whose cells were used in the screening study. Following 1 year of treatment, the patient exhibited improved measures of ASD but worsened symptoms of attention-deficit hyperactivity disorder. Clearly, further, large-scale clinical studies are needed, in addition to basic research on patient-derived iPSCs, to identify candidate targets for exploitation in the treatment of ASD. Combinational efforts utilising animal models and iPSCs will expedite the discovery of critical windows during developmental and/or postnatal life at which interventions are efficacious, as well as dosages and regimens which minimise toxicity.

4 Modelling Affective Disorders Using hiPSC Technologies

4.1 Depression and Anxiety Disorders

Major depressive disorder (MDD) affects approximately 5% of the population worldwide, and the diagnostic criteria include 2 or more weeks of decreased mood

and anhedonia combined with one or more of severe appetite change, disrupted sleep patterns, psychomotor disturbances, excessive guilt and rumination, reduced concentration, or recurrent thoughts of death. Treatments usually involve a combination of psychological, social, and pharmacological interventions, the first line of which is usually treatment with a selective serotonin reuptake inhibitor (SSRI) or serotonin-noradrenaline reuptake inhibitor (SNRI). The focus of pharmacological development has centred on the monoamine oxidase theory looking for genetic variants in the synthesis and signal conduction pathways of monoamines. Anxiety disorders also carry a huge burden and affect approximately 4% of the global population and include panic disorder, generalised anxiety disorder (GAD), obsessive compulsive disorder (OCD), post-traumatic stress disorder (PTSD), and phobic disorders, all characterised by persistent and severe anxiety symptoms with a sense of foreboading thoughts and dread.

In terms of the use of hiPSCs to model these disorders, two independent groups have published methods of generating serotonergic neurons via transdifferentiation from human fibroblasts (Vadodaria et al. 2016; Xu et al. 2016). Vadodaria et al. (2016) also used the model they developed to show that they could screen the effects of the SSRI citalopram in this system. A human ESC and iPSC model has also been developed to generate serotonergic neurons to screen molecules (Lu et al. 2016), and using this system, these researchers used the SSRI escitalopram to establish utility of the patient-derived stem cell system. Deficiencies in brain-derived neurotrophic factor (BDNF) have been proposed as having an important role in neuropsychiatric disorders, including depression and anxiety (Nagahara and Tuszynski 2011), and so cell therapies targeting BDNF deficiencies may hold clinical utility in the future. In this regard it has been shown that mice treated with human iPSC-derived neural progenitors overexpressing BDNF experienced greater neurogenesis than control mice (Liu et al. 2014). Therefore, in the future, it may be possible to use hiPSC-derived cells with inducible BDNF expression as a form of cell-based regenerative therapies, not withstanding many of the current problems and limitations with such techniques.

4.2 Bipolar Affective Disorder

Like SCZ, bipolar affective disorder (BPD) affects approximately 1% of the population and is also highly heritable. Characterised by episodes of major depressive disorder interspersed with manic or hypomanic episodes is the usual clinical picture and is treated with mood-stabilising drugs such as lithium, sodium valproate, or carbamazepine and sometimes also requires treatment with antipsychotics or antidepressants depending on whether the patient is also suffering from psychotic symptoms or depression. Several papers have described the generation of hiPSC-derived neurons from patients with BPD comparing these to healthy controls (Chen et al. 2014; Madison et al. 2015; Mertens et al. 2015; Wang et al. 2014). Differential expression of genes involved in regulating neuronal differentiation, proliferation,

and calcium signalling was found between affected cases and controls from a family affected by BPD (Madison et al. 2015). Neuronal precursor cells generated from these family-derived cases with BPD exhibited impaired differentiation and reduced proliferation, and interestingly both of these cellular phenotypes were rescued by treatment with a selective inhibitor of the enzyme GSK3β, a known target of lithium therapy (Madison et al. 2015). Another study of patients with BPD generated iPSC-derived hippocampal dentate gyrus granule-like neurons, a region known to be particularly affected in this disorder, and showed altered expression of mitochondrial, calcium signalling, and neuronal excitability genes (Mertens et al. 2015). Furthermore, these neurons, when compared to controls, showed a higher frequency of spontaneous action potentials indicating a hyperexcitability phenotype. What is perhaps most exciting, however, for the future utility of iPSC technologies in pharmacogenomics is the fact that a 1-week treatment with lithium partially normalised the earlier observed changes in hyperexcitability and also in mitochondrial gene expression but only in neurons derived from those patients with BPD who had shown an earlier clinical response to lithium therapy (Mertens et al. 2015). This could also help explain differences in molecular mechanisms underlying lithium responsiveness (Harrison et al. 2016) and shows how such hiPSC-derived models can provide platforms of cells for high-throughput screening of candidate molecules for future drug development. One such study has utilised hiPSC-derived neurons from patients with BPD to help validate molecules targeting the Wnt/GSK3β signalling system through which lithium is believed to modulate (Zhao et al. 2012).

5 Human In Vitro Stem Cell Models: Advantages and Limitations

Human iPSC technologies are allowing researchers to interrogate human cortical development in health and disease and provide unlimited platforms of mature neuronal and glial cellular subtypes and cocultures for downstream studies such as cellular physiology and phenotypic screening and for drug development and screening (Fig. 2). Such human iPSC models confer a number of advantages including the fact that it is possible to model for both coding and non-coding variants, and in fact it is also possible to model disease without actually knowing the causal genetic factor (Brennand et al. 2011; Marchetto et al. 2017). Clearly though knowing the causal/contributory variants confers an advantage to translational studies and a greater understanding of putative mechanisms of disease (Wen et al. 2014; Yoon et al. 2014). It is possible to study the effects of genomic mutations on brain development and in neuropsychiatric disorders using clustered regularly interspaced short palindromic repeats (CRISPR) gene editing technologies whereby it is possible to gene edit the predicted causal mutation and determine whether it is possible to rescue the cellular/molecular phenotype. Proteomics, transcriptomics, signalling, and cell biology analysis of isogenic mutant paired lines at the neuronal stem cell and

differentiated neuron cell state offer unique opportunities to identify potential biomarkers of disease as well as pharmacological strategies to target underlying molecular mechanisms that have gone awry. However, limitations include heterogeneity and reproducibility issues arising from multiple sources, including culture methodology and differences in lines and clones used. Furthermore, these hiPSC cultures produced immature feotal-like neurons, limiting their potential to properly model later developmental stages. This however has become less of an issue as it is now possible to mature cells by coculture and also using advanced organoid cultures (Birey et al. 2017; Pasca et al. 2015), as discussed further below. Improved efficiency of iPSC generation with concurrent advancements in 3D tissue modelling and genome engineering will expedite the clinical translation of novel, iPSC-based findings relating to ciliopathies and autism spectrum disorders. Ultimately, this will satisfy the unmet clinical and therapeutic needs for the millions of patients worldwide affected by these neurodevelopmental disorders.

The alternatives to hiPSC models in the past were of course animal models of disease, and these exist for almost all human genes sequenced. In this regard, coding regions of the genome are especially well conserved and lend themselves to animal models, whereas non-coding DNA, including regulatory elements, shows poor conservation across species. The mouse genome is almost as well characterised as the human, and murine models have become relatively cost-effective, straightforward to produce, and amenable to study at molecular, cellular, circuit, and behavioural levels, unlike rat models, however, which can be expensive to generate and maintain. Zebrafish (*Danio rerio*) and fruit fly (*Drosophila melanogaster*) (McCammon and Sive 2015) models by comparison have the highest output and are relatively inexpensive, but these are less suitable for modelling more complex human behaviours. Care must be given also to which mouse strain is used as genetic background effects are potential confounders to such. However, there are a number of obvious limitations and drawbacks when using such models to study certain aspects of neuropsychiatric disorders: although there are established batteries to phenotype core features of autism in mice (Silverman et al. 2010), mice exhibit profound differences in social behaviour from humans, and furthermore, even within mouse studies, variations in laboratory environments impose further variance. How these truly reflect the human condition is debatable. Doing the same to interpret schizophrenia-like phenotypes in mice, particularly for symptoms such as paranoia and delusional beliefs, is again challenging, and so disordered behaviour can only be inferred indirectly which is a major limitation to such models.

The promise of iPSC technology in patient-specific disease modelling, drug discovery, and regenerative medicine has fortified neuroscientific research since its advent over a decade ago. Recently, a Japanese clinical trial commenced in which iPSCs were derived from patients with macular degeneration and differentiated into retinal pigment epithelium sheets with the aim of autologous transplantation (RIKEN Institute). In the first patient, transplantation stabilised visual acuity; however, genetic variability was observed in iPSCs derived from the second patient. Hence, the trial was halted on the basis of safety concerns (Garber 2015). Indeed, iPSCs presently are hindered by safety (i.e. immunogenicity, teratogenicity, and

insertional mutagenesis) and economic issues. However, in relation to immunogenicity, concerns may be quelled through transplantation into immune-privileged sites (Pearl et al. 2012). Moreover, the development of teratomas (caused by undifferentiated cells in the transplant population) may be prevented through treatment of cell populations with iPSC-selective small-molecule inhibitors, such as the survivin inhibitor YM155, leaving just differentiated cells (Lee et al. 2013). Typically, Yamanaka factors are delivered to cells via integrating viral vectors which may induce mutations (Hawley 2008); however, this may be overcome through the development of non-integrating methodologies, such as episomal DNA or Sendai viruses (Takahashi and Yamanaka 2016). Furthermore, autologous iPSCs cost roughly $800,000 to manufacture per line, with a long production time span during which cells may accumulate karyotypic abnormalities and copy number variants (Bravery 2015). This issue may be rectified through the development of allogeneic stem cell banks; indeed, the macular degeneration trial will proceed with donor iPSCs, although concerns remain regarding potential immunogenicity of allogeneic stem cell transplantation (Pearl et al. 2012). Lastly, iPSC derivation is inefficient, and the reprogramming process itself may lead to de novo changes in copy number variation as well as other genetic alterations (Laurent et al. 2011). This is of particular relevance to ASD research as the aetiologies of many idiopathic or nonsyndromic cases remain undefined. Improvements in reprogramming and differentiation protocols are warranted in order to recapitulate physiological and pathophysiological brain development and further foster ASD and other neuropathological research.

6 Cerebral Organoids: Three-Dimensional Cellular Models of Brain Development

An organoid is a multicellular collection of cells that self-organises and develops from stem cell progenitors to resemble the structure and function of an organ in vivo (Lancaster and Knoblich 2014). In vitro models of the developing brain such as 3D brain organoids offer an unprecedented opportunity to study aspects of human brain development and disease, in particular the ability to follow development over time. Neuronal migration, cortical lamination, projection patterns, and circuit-level organisation are difficult to model in 2D cultures. Tissue engineering and 3D organoid cultures will enable the study of some of these phenotypes. To date rodent models have been heavily used to study the cellular function of many of the genes implicated in these disorders, especially those genes which are proposed to have an important role in fundamental neurodevelopmental processes such as cerebral cortex organisation. However, cortex development and organisation are different in rodents compared to humans, so unsurprisingly neurodevelopmental diseases cannot be consistently recapitulated in animal models. This is all about to change as over the past few years there have been further cutting-edge advances in developmental

neurobiology: we can now grow three-dimensional cerebral organoid cultures from patient-derived stem cells to study the early events of human brain development. Proof-of-principle studies using human pluripotent stem cell-derived three-dimensional organoid cultures have allowed researchers to model human brain development and microcephaly in a dish (Lancaster et al. 2013). These "cerebral organoids" develop various discrete brain regions including a cerebral cortex that produces functional cortical neuron subtypes capable of displaying spontaneous synaptic transmission and producing action potentials. Subsequent studies have also shown it is possible to develop region-specific identities including the neocortex (Kadoshima et al. 2013), telencephalon (Watanabe et al. 2005), cerebellum (Muguruma et al. 2015), neural tube (Ranga et al. 2016), pituitary (Davis et al. 2016), hippocampus (Sakaguchi et al. 2015), optic cup (Eiraku and Sasai 2011), and retina (Kuwahara et al. 2015). Through altering specific culture conditions, it is possible to differentiate iPSC and ESC into a range of neuronal (Tao et al. 2016) and glial subtypes including GABAergic interneurons and glutamatergic neurons (Bartolini et al. 2013; Marin and Muller 2014), dopaminergic neurons (Perrier et al. 2004), motoneuron (Li et al. 2005), and hippocampal and glial progenitors (Tabata 2015; Wang et al. 2013).

Most protocols adopted to generate cerebral organoids depend on stepwise establishment of spatio-temporal strategies using human ESC or iPSC (see Fig. 2). The first stage depends on the reaggregation of iPSCs or ESCs in low-adhesion conditions such as those provided by serum-free embryoid body (EB) protocols, allowing the cells enough time to proliferate and expand (Eiraku et al. 2008; Parr et al. 2017). During this initial stage, the stem cells maintain pluripotency, and the EBs that form exhibit all three germ layers (ectoderm, mesoderm, and endoderm). The next stage involves neural induction where the goal is to drive differentiation to neuroectoderm formation. During these early stages, the initial organoids formed display apical-basal and dorsal-ventral polarities, and further induction can promote regional identity such that it is possible to produce region-specific organoids (Birey et al. 2017). The human cerebral cortex is a well-defined structure with six layers of neurons: superficial and deeper layers are connected to one another yet have distinct structural and functional projections and fates (Anderson and Vanderhaeghen 2014; van den Ameele et al. 2014). One of the greatest challenges in development of the human cerebral cortex is the assembly of circuits composed of glutamatergic neurons, generated in the dorsal forebrain (pallium), and GABAergic interneurons arising in the ventral forebrain (subpallium). However, it has recently been shown for the first time using a 3D differentiation approach using hiPSC to specify neural spheroids and assemble these in vitro to model salutatory migration of human interneurons towards the cerebral cortex and functionally integrate into microcircuits (Birey et al. 2017).

Organoid cultures are however not without limitations: spontaneous self-organisation of cerebral organoids in culture generates significant heterogeneity in cell type and structure, with prolonged neotany in development and differentiation limiting their utility to early studies of brain development. There are also challenges with scalability. However, with modifications to culture systems such as the use of

mini-reactors (Qian et al. 2016) and microfluidics (Vadivelu et al. 2017) combined with improved seeding technologies (e.g. laminin-coated nanoparticles) (Lancaster et al. 2016), it is possible to scale up, improve consistency and robustness, and reduce associated costs plus provide higher throughput for drug screening, etc. The size of the organoids produced is not uniform, and without their own blood supply, the core of the organoid frequently starts to die due to starvation of nutrients relying on diffusion instead. The thickness of the organoid tissue also hampers live imaging and time-lapsed microscopy of the dynamic processes of cell proliferation and migration as the cortex develops. However, it has recently been shown that modifications in the culture conditions that limit the growth of the organoid tissue in the vertical axis produce round and flat "pitta"-shaped organoids with a thin space in the middle that remains well perfused and allows the growth processes to be monitored in real time such as neuronal migration and cytoskeletal organisation (Karzbrun et al. 2018). Furthermore, by allowing individual organoids to be followed in real time in a more refined, homogeneous, scaled system, it might also be possible to increase the throughput of this so-called "brain on a chip" design. In this regard, iPSC-derived 2D and 3D model systems hold potential in the future to screen potential drug targets for pharmaceutical development (Fig. 2).

Fundamentally, however, it is a human in vitro system, and as such in vivo connectivity and external milieu are not preserved; thus findings may not precisely translate to in vivo biology experienced during human feotal brain development. However, recent transplantation experiments of cerebral organoids derived from hiPSC into the mouse brain have overcome some of the limitations of in vitro systems by allowing them to become vascularised and survive longer (Mansour et al. 2018), with the promise of being able to permit the maturation and incorporation of transplanted organoid neurons into the host and potentially also in the future bringing about changes in behaviour such as cognition (Lancaster 2018). It is also important to remember that the human brain develops both in utero and during the postnatal period in an environment with inputs via sensory systems as well as from neighbouring brain areas which collectively helps to shape the cellular environment and circuits that develop. Obviously, in vitro culture systems cannot recapitulate this degree of complexity other than ambient fluctuations in temperature, pH, and chemical gradients. Furthermore, the lack of neuromodulatory inputs to synaptic function may preclude our ability to precisely study the effects of systems such as the monoaminergic system in neuropsychiatric disorders and limit their utility in drug development. Another major drawback of organoids to model neurodevelopmental disorders has been the unanswered questions as to what extent they truly modelled regional complexity, cellular diversity, and circuit functionality of the brain. Gene expression analysis in over 80,000 individual cells isolated from 31 human brain organoids has shown that organoids generate a broad diversity of cells, which are related to endogenous classes, including cells from the cerebral cortex and retina (Quadrato et al. 2017). Some caution should be held however as to the relative quantities of the different cell types generated in these organoid systems and to what extent this reflects the quantities in the human developing embryonic and feotal brain. In Quadrato et al.'s study (2017), only two of ten cell clusters

analysed were found to contain neurons from the cerebral cortex, accounting for approximately 20% of cells examined, somewhat less than what might be expected in vivo. Furthermore, these two cell clusters were found in only 32 and 52% of all organoids examined, and within these populations approximately half of the cells expressed the radial glial marker PAX6 after 6 months, reflecting that they could not truly be classified as wholly mature neurons. However, this study has allayed fears that organoid cultures are limited by immaturity as a proportion of the cells do appear more mature than has been seen previously in culture. This team also elegantly demonstrated that neuronal activity, within the organoid, could be controlled using light stimulation of photosensitive cells which provides further opportunity for the coupled use of optogenetics to probe the functionality of human neuronal circuits and specifically model higher-order functions of the human brain, such as cellular interactions and neural circuit dysfunctions related to neurodevelopmental and neuropsychiatric pathologies.

7 Investigating the Role of Glia in Neuropsychiatric Disorder Using hiPSCs

Unlike in autism, schizophrenia psychosis can be thought of as a neurodevelopmental disorder with psychosis as a late stage of illness even though several population-based studies indicate that the problems are evident much earlier (Insel 2010). In this model of schizophrenia, Insel proposes that reduced myelination could alter connectivity in schizophrenia. There are multiple studies showing white matter changes in schizophrenia [reviewed in Davis et al. (2003) and Kubicki et al. (2005) and specifically in the DISC1 family (Whalley et al. 2015)]. It will be possible using hiPSC from families with rare highly penetrant mutations, such as the DISC1 family and those carrying disease-associated CNVs, to generate oligodendrocytes and astrocytes to study the impact of glia on the pathophysiology. One such recent elegant study from Steven Goldman's group generated glial precursor cells (GPCs) from hiPSC from patients with childhood-onset schizophrenia and showed that after neonatal transplantation into myelin-deficient shiverer mice, the GPCs derived from patients with schizophrenia prematurely migrated into the cortex, leading to reduced white matter expansion and hypomyelination relative to controls (Windrem et al. 2017). Furthermore, when established in myelin wild-type hosts, schizophrenia glial mice demonstrated reduced prepulse inhibition and abnormal behaviour, including excessive anxiety, antisocial traits, and disturbed sleep (Windrem et al. 2017). Insel also argues that the trajectory of cognitive development in children developing schizophrenia could include reduced elaboration of inhibitory pathways and excessive pruning of excitatory pathways leading to an altered excitatory-inhibitory balance in the prefrontal cortex. In this regard it will also be interesting to now use hiPSC-derived GABAergic interneurons from the DISC1 families to specifically look for deficits in inhibitory interneuron activity and NMDA

receptor expression. Protocols are now available to generate GABAergic inhibitory interneurons from hiPSCs (Liu et al. 2013) which can be matured in culture to generated PV^+ interneurons for the electrophysiological study of these cell types in vitro. Furthermore, as discussed earlier, Birey et al. (2017) have recently generated 3D spheroids from hiPSC that resemble either the dorsal or ventral forebrain and contain cortical glutamatergic or GABAergic neurons (Birey et al. 2017). This is a seminal study as it demonstrates for the first time that it is now possible to generate organoids/spheroids with network activity: these subdomain-specific forebrain spheroids can be assembled in vitro to recapitulate the salutatory migration of interneurons observed in the foetal forebrain. These protocols will permit the generation and studies of human forebrain spheroids from hiPSCs from patients with other psychiatric disease-associated mutations.

Despite the importance of studying neurodevelopmental disorders and because data from human embryonic tissue is scarce, there is a real challenge of finding an adequate model system. Rodent models have been heavily used to study the cellular function of several risk-associated genes thought to play an important role in corticogenesis such as the gene *NDE1*. However, cortex development and organisation are very different in animal models and humans. In particular, the outer subventricular zone (OSVZ) which is only present to a limited degree in rodents is populated by a unique stem cell subset termed outer radial glia (oRG) (Fietz et al. 2010; Hansen et al. 2010) that allows for the striking expansion in neuronal output and brain size seen in humans. Therefore, it is not surprising that neurodevelopmental diseases cannot be consistently recapitulated in animal models. However, as discussed earlier, the beauty and utility of hiPSC-derived cerebral organoid will present a wealth of new possibilities to thoroughly study the role of *NDE1* and other genes implicated in neuropsychiatric disorders in cellular proliferation, migration, and differentiation, in real time, in the human cerebral cortex and allow the interrogation of genetic risk factors hypothesised to play important roles in human corticogenesis.

8 Conclusion

The remarkable complexity of the genetic architecture of psychiatric disorders poses formidable challenges for clinicians and scientists aiming to find methods to diagnose, subclassify, prevent, and treat what were until recently considered incurable. Over the last 10 years however a quiet revolution has been in progress: our understanding of key molecular pathways associated with schizophrenia, autism, and affective disorders has increased in leaps and bounds as have methods for modelling these disorders in animals; this has been paralleled by the staggering opportunities presented by hiPSC technologies, especially when combined with CRISPR editing, 3D organoid development, and engraftment of in vitro technologies on to in vivo models; many instances now exist where certain molecular changes of human neurodevelopmental and neuropsychiatric phenotypes can be arrested and/or

reversed at least in non-human animal models and in vitro hiPSC studies making this one of the most exciting areas of current psychiatric research.

Acknowledgements MJ is funded by a Wellcome Trust Clinical postdoctoral research fellowship, The Sackler Foundation, The RS Macdonald Trust, and The Simons Initiative for the Developing Brain. RFH is a Wellcome Trust-funded translational neuroscience PhD student.

Disclosures The authors declare that there is no conflict of interest.

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Neuroimmune Biomarkers in Mental Illness

James W. Herron, Louis Nerurkar, and Jonathan Cavanagh

Contents

Abstract Exploration of neuroimmune mechanisms is vital to the understanding of the pathogenesis and pathophysiology of mental disorders. Inflammatory and immune mechanisms are increasingly understood to underpin a number of neuropsychiatric disorders, with an ever-expanding evidence base drawn from basic science to large-scale epidemiological data. Unravelling of these mechanisms should lead to biomarker discovery and potential new avenues for therapeutics that modulate immunological mechanisms. Identification of neuroimmune biomarkers is vital to improving diagnosis, stratification and treatment of mental disorders. There is an urgent clinical need for

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Curr Topics Behav Neurosci (2018) 40: 45–78
DOI 10.1007/7854_2018_45
Published Online: 6 April 2018

new therapeutic approaches with poor treatment response and treatment resistance a major problem for many psychiatric disorders including depression and schizophrenia. Neurodegenerative psychiatric disorders such as Alzheimer's also have clear neuroimmune underpinnings and manifest an urgent clinical need for improvements in diagnosis and research towards transformative disease-modifying treatments. This chapter provides some background on the role of the neuroimmune system in mental illness, exploring the role for biomarkers, in addition to reviewing the current state of knowledge in this exciting field. We also reflect on the inherent challenges and methodological pitfalls faced by research in this field, including the complexity of conceptualising multidimensional mental disorders and the dynamic shifting sands of the immune system.

Keywords BioMarkers · Chemokine · Cytokine · Diagnosis · Immune system · Inflammation · Mental disorders

1 Introduction

As yet few if any biomarkers have broken through into clinical psychiatric practice. This is in the context of a backdrop in which biomarkers have made immense strides in other areas and are considered essential to modern medical practice. There has been a relative paucity of advances across all areas of psychiatric scientific understanding, therapeutics and practice, in spite of significant advances in the field of neuroscience. Since the advent of the SSRI in the late 1980s and early 1990s, we are still waiting for the next major therapeutic breakthrough.

In many respects the challenges are understandable. The physiological processes of the brain, how they relate to behaviour and change in illness remain in large part to be deciphered. When exploring the biology of mental disorders, one must grapple both with the complexity of the brain, in addition to the myriad complexity of the vast and varied aetiological processes that give rise to mental illness.

Biomarker discovery is important. There is an urgent need for peripherally accessible mechanistically specific biomarkers. The current diagnostic systems leave much to be desired – we currently diagnose mental disorder on the basis of symptom clusters and observation of behaviour as opposed to molecular pathophysiology. In 2008 the National Institute of Mental Health proposed a new way of classifying mental disorder in a way that bridges genetics, neuroscience and behavioural science – the Research Domain Criteria (RDoC). The RDoC integrates many levels of information and from molecular to self-report and aspires to inform our understanding of the pathophysiology of mental disorders (Insel et al. 2010). Central to this effort is the discovery of biomarkers, in particular at the molecular and cellular level.

We need better understanding of the pathogenesis and pathophysiological processes that underpin mental illness. Traditional diagnostic methods lack reliability. We have few if any ways of predicting prognosis and response to treatment. Biomarker research also provides an avenue for discovery of novel therapeutic targets.

Biomarker discovery is also essential for underpinning the development of 'precision medicine' therapeutics (Collins and Varmus 2015).

It has been recognised that the complexity of the brain and mental illness necessitates an expansive multifaceted approach involving systems biology, bioinformatics, genetics, imaging, high-throughput molecular omics data, animal models, in vitro and in vivo tissue cultures and in silico models (Kobeissy et al. 2012).

There is an exciting and expanding evidence base for immunological mechanisms of mood, cognition and behavioural disorders. There has been increasing interest in linking mental disorders with 'stress' biology and establishing a common pathway potentially ameliorable by novel therapeutic approaches.

We know that there is extensive communication between the brain and the immune system, and it appears that dysregulated immune function is key to the development of mental disorder, in at least a subset of patients if not more broadly (Gibney and Drexhage 2013). Several recent reviews have focussed on the role of the neuroimmune mechanisms in a number of disorders (Hodes et al. 2015; Tomasik et al. 2016; Van Eldik et al. 2016). The neuroimmune system is an area in which our understanding is evolving and may provide rich ground for biomarker discovery and future therapeutics.

Here we present a review of the current understanding of neuroimmune biomarkers in mental illness and discuss the methodological challenges and areas for future research development.

2 Biomarkers

Biomarkers are defined as 'a measurable indicator of normal biological process, pathogenic process or biological response to a therapeutic intervention' (Downing 2001).

To be truly transformative, biomarkers should have a mechanistic focus, enabling greater understanding of the disorder and thereby allowing developments in targeted therapeutics (Pine and Leibenluft 2015).

Our proposed 'ideal biomarker' properties are listed below:

- Should reflect some basic pathophysiological process.
- Pathophysiological process should be specific to the disease.
- Should ideally not reflect purely clinical symptoms or secondary consequences of the disease state.
- Should be reliably reproducible.
- Show good sensitivity, specificity and predictive value.
- Practical to use – i.e. peripherally accessible, easy to perform, preferably non-invasive and relatively cheap/cost-effective. Should not cause harm to individual, e.g. brain biopsy.

For clarity and precision, it is useful to categorise biomarkers into distinct subtypes. Davis et al. (2015) proposed six distinct categories of biomarker for the study of neuropsychiatric disorders (Davis et al. 2015):

- Biomarkers of risk – indicate a quantitative index of an individual's predisposition to developing a particular disorder.
- Diagnostic or trait biomarkers – measurable characteristics reflecting the presence of disorder and are both sensitive and specific.
- State or acuity biomarkers – biomarkers that are reflective of severity of pathological process. For example, Kapczinski et al. (2010) found particular neuroimmune signatures including inflammatory markers could be interpreted to differentiate between individuals in a manic state from those who were euthymic among patients with bipolar disorder (Kapczinski et al. 2010).
- Stage biomarkers – these would be particularly relevant in neurodegenerative psychiatric disorders such as the dementias.
- Treatment response biomarkers – used to index the probability of response to a particular treatment. Important for the stratification of patients according to effective treatments and precision medicine.
- Prognostic biomarkers – give a prediction of the likely course of illness. In terms of neuroimmune system, there is evidence that elevated levels of interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-α) are associated with poor response to antidepressant therapy and increase risk of treatment resistance (O'Brien et al. 2007).

The challenges to establishing biomarkers in psychiatry in general, not least neuroimmune, are considerable. Pathological features of mental illness are highly variable in their expression from one individual to another and lack specificity to particular disorders.

The immune system is highly dynamic and sensitive to change. This can make it very difficult to obtain consistent and reproducible associations and findings, with research into neuroimmune changes in mental disorder often providing at times contradictory results. There are a plethora of variables that can alter immune readouts, including environmental influences, temporal/stage variations, effects of psychopharmacological interventions, genetic/epigenetic factors, comorbid illness and other individual patient factors to name but a few.

3 Neuroimmune System

Study of the neuroimmune system exists at the interface of neuroscience and immunology and is of interest to scientists in both fields, in addition to psychiatrists and clinicians with an interest in the brain. The peripheral immune system and brain were once thought to be quite separate with minimal interaction. We now know this to be false, and our understanding of how the nervous system and immune system interact is rapidly developing.

It was traditionally held that a number of factors limited neuroimmune communication. The brain is protected from circulating pathogens and immune cells by structural barriers including the blood-brain barrier (BBB). Certain characteristics peculiar to the central nervous system (CNS) were also thought to support this

phenomena – absence of antigen-presenting cells (APCs), low expression of major histocompatibility complex (MHC) class I and II expression and the supposed absence of typical lymphatic drainage from the CNS (Louveau et al. 2015a). However, in recent years, a much more dynamic picture has emerged indicating that the brain does not operate in immunological isolation but rather exists in constant communication with the periphery and is highly immunologically active with its own repertoire of complex innate immune responses (Lampron et al. 2013).

Within the CNS microglia play an important role in the neuroimmune system acting as resident macrophages and are vital for regulating neuronal proliferation and differentiation (Ginhoux et al. 2013). Microglia are the principal producers of inflammatory mediators, including cytokines, within the CNS (Hanisch 2002). Microglia also play a role in synaptic pruning, synaptogenesis, monitoring synaptic integrity and neuronal apoptosis (Wake et al. 2011). Astrocytes are another central player in the neuroimmune system within the CNS, regulating the blood-brain barrier in addition to being thought to play a role in immune responses, via chemokine and cytokine release, to influence effector cells (Jensen et al. 2013).

A number of discoveries have challenged the traditionally held concept of immune privilege and informed our understanding of neuroimmune communication, and the concept of immune privilege is being redefined. It has been demonstrated using laboratory animals that brain extracellular fluid containing CNS-derived antigens drains across arachnoid villi and into lymphatics draining to deep cervical lymph nodes (Cserr et al. 1992). More recently the function of these lymphatic systems has been delineated. A functional lymphatic system located within the meninges and draining to the deep cervical nodes is now known to carry both fluid and immune cells from the CNS (Louveau et al. 2015b). This finding is contrary to the long-held belief that there was no lymphatic system serving the CNS – a major pillar of its supposed immune privilege.

We know from a range of evidence that the peripheral immune system can influence brain function and behaviour. Cytokines and chemokines and their receptors are expressed both within the CNS and the periphery. Peripheral cytokines can either cross the blood-brain barrier or signal across it by a number of mechanisms (Capuron and Miller 2011):

- Humoral pathway – pro-inflammatory cytokines released by activated peripheral immune cells can cross the BBB and access the brain via fenestrated regions including choroid plexus and circumventricular organs.
- Neural pathway – activated peripheral immune cells release pro-inflammatory cytokines that stimulate afferent fibres of the vagus nerve which relay signals back to the brain.
- Cellular pathway – pro-inflammatory cytokines such as TNF-α can stimulate the brain's innate immune cells such as microglia to produce chemokines which can recruit peripheral immune cells to the brain.

Cytokines can directly affect a number of pathways linked to mental disorder including the hypothalamic-pituitary-adrenal (HPA) axis, hippocampal neurogenesis and neurotransmission including monoaminergic and glutamatergic pathways

(Krishnadas and Cavanagh 2012). The role of cytokines such as interleukin 1-beta (IL-1β), IL-6 and TNF-α in so-called 'sickness behaviour' is well established (Konsman et al. 2002; McCusker and Kelley 2013), and chronic dysregulation of this system is implicated in a number of mental health disorders (Gibney and Drexhage 2013).

Prinz and Priller (2017) recently reviewed the role of peripheral immune cells including monocytes, neutrophils, T cells and B cells which are thought to cross the BBB under pathological conditions and contribute to disease progression in a number of neuropsychiatric disorders (Prinz and Priller 2017). These are thought to traverse the BBB in a similar manner to signalling proteins – either by traversing a leaky inflamed BBB or by recruitment and active transport. The permeability of the BBB is dynamic and is modulated by cytokines and chemokines.

Well-balanced and regulated neuroimmune function is essential for normal and efficient brain functioning. However, when pathological dysregulation of this system becomes established, it can initiate cascade processes which lead to neurobiological sequelae and can manifest in psychiatric illness as discussed later in the chapter.

4 Summary of Neuroimmune Mechanisms and Potential Neuroimmune Biomarkers in Specific Mental Disorders

4.1 Depression

Depression represents the single greatest burden of disease in developed countries (Mathers and Loncar 2006) and is among the leading causes of global disease burden worldwide (Whiteford et al. 2013). Among the challenges in treating depression are the fact that up to 60% of sufferers will experience treatment resistance to a degree which prolongs or worsens their illness (Fava 2003). In this context biomarkers could lead both to new therapeutic targets in addition to having a role in stratifying patients into more homogeneous subgroups leading to more precise and effective treatments.

4.1.1 Neuroimmune Features of Depression

Among the earliest indicators that depression may be an inflammatory disorder arose as a result of studies which examined psychiatric complications in patients treated for hepatitis C infection with interferon-alpha (IFN-α) therapy, a subset of whom went on to develop a depressive illness (Renault et al. 1987). A review by Sockalingam in 2011 reported the prevalence of IFN-α-induced depression was in the range of 10–40% on the basis of nine prospective studies (Sockalingam et al. 2011). More recently Chiu et al. (2017) reported on the long-term recurrence of depression after IFN-α treatment and found that IFN-α-induced depression was associated with increased risks of

recurrent depression (Chiu et al. 2017). These findings may suggest that IFN-α in some way primes or sensitises some individuals to future depressed mood states in the longer term. In 1991 Smith proposed the 'macrophage theory' of depression based on the observation that IL-6 and IL-1β can induce depression in nondepressed subjects (Smith 1991). It has also long been noted that rates of depression are significantly higher among individuals with chronic inflammatory medical conditions, both in disorders primarily affecting the CNS such as multiple sclerosis (Lo Fermo et al. 2010) and those affecting the periphery such as rheumatoid arthritis (Dickens et al. 2002) and inflammatory bowel disease (Graff et al. 2009). The body of evidence that has since been established linking depression to inflammation and disordered immune function is substantial.

Significant alterations in serum and CSF levels of pro-inflammatory mediators such as cytokines and chemokines and acute-phase proteins have been consistently demonstrated in the literature. Systemic inflammation is known to provoke 'sickness behaviour', a syndrome which shares significant overlap with the behavioural manifestations of depression including disturbed sleep and appetite, lethargy and malaise (Dantzer et al. 2008). These phenomena are notable across mammalian species and are likely to convey some evolutionary advantage. Injecting rodents with lipopolysaccharide (LPS) promotes cytokine release, provokes 'sickness behaviour' and activates the HPA axis (Bluthé et al. 1992). The effects of LPS are mediated via toll-like receptors (TLRs) to trigger a cascade leading to increased expression and release of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α (Akira and Takeda 2004). In humans, administration of typhoid vaccination which causes a similar acute inflammatory stimulus has been shown to cause a transient depressed mood state (Strike et al. 2004).

Strong evidence supports the fact that patients who suffer from depression have elevated levels of multiple inflammatory cytokines including TNF-α, IL-6 (Dowlati et al. 2010) and IL-1β (Maes et al. 1997a). Depression is also associated with leucocytosis (Maes et al. 1992). Meta-analysis has also found there to be a significant association between depression and elevated levels of the chemokine monocyte chemoattractant protein-1 (MCP-1 a.k.a. CCL2) (Eyre et al. 2016). Howren et al. (2009) demonstrated by meta-analysis that not only was there an association between inflammatory markers with depression but also that there was a dose-response relationship (Howren et al. 2009). A possible causative role for peripheral inflammation in the development of depression is further supported by the recent finding that inflammation predates depression, in a study which showed that children with elevated IL-6 levels at age 9 had a 10% increased risk of developing major depressive disorder in comparison to controls (Khandaker et al. 2014).

C-reactive protein (CRP), a major non-specific acute-phase protein, is reliably found to be elevated in association with depression (Haapakoski et al. 2015). CRP is routinely and cheaply measurable in peripheral blood samples. Studies using CRP to differentiate depressed patients have provided some indications that baseline CRP levels may predict likelihood of treatment response, though the results have shown some inconsistency – Harley et al. (2010) reported high CRP predicting poor response to psychological therapy but good response to nortriptyline or fluoxetine (Harley et al. 2010),

whilst Uher et al. (2014) found the opposite effect for escitalopram (Uher et al. 2014). Overall, composite measures of inflammatory markers appear to indicate that non-response to treatment is more likely when these are elevated (Strawbridge et al. 2015).

Interestingly, treatment with antidepressant medication appears to be associated with subsequent reductions in inflammatory markers including IL-6, IL-1β, IL-10 and CRP (Strawbridge et al. 2015; Hannestad et al. 2011; Hiles et al. 2012).

How disordered inflammatory signalling may mechanistically cause depressive symptoms is not fully understood. It is hypothesised that cytokines may stimulate changes in brain structure and function, resulting in depressive symptoms. As discussed above cytokine release can originate within the brain (e.g. from microglia) or can access or influence the brain from the periphery, by either traversing the BBB or via vagus neural feedback. A proposed summary of how cytokines may mediate mechanisms of depression is illustrated in Fig. 1.

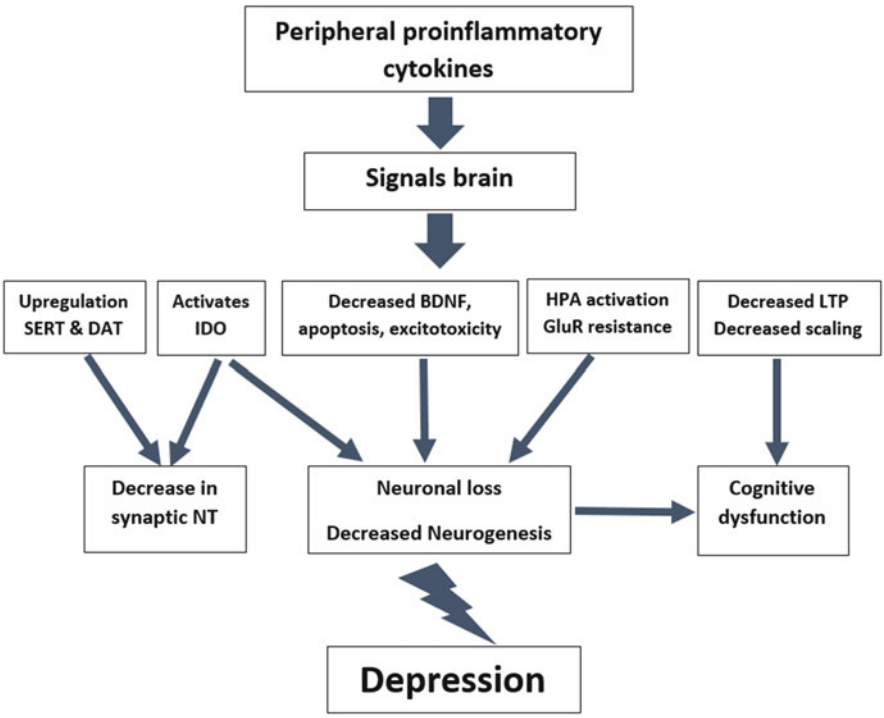

Fig. 1 Cytokine-mediated mechanisms of depression. Peripheral inflammatory signalling to the brain can result in a number of pathological mechanisms being activated. There is a decrease in synaptic monoaminergic neurotransmitter availability as a result of upregulation of serotonin transporter (SERT) and dopamine transporter (DAT), in addition to activation of indoleamine 2,3-dioxygenase (IDO) which depletes tryptophan, a necessary substrate for serotonin (Christmas et al. 2011). IDO activation also produces toxic metabolites which along with reduced BDNF, propensity to apoptosis and excitotoxicity, HPA axis activation and glutamate receptor (GluR) resistance result in neuronal loss and disrupted neurogenesis

The HPA axis plays a pivotal role in the stress response, and abnormalities of the HPA axis are understood to be associated with depression (Pariante 2003). Inflammatory cytokines including TNF-α may induce glucocorticoid receptor resistance and thereby induce HPA axis overactivity (Pace et al. 2007). Pro-inflammatory cytokines are also thought to affect monoaminergic neurotransmission, with evidence that they influence their synthesis and reuptake (Christmas et al. 2011; Cavanagh et al. 2010). Dysregulated glutamate neurotransmission and associated neurotoxicity is understood to be implicated in a number of psychiatric disorders including depression (Lee et al. 2002). Inflammatory mediators have been shown to be associated with this process by (1) upregulation and augmentation of N-methyl-D-aspartate (NMDA) receptors (Bernardino et al. 2005), (2) increased release and inhibited reuptake of glutamate (Hu et al. 2000), (3) alteration of AMPA receptors to facilitate calcium influx and sensitise to excitotoxicity (Stellwagen et al. 2005) and (4) activation of the kynurenine pathway (Maes 2008).

Hippocampal neurogenesis is important in learning and memory and understood to be abnormal in depression. Enhanced neurogenesis has been shown to be associated with antidepressant treatment response (Sahay and Hen 2007), whilst a significant body of evidence, in part derived from animal models, suggests that inflammation attenuates neurogenesis (Monje et al. 2003; Kaneko et al. 2006; Iosif et al. 2006).

4.1.2 Immunomodulatory Treatments in Depression

Exploration of the immunological processes in depression has led to the trialling of various immunomodulatory drugs which have shown to be efficacious in the treatment of depressive symptoms. Infliximab, an anti-TNF-α antibody usually used in the treatment of autoimmune inflammatory conditions, has been shown to have an effect on reducing depressive symptoms in individuals who were resistant to standard antidepressant therapies and who had raised inflammatory markers at baseline (Raison et al. 2013). An improvement in depressive symptoms in individuals with psoriasis treated with etanercept has also been reported, with mood improvements being shown to be independent of improvements in psoriatic symptoms (Tyring et al. 2006). There is also interesting evidence to suggest efficacy of ketamine in treatment-resistant depression (Murrough et al. 2013). Ketamine is known to have significant anti-inflammatory effects and antagonises the NMDA receptor. Further discussion of the potential mechanisms of ketamine as an antidepressant is highlighted by Slaney et al. (this volume).

Evidence for tackling inflammation more broadly in order to treat depression is somewhat inconsistent, however, with evidence from animal studies and statistical analysis of the large-scale STAR-D study showing that anti-inflammatory treatment, given in combination with standard antidepressant therapy, can have the effect of attenuating rather than enhancing response (Warner-Schmidt et al. 2011).

4.1.3 Neuroimmune Biomarkers of Depression

Translating what we understand about the neuroimmune mechanisms of depression into tangible and clinically useful biomarkers and therapeutics poses a significant challenge. Depression is a highly heterogeneous condition, and the complex aetiopathogenesis, including neuroimmune mechanisms, remains incompletely delineated.

Although the evidence of immunological abnormalities is convincing, it is important to bear in mind that inflammation is neither necessary for the development of depression nor is it sufficient to be the sole aetiological mechanism. It should also be remembered that many of the inflammatory and immunological phenomena implicated are not specific to depression, with similar associations having been found in a range of psychiatric conditions. It is likely that neuroimmune processes and their mediators play a role as either part of the physiological response to depression or act as a trigger of a cascade of events leading ultimately to depressed mood states.

How then to establish biomarkers that are mechanistically valid, specific, reproducible and clinically useful? One way forward may be in the stratification of depressed individuals. The possibility of a 'biomarker panel', including neuroimmune measures, which could be used to predict treatment response has been suggested (Schmidt et al. 2011). It is becoming increasingly clear that there is a definite subset of patients in whom the inflammatory processes are particularly important, and a number of studies have provided evidence to suggest that elevated inflammatory markers may be a marker for poor treatment response or treatment resistance (O'Brien et al. 2007; Lanquillon et al. 2000; Eller et al. 2008).

Review of the inflammatory biomarkers of depression suggests that there are four main observations that can be deduced and supported by good evidence (Strawbridge et al. 2017). Firstly there is strong evidence that pro-inflammatory markers are higher in depression than controls (Haapakoski et al. 2015). Markers of elevated inflammatory response appear to reduce in response to antidepressant treatment (Hiles et al. 2012). An aberrant inflammatory profile appears to be associated with poor treatment response/treatment resistance (Strawbridge et al. 2015). Lastly, some anti-inflammatory treatments appear to reduce depressive symptoms (Köhler et al. 2014).

4.1.4 Summary of Selected Potential Neuroimmune Biomarkers for Depression

A summary of selected potential neuroimmune biomarkers for major depressive disorder is shown in Table 1.

4.2 Schizophrenia

Schizophrenia is probably best conceptualised as a cluster of conditions which share a number of common clinical manifestations and are therefore grouped together as a 'syndrome', such is the heterogeneity of the condition. As in other areas of

Table 1 Selected potential neuroimmune biomarkers for depression

Biomarker	Strength of association	Translation potential	Exemplar references
IL-6	Supported by meta-analysis	Part of diagnostic array or stratification marker; treatment response indicator	Maes et al. (1997a), Haapakoski et al. (2015), Liu et al. (2012) and Dowlati et al. (2010)
CRP	Supported by meta-analysis	Stratification marker	Haapakoski et al. (2015)
TNF-α	Supported by meta-analysis	Stratification marker; treatment response indicator	Strawbridge et al. (2015), Liu et al. (2012) and Dowlati et al. (2010)
IL-1β	Meta-analytical data variable	Stratification marker; treatment response indicator	Kaestner et al. (2005), Farooq et al. (2017) and Haapakoski et al. (2015)
MCP-1/CCL2	Supported by meta-analysis	Part of diagnostic array; stratification marker	Eyre et al. (2016)
IL-8/CXCL8	Meta-analytical data uncertain	Part of diagnostic array; stratification marker	Eyre et al. (2016)
IFN-γ	Meta-analytical data uncertain	Stratification marker	Simon et al. (2008) and Dowlati et al. (2010)
IFN-α, IL-4, IL-5, IL-7, IL-10, IL-12, etc.	Data uncertain/ inconclusive	More investigation required to establish relationships more fully	

psychiatry, the diagnosis is based entirely on clinical symptoms and behavioural observation due to the absence of an established diagnostic biomarker.

Until recently much of the research aiming to understand the pathophysiology of schizophrenia has focussed on the dopaminergic neurotransmitter system, in spite of the fact that many patients fail to recover or suboptimally respond to medications that are aimed at restoring normal function to this neurotransmitter system. Additionally these medications tend to have little or no effect on the 'negative' and cognitive symptoms of the disorder (Leucht et al. 2009). Since the advent of second-generation antipsychotics and clozapine in the 1980s, there has been little in the way of new therapeutic interventions, despite the significant unmet clinical need.

There has been increasing interest in other aspects of the neurobiological changes that are associated with schizophrenia, including the immunological perturbations that can be observed. There has been increased emphasis on better understanding the complex aetiopathogenesis, including the molecular signatures that underpin the disorder, with a view to improving diagnosis, stratification and treatment (Guest et al. 2013).

4.2.1 Neuroimmune Features and Biomarkers in Schizophrenia

There are a number of associations that support the role of a dysregulated immune system in schizophrenia. Autoimmune disorders appear to share a number of genetic

and epidemiological associations (Wright et al. 1996; Benros et al. 2011). This association was further strengthened by Corvin and Morris' (2014) recent review on genome-wide association studies (GWAS) which reported data supporting involvement of the major histocompatibility complex (MHC) in the aetiology of schizophrenia (Corvin and Morris 2014).

The association with prenatal/perinatal infections has long been known and further supports a role for immunological factors (Buka et al. 2002; Brown et al. 2005; Brown 2006; Khandaker et al. 2013). There appears to be an association between maternal inflammatory cytokine abnormalities and future risk of schizophrenia in offspring (Buka et al. 2001).

Autoimmune limbic encephalitis deserves particular consideration. Autoimmune limbic encephalitis occurs as a result of autoantibodies reactive to either NMDA receptors or voltage-gated potassium channel (VGKC) receptors and is characterised by a presentation including acute psychotic symptoms and marked behavioural disturbance (Wandinger et al. 2011). It is highly treatable, particularly if identified early in its course. This disorder is one of the very few neuropsychiatric disorders for which we have relatively reliable diagnostic biomarkers (serum assays to detect anti-NMDA or anti-VGKC antibodies) and can provide a paradigm for how we might investigate and understand the neuroimmune processes in schizophrenia and psychosis.

Within the CNS of individuals with schizophrenia, there is convincing evidence of altered expression of genes involved in immune regulation, in areas including the prefrontal cortex, temporal cortex and hippocampus (Arion et al. 2007; Fillman et al. 2013; Hwang et al. 2013).

A number of studies indicate evidence of immune activation within the CNS of schizophrenia sufferers including elevated expression of neuroinflammatory markers such as TNF-α and microglia marker CD11b (Rao et al. 2013), in addition to post-mortem immunohistochemical studies showing increased microglial density (Radewicz et al. 2000). Microglial activation has also been demonstrated in vivo in studies that utilised positron emission tomography (PET) imaging with radiolabelled ligands targeted to the mitochondrial membrane of microglia (van Berckel et al. 2008; Doorduin et al. 2009).

Studies examining the CSF of patients with schizophrenia have identified changes in various inflammatory cytokine levels suggestive of glial cell activation including IL-1β, IL-6, TNF-α, transforming growth factor-beta (TGF-β), IL-10 and IL-12 (Tomasik et al. 2016).

Quinolinic acid and kynurenic acid, which are markers of glial cell activity, are also known to be elevated in the CSF and are known to activate NMDA receptors (MÜller and Schwarz 2006).

The usefulness of CNS biomarkers can be limited by their accessibility – many are only possible to interrogate post-mortem or by lumbar puncture. However, advances in neuroimaging and novel ligands that can bind to CNS inflammatory targets, such as the PET imaging protocol mentioned above, may offer promise for the future.

A review by Chan et al. (2011) identified over 100 potential peripheral biomarkers for schizophrenia, of which 75 related to immune or inflammatory markers (Chan et al. 2011). Peripheral inflammatory mediators, including cytokines, can either cross the

BBB or indirectly communicate with the CNS by various mechanisms as outlined above, and it may be that the CNS immune alterations that are observed originate from peripheral stimuli and vice versa by processes of neuroimmune communication.

A number of immune markers have been shown to be elevated in peripheral blood including both pro- and anti-inflammatory. These include IL-1β, IL-1RA, IL-6, IL-10, IL-12B, s100B and TNF-α (Tomasik et al. 2016). Peripheral biomarkers can be shown to be either stable trait markers or state markers that may normalise with treatment (Miller et al. 2011).

Though not typically considered a neuroimmune marker, brain-derived neurotrophic factor (BDNF) has been found to be reliably reduced in schizophrenia, particularly in drug-naïve patients (Chan et al. 2011). BDNF is a marker for neurogenesis, a process which is significantly affected by inflammatory mechanisms. The protein S100B is an acidic calcium-binding protein that predominates in the brain but can be detected peripherally and is thought to be an important marker for cellular integrity within the brain, which is raised in CSF and plasma in response to a number of neurological insults (Sindic et al. 1982). S100B is found to be elevated in unmedicated schizophrenia and can reduce in some cases in response to antipsychotic treatment (Rothermundt et al. 2001; Chan et al. 2011).

Peripheral leukocyte landscape has also been found to be altered in schizophrenia. Miller et al.'s (2013) meta-analysis reported increased total lymphocytes, CD3+ T lymphocytes, CD4+ helper T cells and CD4/CD8 ratio in drug-naïve first-episode psychosis (Miller et al. 2013). CD4/CD8 ratio appears to reduce with antipsychotic treatment, and Miller suggested that increased CD4/CD8 ratio may be a state marker for acute psychosis (Miller et al. 2013). The mononuclear phagocyte system has also been implicated in schizophrenia, and changes in inflammatory gene expression in monocytes have been reported (Drexhage et al. 2010).

Various attempts have been made to identify a unique molecular profile associated with immune disturbance in schizophrenia. Herberth et al. (2013) reported nine molecules showing altered expression in first-episode antipsychotic-naïve schizophrenia, including those with roles in endothelial cell function, inflammation, acute-phase response and fibrinolysis pathways (Herberth et al. 2013). There are various possible confounders for the immunological abnormalities that are observed in schizophrenia, including obesity, metabolic syndrome and smoking, all of which are common in schizophrenia and can give rise to low-grade inflammation, in addition to the effects of antipsychotic medication. This is in part what makes studying drug-naïve, first-episode schizophrenia attractive in attempting to measure a 'clean slate'.

For a more detailed discussion of immunological processes in schizophrenia as potential biomarkers, see Notter (this volume).

4.2.2 Immunological Approaches in Treating Schizophrenia

As in depression, inflammatory pathways represent an important potential target for future interventions involving immunomodulatory treatments. Indeed some of our most potent current treatments have been shown to have these effects – clozapine is

known to mediate immunosuppression (Maes et al. 1997b) and attenuate microglial activity (Hu et al. 2012). There is little strong evidence so far to support existing immunomodulatory treatments with meta-analysis failing to show any significant effect of adjunctive celecoxib, minocycline and fatty acids, though aspirin may have some effect on improving PANSS scores (Sommer et al. 2013).

4.2.3 Summary of Selected Potential Biomarkers for Schizophrenia

A summary of potential biomarkers for schizophrenia is summarised in Table 2.

4.3 Bipolar Disorder

In comparison to major depressive disorder and schizophrenia, there is relatively less research data reporting the neuroimmune changes observed in bipolar disorder. The field is complicated by the heterogeneity of the disorder in addition to the temporal variation of the disorder with periods of severe depression and acute mania, each with markedly different clinical presentations and physiological milieu. In spite of this, over the years an increasing body of evidence has been accumulated to support immune and inflammatory processes as integral players in the pathophysiology of bipolar disorder (Goldstein et al. 2009). However, a significant amount of the evidence is based on relatively small samples, has a number of methodological limitations and is at times conflicting. Bipolar disorder is often characterised by initially inaccurate diagnosis and delay in treatment, making biomarkers potentially extremely

Table 2 Selected potential neuroimmune biomarkers for schizophrenia

Biomarker	Strength of association	Translation potential	Exemplar references
BDNF	Supported by meta-analysis	Could inform a diagnostic battery of tests; medication response	Green et al. (2011)
S100B	Supported by meta-analysis	Could inform a diagnostic battery of tests; prognosis/treatment response	Aleksovska et al. (2014)
IL-6	Supported by meta-analysis	Diagnostic array; marker of disease state; medication response	Potvin et al. (2008), Miller et al. (2011) and Upthegrove et al. (2014)
TNF-α	Supported by meta-analysis	As above	Miller et al. (2011) and Upthegrove et al. (2014)
IL-1β	Supported by meta-analysis	As above	Miller et al. (2011) and Upthegrove et al. (2014)
IL-1RA	Supported by systematic review	As above	Potvin et al. (2008)

important in improving timely and appropriate management. There are significant individual variations in response to mood-stabilising treatment and a pressing clinical need for stratification biomarkers.

4.3.1 Neuroimmune Features of Bipolar Disorder

Both depressed and manic mood states in bipolar disorder are associated with detectable immunological read-outs including increased levels of pro-inflammatory cytokines and CRP (Uyanik et al. 2015; Kim et al. 2007). The inflammatory cascade in bipolar disorder is thought to involve IL-1β and TNF-α-mediated activation of nuclear factor-kappa-beta (NFκβ) and subsequent IL-6, IL-8 and interferon-gamma (IFN-γ) activation and thence upregulation of CRP/acute-phase proteins (Berk et al. 2011). Some of these inflammatory changes are found to improve upon treatment (Uyanik et al. 2015). IL-6, for example, has been shown to be associated with manic states, returning to normal levels upon remission (Kim et al. 2007). IL-1 and IL-6 changes correlate with HPA axis disturbances in bipolar disorder, in a way that is also observed in major depression (Knijff et al. 2006; Padmos et al. 2008). The complement system has also been shown to be upregulated in mania (Wadee et al. 2002).

BDNF plays a key role in synaptic plasticity and neurogenesis and is peripherally accessible, crossing the BBB readily (Poo 2001). BDNF has been extensively studied in bipolar disorder and has been shown in systematic review and found to be reduced in both manic and depressed episodes in bipolar and appears to be inversely correlated with manic symptoms severity (Fernandes et al. 2011).

Persistent low-grade inflammatory states are observed even during periods of euthymia (Bai et al. 2014). Breunis et al. (2003) reported elevated circulating T cells and increased expression of IL-2 receptor in bipolar disorder during periods of euthymia in addition to mania/depression (Breunis et al. 2003). Various earlier studies had also detected this increase in IL-2R (Tsai et al. 1999, 2001), but IL-2 levels have been found to be either reduced (Ortiz-Domínguez et al. 2007) or unchanged between mood states (Kim et al. 2007).

Various genetic studies have examined the role of polymorphisms of genes involved in regulation of the immune system, though many have involved relatively low numbers of subjects. However, the findings tend to coalesce around an association with IL-1 and IL-6 genetic polymorphisms (Goldstein et al. 2009).

As in other areas of psychiatric disorders, the relationship between inflammation as a cause and as an effect is complex and not entirely clear. Stress and chronic inflammation may be implicated in the triggering of relapse as in other areas of psychiatry (Barbosa et al. 2014). Similar to other mental disorders, the stress-diathesis and allostatic load hypotheses are particularly relevant to bipolar disorder (Kapczinski et al. 2008). Neuroimmune mechanisms may play an important role in the remodelling and dysfunction of emotional and mood-regulating brain regions which result in increased vulnerability to recurrent episodes.

4.3.2 Neuroimmune Biomarkers in Bipolar Disorder

What would a neuroimmune biomarker for bipolar disorder look like? So far the numerous associations between neuroimmune markers do not point to any reliable or clinically useful biomarker of diagnosis, state, stratification or prognosis. Whilst associations may be statistically significant, this is very different from clinical significance. Certainly there is a need for markers in bipolar disorder. As discussed above, a particular problem in bipolar disorder is inaccurate diagnosis and inappropriate initial management, e.g. bipolar first presenting as depression.

A summary of selected potential biomarkers drawn from the literature is presented in Table 3.

4.4 Alzheimer's Disease

The need for effective biomarkers in Alzheimer's disease (AD) is multifaceted. As in other areas of psychiatry, accurate diagnosis is important. In AD this may be particularly so if disease-modifying therapies come to light. So far the rate of success in clinical trials of therapeutics that target AD has been abysmal, and it is hypothesised that this may in part be as a result of initiating treatments at a stage when so much damage has occurred that it is impossible to slow or reverse the process. By that token, biomarkers that can detect the earliest prodromal stages of the disorder may become particularly important.

4.4.1 Neuroimmune Features of Alzheimer's Disease

Neuroimmune processes play a pivotal role in the pathophysiology of almost all neurodegenerative disorders including AD and Parkinson's disease.

Nevertheless, the understanding of the genetics of Alzheimer's disease, whilst still at a relatively tentative stage, has focused mainly on genes involved in amyloid physiology or apolipoprotein E (ApoE) polymorphism rather than immune mechanisms.

The principle pathological features of Alzheimer's include amyloid-β deposition and plaque formation, aggregation of neurofibrillary tangles of hyperphosphorylated

Table 3 Selected potential neuroimmune biomarkers for bipolar disorder

Biomarker	Strength of association	Translation potential	Exemplar references
BDNF	Supported by meta-analysis	Part of diagnostic battery, can aid distinguishing BD from MDD, monitoring treatment response and disease state	Fernandes et al. (2011, 2015) and Polyakova et al. (2015)
IL-6, TNF-α, IL-2, IL-8/CXCL8	Supported by meta-analysis (variable)	Disease state indicators, treatment response	Modabbernia et al. (2013) and Munkholm et al. (2013a, b)

tau protein, synaptic dysfunction and neuronal loss. These features are all either mediated and modulated by or induce reaction from the neuroimmune system. Cellular responses within the CNS including glial cells are mainly consistent with innate immune responses, whilst evidence is emerging for the role of the peripheral immune system and adaptive immune responses relating to neuroinflammation (Prinz and Priller 2017).

The role of neuroimmune mechanisms and neuroinflammation has long been an area of debate – is immune activation in Alzheimer's disease beneficial, harmful or simply a secondary bystander phenomenon? In truth the picture is complex, which is in part why it has so far defied adequate and complete explanation. For example, the same inflammatory processes may be either adaptive and beneficial or pathological and detrimental at different time points within the disease process in the same individual.

Deposition of extracellular amyloid-β is known to be associated with neuroinflammatory responses including release of cytokines, activation of the complement system and increased levels of acute-phase proteins (Akiyama et al. 2000). Amyloid-β molecules are recognised by toll-like receptors (TLRs) on microglia and astrocytes to be danger-associated molecular patterns (DAMPs), and activation can induce production and release of pro-inflammatory cytokines including TNF-α and IL-6 and chemokines such as IL-8/CXCL8 (Bsibsi et al. 2002). Cytokine release is likely to be a key factor in AD pathogenesis. Microglial activation results in cytokine release, and microglial activity is modulated by cytokines, and it may be that the role of microglia in amyloid deposition and phagocytosis is mediated via these molecules (Meyer-Luehmann and Prinz 2015). Elevated levels of TNF-α, IL-6 and IL-1 have been found in amyloid precursor protein (APP) transgenic mice, and the levels appear to correlate with amyloid load (Patel et al. 2005). Elevated pro-inflammatory cytokine levels have been found in the CSF of human AD samples (Cacabelos et al. 1991; Blum-Degena et al. 1995). APP/PS1 transgenic mice deficient in IL-12 and IL-23 demonstrate reduced amyloid-β plaque burden and reduced behavioural deficits (Vom Berg et al. 2012). The inflammatory cytokine TGF-β1 has been found to be associated with potent microglial activation and reduced plaque burden in transgenic mice (Wyss-Coray et al. 2001).

Microglia tend to be found in close association with amyloid plaques. It has been demonstrated in transgenic mouse model of AD that microglia are found to be enlarged and clustered around plaques suggesting activation around plaques and increased microglial density and size around areas of particular plaque formation such as the hippocampus and frontal lobes (Frautschy et al. 1998). The role of microglia in plaque formation is complex and the evidence has been contradictory. A review by Meyer-Luehmann and Prinz (2015) encapsulated this issue and explored whether myeloid cells were 'Culprits, victims or innocent bystanders?' (Meyer-Luehmann and Prinz 2015). Microglia have been put forward as the 'driving force' in plaque formation, with astrocytes being crucial to plaque degradation (Wegiel et al. 2000), though later evidence appeared to contradict this (Stalder et al. 2001). Microglia have been shown to be rapidly activated and recruited to sites of amyloid-β plaques – within 24–48 h (Meyer-Luehmann et al. 2008) – but are not necessarily involved in plaque clearance. Some evidence suggests that whilst early microglial recruitment may promote amyloid-beta clearance, as the disease progresses, pro-inflammatory cytokines

produced in response to amyloid-beta deposition downregulate genes involved in clearance and thereby promote clearance (Hickman et al. 2008). This suggests that the role of microglia is dichotomous and complex – microglia may be unable to phagocytose amyloid under pathological conditions (Meyer-Luehmann and Prinz 2015).

The fractalkine receptor (CX3CR1) whilst normally promoting phagocytosis of apoptotic cells appears to modulate microglia in such a way as to reduce phagocytosis of fibrillary congophilic amyloid-beta (Liu et al. 2010). Mice deficient in CX3CR1 have been shown to have lower levels of amyloid-β30 and 42 in the brain and reduced amyloid deposits (Liu et al. 2010), suggesting exploration of chemokines and their influence on immune cell-plaque dynamics may be important in the search for new therapeutics. The potentially crucial role for CX3CR1 in amyloid pathology was further inferred by the finding (using two-photon in vivo imaging) of reduced neuron loss in triple-transgenic mice that are CX3CR1-deficient (Fuhrmann et al. 2010). The study found that microglia were recruited prior to neuron loss, not after. Neuronal loss appears to require communication between microglia and neuron via the fractalkine receptor (Fuhrmann et al. 2010). The role of CX3CR1 in non-AD neuroinflammatory disorders is not fully understood – it may have different roles in different conditions. CX3CR1 knockout in a toxic model of PD and a transgenic model of amyotrophic lateral sclerosis (ALS) appears to show increased neuronal cell death, suggesting augmentation of CX3CR1 signalling may be therapeutic and that CX3CR1 antagonists could increase neuronal susceptibility to damage/loss (Cardona et al. 2006). In cerebral ischaemia models, CX3CR1 deficiency appears to reduce neuron loss, limit the size of infarct spread through the penumbra and enhance functional recovery (Dénes et al. 2008). Some evidence has shown that the absence of CX3CR1 does not impair neuronal-glial cell communication and that it may have little effect on modulation of neuron loss (Jung et al. 2000).

Monocyte chemoattractant protein-1 (MCP-1 a.k.a. CCL2) which is the ligand for chemokine receptor-2 (CCR2) plays an important role in the chronic neuroinflammatory process that takes place in the AD brain (Sokolova et al. 2009). CCR2 deficiency has been suggested to accelerate progression of amyloid pathology in AD transgenic mice as a result of impaired amyloid phagocytosis by microglia (El Khoury et al. 2007). CCL2 may be involved in the recruitment of CCR2-expressing myeloid cells into the CNS of AD transgenic mice (Mildner et al. 2011). Recruitment of perivascular macrophages from bone marrow to cerebrovascular sites of amyloid-β deposits has been shown to occur independently of CCR2, but amyloid-β clearance appears to be CCR2-dependent (Mildner et al. 2011).

The complement system is a key cornerstone of immunity and is involved in cytolysis, phagocytosis and potentiating inflammation. Complement can be produced peripherally in the liver but also by glial cells and neurons within the CNS, the production of which is upregulated in brain injury and neurodegeneration (D'Ambrosio et al. 2001). Amyloid-β appears to activate the complement cascade in the AD brain (Shen et al. 2013). Complement opsonins C1q, C3b and iC3b promote phagocytosis, whilst C3a and C5a initiate inflammatory responses that contribute to tissue protection and healing. Inappropriate or dysregulated complement activation is proposed as a mechanism in AD via induction of neuronal apoptosis, release of pro-inflammatory

cytokines and neuronal and synaptic lysis (van Beek et al. 2003). C1q in particular has been shown to be important in mediating the toxic effects of soluble amyloid-β oligomers on synapses, and microglial synaptic pruning is dependent on complement receptor 3 (CR3) (Hong et al. 2016), making modulation of the complement system a potentially attractive therapeutic target.

Attempts at therapeutically targeting immunological mechanisms have proven disappointing. Vaccination trials to induce cellular immunity proved to have no effect on clinical symptoms or disease progression (Kohyama and Matsumoto 2015). Though a meta-analysis of epidemiological studies appeared to show that the long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) was associated with a significantly reduced risk of developing AD (McGeer et al. 1996), subsequent clinical trials proved disappointing (Szekely et al. 2007).

4.4.2 Neuroimmune Biomarkers for AD

Although the neurobiology of AD and neurodegenerative disease is perhaps less heterogeneous and better delineating in comparison to other neuropsychiatric disorders, there remains a significant paucity of tractable mechanistic biomarkers.

CSF sampling by lumbar puncture has been used to investigate potential biomarkers in AD, though the clinical utility of this approach is debatable given the technical challenges that routinely performing lumbar puncture would present in clinical practice in addition to the associated risks and complications.

As discussed above, amyloid-β is thought to be of central importance in the pathophysiology of AD. There is significant evidence to support the hypothesis that amyloid-β levels in CSF may be lower in AD due to reduced clearance and efflux into CSF. Consequently there has been extensive study of its presence within the CSF. Kapaki et al. (2003) examined amyloid-beta 42 levels in the CSF comparing AD to normal ageing and non-AD dementia (Kapaki et al. 2003). They found a 0.5-fold reduced CSF level of amyloid-β42 in CSF of AD patients compared to controls, a finding that was broadly replicated with reasonable sensitivity in further studies (de Jong et al. 2006; Mulder et al. 2010). Later studies examined amyloid-β42/amyloid-β40 ratio as a biomarker and found they could differentiate AD from non-AD with equally effective sensitivity/specificity to amyloid-β42 alone but could reduce the number of indeterminate CSF profiles by approximately 50% (Dumurgier et al. 2015).

Neurofibrillary tangles of tau protein are another hallmark of AD pathology, and tau can be measured in the CSF. Elevated CSF tau levels have been found to be correlated with progression from mild cognitive impairment (MCI) to AD (Hansson et al. 2006), suggesting utility as a prognostic biomarker. Combining CSF measures of amyloid-β and tau has been shown to be promising, with impressive sensitivity, specificity and positive predictive values (in discriminating between AD and controls) (Huynh and Mohan 2017).

Peripheral blood biomarkers are by far the easiest to access. Ray et al. put forward an array of 18 distinct signalling proteins detectable in peripheral blood that could be used to classify AD and controls with up to 90% accuracy (Ray et al. 2007). These

proteins were associated with dysregulation of diverse systems including haematopoiesis, immune responses, apoptosis and neuronal support. Other protein arrays have also shown promise as biomarkers, particularly when combined with markers of genetic risk such as apolipoprotein E4 (ApoE4) allele (Hye et al. 2014).

Cortical amyloid burden is associated with risk of developing AD in addition to disease severity. Amyloid burden can now be detected using PET imaging (Clark et al. 2011). Reduced plasma amyloid-β42/40 ratio appears to be associated with increased brain load (Rembach et al. 2014). Ashton et al. recently reported that measurement of fibrinogen gamma chain (FGG) combined with age can be used to predict amyloid burden with 59% sensitivity and 78% specificity (Ashton et al. 2015).

4.4.3 Summary of Selected Potential Neuroimmune Biomarkers for Alzheimer's Disease

A summary of potential biomarkers is presented in the Table 4.

5 Methodological Approaches to Neuroimmune Biomarker Discovery

Research into neuroimmune mechanisms of mental illness and their biomarkers requires exploitation of a diverse range of methodological approaches from research at the cellular level to big-data epidemiological studies.

Current animal models vary depending on the disorder which is being investigated. A number of animal models exist which display both plausible behavioural and neuroimmune read-outs. Though often termed 'models of depression', it is more accurate to term them as models of behaviour that relate to human depression phenotypes.

Table 4 Selected potential biomarkers for Alzheimer's disease

Biomarker	Strength of association	Translation potential	Exemplar references
CSF: amyloid-β1–42; amyloid-β1–40/1–42 ratio; tau	Supported by meta-analysis (variable)	Diagnostic, staging/disease burden, treatment response, prognosis	Diniz et al. (2008), Mitchell (2009) and Olsson et al. (2016)
Plasma/serum: amyloid-β; amyloid-β peptide ratios	Meta-analysis data equivocal, relationship required further exploration	As above	Olsson et al. (2016), Koyama et al. (2012) and Song et al. (2011)
Amyloid PET imaging	Supported by meta-analysis	As above	Johnson et al. (2013) and Ossenkoppele et al. (2015)
IL-8/CXCL8, TNF-α, TGF-β, IL-1β, IL-6	Meta-analysis suggests relationship (variability)	Diagnosis, stratification	Swardfager et al. (2010)

The ‘chronic mild stress’ model involves exposure of animals to a varied and unpredictable series of stressors over a period of time in a manner which induces a depression-like state (Willner 2005). Mice exposed to this model show behavioural changes including reduced grooming, reduced libido and reduced reward seeking (e.g. sucrose consumption) in addition to inflammatory changes such as increase IL-1β, IL-6 and TNF-α (Krishnan et al. 2008).

Other animal models include ‘learned helplessness’ where animals are either exposed to controllable or uncontrollable stressors such as foot or tail shocks (Pryce et al. 2011). Around 20% of the animals exposed to the uncontrollable shocks develop so-called ‘learned helplessness’ and display behavioural manifestations consistent with depression and an inflammatory cytokine profile (Yang et al. 2015). Given that 80% of exposed mice appear to show resilience to the experiment effects, this model may give interesting insights into molecular variants that bestow this fascinating trait.

The model of ‘repeated social defeat stress’ involves placing an experimental mouse in the cage of a larger, dominant and aggressive mouse for a 10-day period (Golden et al. 2011). Around two-thirds of exposed mice develop depression-like behavioural phenotypes in addition to pro-inflammatory immune profiles including elevated IL-6 (Hodes et al. 2014).

Animal-based research, particularly involving rodent models, remains integral to understanding the essential pathophysiology of mental disorders, including the role of the neuroimmune system, and is likely to do so for the foreseeable future. However, these models are not without significant limitations, not least the inherent difficulty of modelling complex, multidimensional psychopathological syndromes of human cognition, emotion and behaviour in a mouse – an animal that we are separated from by millions of years of mammalian evolution.

Nevertheless, considerable advances are being made in the development of preclinical models with improved translation, over and above the models noted above. In particular models that reflect the cognitive affective bias seen in major depressive disorder are feasible in rodents (see Slaney et al. this volume).

At the human level, proteomics and transcriptomics can be used to detect changes in proteins involved in regulating the immune system. To date, numerous significant associations have been discovered, but none has reached major ‘clinical’ significance. However, machine learning and other pattern recognition approaches to large volumes of data may provide us with arrays or molecular signatures that can be useful in diagnosing and stratifying individuals with mental disorder.

Machine learning and other forms of analysis of various ‘big data’ to expand our understanding of mental illnesses represent an interesting and promising avenue of research (Lin and Lane 2017; Orrù et al. 2012). This can include imaging, genomic, proteomic/transcriptomic and clinical data with a view to identifying disease-specific mechanisms and achieve valid and useful clinical predictions that are translatable to the individual level (McIntyre et al. 2014). The combination of the advent of massive databases that can be readily interrogated along with adequate computing power to handle huge volumes of data is likely to mean that this area of research will intensify.

6 Methodological Challenges and Limitations in Neuroimmune Biomarker Research

Whilst beginning to reveal promising targets, current literature studying neuroimmune biomarkers in mental illness faces several methodological challenges. These include (1) limitations of study design, (2) heterogeneity of patient groups, (3) confounding of immune markers by demographic differences and (4) assay variability.

Studies of neuroimmune biomarkers often use a cross-sectional design; this approach prevents the need for follow-up and allows for the use of archived sample material. In addition, for studies that utilise a post-mortem approach, as is commonly employed in neurodegenerative disease, this may be the only design that is appropriate. However, whilst these practical considerations have merit, this methodology is limited by the fact it provides only a snapshot of a single time point. This means that drawing conclusions on directionality or mechanism is difficult. In contrast, longitudinal studies, whilst not entirely able to overcome this difficulty, begin to provide some evidence that immune markers are functionally linked to disease states. For example, Köhler et al. (2017) in the recent meta-analysis of blood cytokine networks in mood disorders identified a normalisation of several immune markers in response to treatment (Köhler et al. 2017). Goldsmith et al. (2016) looked at this more broadly in their meta-analysis of blood cytokine network alteration in schizophrenia, bipolar disorder and major depression (Goldsmith et al. 2016). Whilst not conclusive, this provides more robust evidence that these molecules may have pathophysiological relevance.

Another challenge of neuroscience biomarker study design is the small sample size many studies employ and the often underpowered nature of studies (Button et al. 2013). Underpowered studies can both find effects where there are none and fail to identify an effect that is present. As the study of neuroimmune biomarkers has grown, a wealth of data has become available that would allow for reasonable estimates of required group sizes to achieve an appropriate level of power. Whilst it may not always be feasible to recruit sufficient participants for a desired power level of 80% or 90%, it would be strongly beneficial for power calculations to be presented and limitations discussed in the absence of sufficient power.

Many of the conditions that are studied in the fields of mental illness and neuroimmunology are heterogeneous pathologies defined more by clinical symptomatology than objective diagnostic criteria. This is particularly true in major depressive disorder, where patients likely have multiple differing aetiologies of disease and studies that have stratified patients on the basis of baseline immune alterations have identified a treatment response in subsets of patients (Subramaniapillai et al. 2017). These findings suggest that in complex neuroimmune diseases, stratification of subjects based on evidence or well-reasoned rationale can aid in identification of both patient subgroups and potential biomarkers.

Studying neuroimmune biomarkers is further complicated by the complex nature of immune confounders. Numerous immune confounders exist and reporting of these factors is often limited. Immune differences based on age (Brüünsgaard and Pedersen 2003), sex (Aulock et al. 2006) and body mass index (Fontana et al. 2007)

are well known, and study reporting of these demographic differences is relatively well established. However such diverse factors including smoking (Sopori 2002), circadian rhythm (Lange et al. 2010), blood pressure (Dzielak 1992) and socioeconomic status (Owen et al. 2003) also appear to influence immune status, and limited reporting of clinical demographics can make assessment of these confounders highly challenging. This renders studies hard to synthesise when this information is lacking. This is particularly true when many of these immune confounders are also associated with mood disorders, making it more likely that differences will exist between patient and control groups.

One question that remains to be definitively answered in the field of neuroimmunology is the relevance of peripheral biomarkers to the central compartment. Whilst post-mortem studies and studies that use imaging approaches may overcome this, many studies examine circulating immune biomarkers. Whilst there is evidence that circulating cytokines gain access to the CNS or convey signalling across the BBB (Banks and Erickson 2010), few studies have compared the peripheral and central compartment in detail. The relevance of these peripheral biomarkers remains equivocal. Moving forward it would be beneficial for studies to attempt to attain samples that may reflect the CNS compartment more accurately, such as by sampling CSF, and where possible, to compare this to alterations in peripheral samples such as plasma or serum. Newer imaging techniques using PET or SPECT imaging of receptors within the CNS or using novel imaging paradigms such as magnetic resonance spectroscopy may also help to overcome these challenges.

Whilst great strides have been taken in the field of neuroimmune biomarker research, various challenges remain to overcome the methodological limitations that are reflected in much of the literature to date. Hopefully the section above has highlighted a number of points for consideration when designing or performing studies of this nature. Whilst highly powered longitudinal studies that can directly measure a central biomarker alongside appropriately matching or adjusting for all possible immune confounders are likely not possible, even simple steps such as attempting to report on clinical demographics associated with changes in immunity can go a long way to providing greater clarity within this field of research.

7 Conclusions

Understanding the role of the neuroimmune system is vital to elaborating the mechanisms of mental illness, identifying biomarkers and exploring new therapeutic approaches. A plethora of potential biomarkers have been implicated by research so far, but as yet their role in the pathophysiology of mental disorders remains to be fully elucidated and clarified.

If we are to unravel the pathophysiology of mental disorders, then significant funding and research commitment is likely to be required. Mental illness represents among the highest disease burden of any medical diagnostic category (Whiteford et al. 2013), and yet funding for research lags significantly behind other areas such as cancer biology or infection (Kingdon and Wykes 2013). One parsimonious potential

solution to this issue which is feasible in neuroimmune research would be to dovetail with existing immune-therapeutics research. Significant sums are invested in research targeting immune mechanisms in a number of physical disorders including cancer and autoimmune diseases. Repurposing of compounds which have already undergone experimentation in human subjects for other conditions may yet yield significant results.

Neuroimmune mechanisms are increasingly seen as an attractive target for therapeutic intervention. In some disorders, for example, dementia and neurodegenerative diseases, modulation of neuroimmune mechanisms may prove transformational. However, in other areas such as in mood disorders or schizophrenia, therapeutics targeting the neuroimmune system are perhaps more likely to provide incremental treatment benefits, e.g. adjunctive treatment for cases in which there is poor response to conventional treatment. Stratification utilising neuroimmune biomarkers may make this sort of targeted treatment feasible.

Key Points

- Discovery of biomarkers is essential for transforming diagnosis and treatment of mental disorders.
- Neuroimmune mechanisms represent an exciting avenue of research in understanding the pathophysiology of mental disorders.
- Neuroimmune biomarkers hold the potential for disease stratification, personalised treatments and may inform development and use of immunologically targeted therapeutics.
- Methodological approaches to neuroimmune biomarker discovery encompass research ranging from molecular studies in animal models to machine learning and data science.
- Methodological limitations include problems with study designs, patient heterogeneity, assay variability and immune confounders.
- Discovery of neuroimmune mechanisms and subsequent design of therapeutic approaches may prove transformative in some areas such as neurodegenerative disorders, whilst in other areas such as mood or psychotic disorders, we may expect incremental improvements in treatment or adjunctive/targeted treatments for patient subsets.
- Inherent challenges include the heterogeneity of psychiatric disorders and the dynamic mutability of the immune system. However, we must not be pessimistic – these challenges are common to biomarker research in much of biomedicine, where great progress has already been made.

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Network Neuroscience: A Framework for Developing Biomarkers in Psychiatry

David M. Lydon-Staley and Danielle S. Bassett

Contents

Abstract Psychiatric disorders are disturbances of cognitive and behavioral processes mediated by the brain. Emerging evidence suggests that accurate biomarkers for psychiatric disorders might benefit from incorporating information regarding multiple brain regions and their interactions with one another, rather than considering local perturbations in brain structure and function alone. Recent advances in the field of applied mathematics generally – and network science specifically – provide a language to capture the complexity of interacting brain regions, and the application of this language to fundamental questions in neuroscience forms the emerging field of network neuroscience. This chapter provides an overview of the use and utility of network neuroscience for building biomarkers in psychiatry. The chapter begins with an overview of the theoretical frameworks and tools that encompass network neuroscience before describing applications of network neuroscience to the

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Curr Topics Behav Neurosci (2018) 40: 79–110
DOI 10.1007/7854_2018_41
Published Online: 6 April 2018

study of schizophrenia and major depressive disorder. With reference to work on genetic, molecular, and environmental correlates of network neuroscience features, the promises and challenges of network neuroscience for providing tools that aid in the diagnosis and the evaluation of treatment response in psychiatric disorders are discussed.

Keywords Cognitive neuroscience · Depression · Graph theory · Network neuroscience · Schizophrenia

1 Introduction

Psychiatric disorders are disturbances of cognitive and behavioral processes mediated by the brain. Biomarkers for such disorders are objective indications of medical state; their clinical utility lies in the potential to diagnose the disorder, to determine its prognosis, and to predict and monitor a patient's response to interventions (Biomarkers Definitions Working Group 2001; Strimbu and Tavel 2010). Promising approaches for the development of biomarkers include noninvasive neuroimaging techniques, which have the capacity to capture the structure and function of the brain in health and disease, without requiring the injection of contrast agents or radiation exposure that may not be particularly well-tolerated by patient populations with mental illness. Historically, such approaches have focused on delineating regions whose anatomy or physiology – as estimated from imaging measurements – is altered in the disease. Yet, emerging evidence suggests that accurate biomarkers might benefit from incorporating information from both imaging and non-imaging modalities regarding not a single region but multiple regions and their interactions with one another. The complexity of building such a biomarker can initially seem quite daunting, largely because it requires the development of a language in which to describe, quantify, and predict multi-region, multimodal, interacting networks.

Recent advances in the field of applied mathematics generally – and network science specifically – have begun to provide just such a language. Their application to fundamental questions in neuroscience forms the emerging field of network neuroscience (Bassett and Sporns 2017). There are several features that render network neuroscience approaches to neuroimaging data exquisitely suited to the task of identifying mental illness (Fornito and Bullmore 2015). First, many psychiatric disorders have a genetic component, with heritability estimates as high as 80–90% observed for some disorders (e.g., schizophrenia and bipolar disorder; Cannon et al. 1998; McGuffin et al. 2003). The association between genetic variants and liability for mental illness is complex, arising from the combined effects of many genes exerting small effects. As such, identifying genetic markers of risk is difficult. Given that genes exert their effects on behavior via their influence on brain regions and their complex patterns of interactions (Esslinger et al. 2009; Richiardi et al. 2015), neuroimaging and network approaches to it provide a way forward by allowing the examination of intermediate phenotypes through which genetic risk for disorder is conferred (Meyer-Lindenberg and Weinberger 2006). Second, healthy brain function depends on complex interactions among distributed brain

regions (Sporns 2014), and psychiatric disorders are conceptualized as dysfunctions in the dynamics across regions of the brain (Friston et al. 2016; Kana et al. 2011; Woodward and Cascio 2015), rather than resulting from pathological perturbations to individual regions (Fornito et al. 2015). Methodological approaches such as network neuroscience, which are capable of capturing aberrant connectivity across regions of the brain, provide a good match to theories of psychiatric disorders.

In this chapter, we provide an overview of the use and utility of network neuroscience for building biomarkers in psychiatry. We begin by describing the fundamentals of the field of network neuroscience. We then turn to a brief review of the application of theoretical frameworks and mathematical tools from network neuroscience to the study of schizophrenia and major depressive disorder. We close by highlighting the value of the network neuroscience approach for understanding the biological underpinnings of psychiatric disorders more generally and the construction of novel biomarkers specifically.

2 Network Neuroscience: A Primer

The emerging field of network neuroscience pursues new ways to map, record, analyze, and model the elements and interactions of neurobiological systems (Bassett and Sporns 2017). Many types of elements and their interactions are examined in network neuroscience, reflecting the multi-scale nature of brain networks (Betzel and Bassett 2017). However, the tools available for observing the brain and its constituent parts place limits on the scales that may be examined. Imaging connectivity approaches often make use of magnetic resonance imaging (MRI) data. From that data, one can construct a graph, which is a simple mathematical representation of a network composed of nodes representing system elements and edges representing element relations or interactions. In imaging-derived networks, the nodes are typically parcels of gray matter voxels, ranging from single voxels to the entire brain. Associations among nodes (edges) may be established in a number of ways, which are typically categorized into structural or functional connectivity approaches.

Structural connectivity approaches aim to understand the network architecture of anatomically connected regions. There are two main approaches available for constructing anatomical networks. Diffusion imaging tractography aims to reconstruct the trajectory of axonal tracts using indices of the diffusion of water molecules within neural fibers (Li et al. 2016; Mukherjee et al. 2008). In this approach, edges reflect estimates of the probability with which a node is physically connected to another via a white matter tract. Structural connectivity may also be established via structural covariance analysis (Mechelli et al. 2005). In this approach, the covariance between morphometric features (e.g., gray matter thickness) of each possible pair of nodes in an anatomical network is estimated. In structural covariance networks, edges represent shared morphometric features between nodes that are thought to indicate physical connectivity of white matter tracts or functional connectivity related to synchronous neural activation between regions (Alexander-Bloch et al.

2013). Functional connectivity, in contrast, can be used to define network edges based on statistical similarities in the time series of nodes at rest or during task performance (Friston 2011). The edges in functional brain networks represent communication or coordination between nodes. With appropriate analytic techniques, causal relations between nodes can also be established. This form of connectivity is typically referred to as effective connectivity (Stephan and Friston 2010).

Once nodes and edges have been estimated, structural and functional connectivity networks are represented with an adjacency matrix **A** (see Fig. 1). For an unweighted and undirected graph, the element A_{ij} indicates the presence (1) or absence (0) of an edge between node i and node j. For a weighted graph, the element A_{ij} takes on a value corresponding to the strength of the association between node i and node j. The adjacency matrix for an undirected graph is symmetric, but in a directed graph, in which the direction of the associations between node i and node j are specified, the adjacency matrix may not be symmetric. In this case, A_{ij} represents the edge weight from node j to node i. This general graph construction can be used to represent a time-invariant network, one that describes network organization across the entire length of the scan, which is one typical object of study in network neuroscience. Yet, more recently the time-varying nature of network organization has been increasingly recognized (Calhoun et al. 2014), and tools with which to examine the changing organization of the brain over time have emerged (Khambhati et al. 2017; Mucha et al. 2010; Sizemore and Bassett 2017). In the case of time-varying networks, multiple adjacency matrices may be constructed, by applying a sliding window across smaller sections of imaging time series to extract a time-ordered graph ensemble, providing the basis for analyses focused on capturing changes in network organization across time (De Domenico 2017).

Following adjacency matrix construction, graph theory is applied to examine the properties of brain networks (for a recent overview, see Fornito et al. 2016). The application of graph theory to imaging data has led to the discovery of fundamental

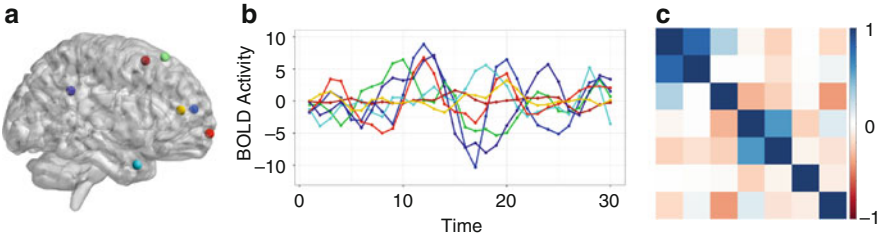

Fig. 1 Panels (**a**) through (**c**) provide an overview of the main steps involved in transforming functional brain data into an adjacency matrix that encodes the associations among brain regions. In panel (**a**), nodes are denoted by colored spheres. Spherical node parcellations are commonly used (e.g., Power et al. 2011) although other options exist (e.g., areal pacellations; Gordon et al. 2014). In panel (**b**), the mean time series of the BOLD response across the length of the scan is depicted for each node of panel (**a**). Edges, or an estimation of the extent or strength of connectivity between nodes, are created by estimating pair-wise correlations (or alternative statistical indices of association, e.g., coherence; Bassett et al. 2011) among the time series of all node pairs. Nodes and edges of the network are parsimoniously represented as an N-by-N adjacency matrix (where N is the number of nodes) in panel (**c**); here color indicates edge strength

organizational features of the brain. Structural and functional brain networks show a small-world architecture, characterized by a combination of high clustering with short characteristic path length (Watts and Strogatz 1998). The clustering coefficient captures the extent to which neighboring nodes of a network tend to be densely interconnected, or *cluster,* together. More formally, clustering in a binary graph indicates the probability that nodes *j* and *k*, which are both connected to node *i*, are also connected to each other (Chalancon et al. 2013). The shortest path length between node *i* and node *j* describes the minimum number of edges that must be traversed to travel from node *i* to node *j* in the graph. The characteristic path length of a network is then defined as the average shortest path lengths across all possible pairs of nodes in the network (Schreiber 2013). Based on the clustering coefficient and the characteristic path length of a network, a network can be likened to a regular graph (high local clustering and long path length), small-world graph (high local clustering and short path length), or random graph (low local clustering and short path length).

Small-world architecture has been observed in human structural and functional brain networks across a number of imaging modalities (for reviews, see Bassett and Bullmore 2006, 2016), as well as across a number of methods for network construction. A few pioneering early examples of such studies examined connectivity patterns of cortical thickness across the cerebral cortex using MRI (He et al. 2007), white matter connection probabilities between gray matter volumes using diffusion-weighted imaging (Iturria-Medina et al. 2008), and functional connectivity at rest (Achard et al. 2006; Salvador et al. 2005) as well as across task conditions (Eguíluz et al. 2005). Small-world architecture, as evidenced by a combination of high clustering and short path length, is thought to confer the capacity for specialized processing in local regions as well as the ability to integrate processes across the entire network, mapping onto the functional segregation and integration thought to enable efficient cognition (Sporns et al. 2004).

In addition to exhibiting small-world characteristics, the brain exhibits community structure, such that the large-scale network of the brain can be decomposed into communities or modules. Modules are made up of nodes with dense connectivity with each other and sparse connectivity with nodes in other modules. Both structural and functional graphs of human brains exhibit modularity (Bassett et al. 2010; Chen et al. 2008; Meunier et al. 2009). Functional connectivity studies, for example, have uncovered multiple functional modules at rest characterized by relatively dense internode connectivity (Nelson et al. 2010; Power et al. 2011). Named to reflect the functions typically associated with the constituent nodes, these functional systems include salience, central executive, default mode, dorsal attention, ventral attention, subcortical, cingulo-opercular, memory, visual, auditory, motor, and cerebellar systems (although the systems are not consistently labeled). Modular organization is thought to confer significant advantages to cognitive functioning (Meunier et al. 2010; Sporns and Betzel 2016). From an evolutionary perspective, modular organization allows adaptation of the system in response to changing environments one module at a time, allowing for system change without risking loss of function in already well-adapted modules (Simon 1962). In terms of its relevance to cognition, modular organization contributes to efficient local

information processing within functionally specialized modules as well as to the rapid exchange of information between modules, allowing for a balance between the functional segregation and integration important for cognition (Cohen and D'Esposito 2016; He et al. 2009).

The availability of tools to examine time-varying aspects of network organization has provided insight into changes in network organization over time and how these changes relate to cognitive performance (for a recent review, see Cohen 2017). Brain network organization varies across task contexts but also within scan sessions over the course of seconds (Calhoun et al. 2014; Medaglia et al. 2015a). Dynamics in brain network organization at these timescales have implications for cognition and behavior. In an auditory detection task, for example, reduced modularity of the brain was observed prior to trials on which the target was missed relative to trials on which the target was heard (Sadaghiani et al. 2015). In addition, performance on a broad range of cognitive tasks was related to the flexibility with which the salience system interacted with other modules over time at rest (Chen et al. 2016). The salience system is a module involved in facilitating access to executive functions by signaling the engagement and disengagement of task-relevant and task-irrelevant modules, respectively (Menon and Uddin 2010; Sridharan et al. 2008). These and similar data further highlight the role of dynamic changes in brain organization in cognition and behavior. Emerging frameworks linking dynamic features of brain organization to both behavior and cognition emphasize that a brain that can flexibly traverse many states of organization while also maintaining a preference for a few states will support consistently accurate but also adaptable behavior (Medaglia et al. 2015b).

3 Network Features as Biomarkers of Disease

Network neuroscience has revealed organizational principles of healthy brains (e.g., small-world architecture and modularity) that allow for efficient, flexible, and robust information processing. The fundamental insights into brain network organization conferred by network neuroscience hold great promise for providing biomarkers of disease. Studies comparing the networks of individuals with psychiatric disorders to those of healthy controls have observed disease-related deviations from the network topology that defines healthy networks. In this section, we present an overview of findings in schizophrenia and major depression disorder that have emerged from graph theory applications to neuroimaging data.

Schizophrenia Schizophrenia is a mental disorder characterized by positive symptoms, including delusions and hallucinations, and negative symptoms, such as flattened affect, as well as deficits in cognitive functions (Kahn et al. 2015). It has an average lifetime prevalence of approximately 1% (Perälä et al. 2007) and is associated with a shorter life-span relative to the general population (McGrath et al. 2008). Brain network features may be particularly well suited as biomarkers

of schizophrenia given current, and longstanding, dysconnectivity hypotheses of schizophrenia (Andreasen et al. 1998; Friston and Frith 1995; Wernicke 1906; Stephan et al. 2009). From the dysconnectivity perspective, it is an abnormal functional integration between distinct brain regions, rather than simply focal brain abnormalities, that are thought to underpin the disorder.

The advent of techniques capable of capturing connectivity disturbances provided early evidence for dysconnectivity in schizophrenia (Volkow et al. 1988). Since then, dysconnectivity in the organization of brain networks has been observed across a range of scales in schizophrenia. Small-world network architecture has been observed in people with schizophrenia as well as healthy volunteers using inter-regional covariation of gray matter volume (Bassett et al. 2008) as well as resting state functional connectivity (Liu et al. 2008; Lynall et al. 2010), suggesting that small-world organization is conserved across individuals with schizophrenia and healthy controls. However, quantitative differences in small-world network architecture among people with schizophrenia and healthy controls have emerged. Small-worldness is significantly reduced in people with schizophrenia relative to healthy controls across rest and task states (Liu et al. 2008; Lynall et al. 2010; Ma et al. 2012), and the extent of this reduction may be associated with the length of illness (Fornito et al. 2011a). Significant reductions in clustering (and the related notion of local efficiency) have also been observed in people with schizophrenia (Liu et al. 2008; Wang et al. 2010; Zhu et al. 2016) and some evidence for increased global efficiency (the harmonic mean of the inverse of the average shortest path) in schizophrenia has emerged, although this is a less consistent finding (Alexander-Bloch et al. 2012, 2010; He et al. 2012).

Analyses of topological disturbances in structural connectivity related to schizophrenia have not mapped onto the functional network findings for schizophrenia in a straight-forward fashion, reinforcing the complex relationship between structural and functional connectivity observed in the field more broadly (Honey et al. 2010; Damoiseaux and Greicius 2009). Few differences in the overall topology of structural brain networks, as operationalized through clustering coefficients and path length, were observed between people with schizophrenia and healthy controls (van den Heuvel et al. 2010). A diffusion tensor imaging study observed reduced global efficiency in people with schizophrenia relative to healthy controls (Wang et al. 2012), a result that differs from functional connectivity findings. In sum, schizophrenia is characterized by differences in the small-world architecture of functional brain organization, marked by a subtle randomization of network topology (Rubinov et al. 2009), although findings for structural networks are not as clear.

A variation on the dysconnectivity hypothesis of schizophrenia specific to brain network modularity was proposed by David (1994) and focused on abnormalities in the segregation of specialized processing regions. From this perspective, symptoms of schizophrenia reflect a breakdown in the encapsulation of brain systems that are specialized to carry out different processes. Hallucinations, for example, may result from cross-communication between inner speech and auditory modules. In line with this hypothesis, people with childhood-onset schizophrenia exhibit reduced

modularity in resting state functional connectivity networks relative to healthy controls (Alexander-Bloch et al. 2010). Indeed, some studies have reported more and smaller modules in people with schizophrenia relative to healthy controls (Yu et al. 2012), again providing evidence for altered modular architecture in the functional networks of schizophrenia. Recent work examining community structure in individuals with schizophrenia and healthy controls at rest, paired with rigorous preprocessing techniques to minimize the effects of motion, have also observed alterations in community structure in individuals with schizophrenia relative to controls (Lerman-Sinkoff and Barch 2016).

Alongside dysconnectivity in static graphs, deficits in the coordination of large-scale networks across time have also been proposed to underlie schizophrenia (Uhlhaas 2013). Braun et al. (2016) examined the reconfiguration of large-scale brain networks during a working memory paradigm in people with schizophrenia, unaffected first-degree relatives, and healthy controls. Dynamic changes in the interactions among brain regions with other regions were captured using a network flexibility measure that indicated the frequency with which a brain region changed its allegiance to a community of nodes over the course of the scan. Both patients with schizophrenia and their relatives showed increased brain-wide, network flexibility relative to controls. Findings suggest an excess of network flexibility in schizophrenia and deficits in the temporal coordination of large-scale networks that underpin efficient cognitive function. Further evidence for dysconnectivity in dynamic brain network organization in people with schizophrenia relative to healthy controls has been observed during resting state scans (Damaraju et al. 2014).

In sum (see Table 1 for overview), network neuroscience has provided tools to test dysconnectivity hypotheses of schizophrenia across multiple levels of brain organization. Findings indicate a greater randomization of large-scale brain networks in schizophrenia relative to healthy controls as well as alterations in the modularity of both static and time-varying networks. Notably, approaches aiming to characterize patients with schizophrenia relative to healthy controls based on network organization indices (e.g., clustering coefficient) show promising levels of classification accuracy (Anderson and Cohen 2013), suggesting that network neuroscience indices may have future clinical utility as biomarkers of schizophrenia.

Major Depressive Disorder Major depressive disorder is a prevalent psychiatric disorder associated with extensive personal and societal costs (Greenberg et al. 2015; Kessler 2012), affecting approximately 6% of the adult population worldwide each year (Bromet et al. 2011). Depressed mood and diminished interest or pleasure are core symptoms of depression, with other symptoms including diminished ability to concentrate, recurrent thoughts of death, and psychomotor agitation or retardation (see Otte et al. 2016 for a recent review). Contemporary models of major depressive disorder emphasize dysfunctional interactions between brain networks that are critical for the regulation of mood, as well as general cognitive, motor, and somatic behaviors (e.g., Mayberg 1997).

Table 1 Summary of network neuroscience findings for schizophrenia

Study	Modality	Sample	Main findings
Bassett et al. (2008)	sMRI	203 SZ 259 HC	• Small-world properties similar in SZ and HC
Van den Heuvel et al. (2010)	DTI	40 SZ 40 HC	• Small-world properties similar in SZ and HC
Wang et al. (2012)	DTI	79 SZ 96 HC	• Small-world properties reduced in SZ relative to HC
Alexander-Bloch et al. (2012)	rsfMRI	19 SZ 20 HC	• More connections between modules and fewer connections within modules in SZ relative to HC
Alexander-Bloch et al. (2010)	rsfMRI	13 SZ 19 HC	• Small-world properties reduced in SZ relative to HC • Reduced density of intra-modular connections in SZ relative to HC
Damaraju et al. (2014)	rsfMRI	151 SZ 163 HC	• SZ spend more time in more sparsely connected brain states relative to HC
Lerman-Sinkoff and Barch (2016)	rsfMRI	44 SZ 41 HC	• Small changes in modularity across SZ and HC. Differences in node community participation in subcortical, somatosensory, auditory, default mode, and salience networks
Liu et al. (2008)	rsfMRI	31 SZ 31 HC	• Small-world properties reduced in SZ relative to HC
Lynall et al. (2010)	rsfMRI	12 SZ 15 HC	• Small-world properties reduced in SZ relative to HC
Yu et al. (2012)	rsfMRI	24 SZ 24 HC	• More numerous and smaller modules in SZ relative to HC
Zhu et al. (2016)	rsfMRI	26 FSZ 26 SSZ 26 HC	• Small-world properties reduced in FSZ relative to SSZ and HC
Braun et al. (2016)	Task	28 SZ 37 UR 139 HC	• Increased flexibility of dynamic community structure in SZ and UR relative to HC
Fornito et al. (2011a)	Task	23 SZ 25 HC	• Small-world properties similar in SZ and HC
He et al. (2012)	Task	35 SZ 35 HC	• Small-world properties reduced in SZ relative to HC at medium difficulty • Small-world properties more variable across conditions in SZ than HC
Wang et al. (2010)	Task	23 SZ 33 HC	• Small-world properties reduced in SZ and HC
Ma et al. (2012)	Task; rsfMRI	28 SZ 28 HC	• Small-world properties reduced in SZ relative to HC

sMRI structural MRI, *DTI* diffusion tensor imaging, *rsfMRI* resting state fMRI, *SZ* schizophrenia, *HC* healthy controls, *FSZ* familial schizophrenia, *SSZ* sporadic schizophrenia, *UR* unaffected first-degree relatives

While small-world organization has been observed in participants with major depressive disorder as well as healthy controls, findings have been mixed as to whether there are quantitative differences between patients and controls based on whether functional or structural networks are under consideration. Indications of

reduced small-world architecture and a shift to randomization in brain networks have been observed in the functional networks of participants with major depressive disorder relative to healthy controls (Jin et al. 2011; Zhang et al. 2011). For structural networks, in contrast, no significant differences in small-world organization or associated features (e.g., global efficiency, path length, clustering coefficient) were observed between individuals with major depressive disorders and non-depressed controls in two studies (Korgaonkar et al. 2014; Sacchet et al. 2014).

While findings are mixed for whole-brain topology in major depressive disorder across functional and structural networks, examinations of connectivity within and between modules associated with both emotional and cognitive functions are providing insight into major depressive disorder (Ye et al. 2015). Interactions among three modules in particular have been the focus of much attention in network neuroscience studies of major depressive disorder. These modules include the salience system (SN), central executive system (CEN), and the default mode system (DMN). The DMN (for review see Buckner et al. 2008) is characterized by deactivation during task and activation during both rest and self-referential tasks (Mazoyer et al. 2001; Shulman et al. 1997) and encompasses many regions, including posterior cingulate cortex, precuneus, medial prefrontal cortex, orbital frontal gyrus, anterior cingulate cortex, inferolateral temporal cortex, parahippocampal gyrus, and bilateral parietal cortex (Raichle et al. 2001; Thomason et al. 2008; Van Den Heuvel et al. 2009). In contrast to the DMN and its characteristic deactivation during tasks and activation at rest is the CEN. The fronto-parietal CEN is characterized by nodes showing increased, rather than decreased, activation during the performance of cognitive tasks. The nodes of the CEN have established roles in a range of executive functions, including sustained attention and response suppression (Curtis and D'Esposito 2003; Jiang and Kanwisher 2003; Ridderinkhof et al. 2004). Due to the associations between its nodes and executive functions, the CEN is viewed as essential for guiding goal-directed behavior. Core nodes of the CEN include the dorsolateral, dorsomedial prefrontal cortex, and the posterior parietal cortex. A core function of the SN is salience detection, with nodes of the SN activating in response to different forms of salient stimulation (Uddin 2015; Menon 2015). The SN is also thought to facilitate access to executive functions by signaling the engagement of the CEN while suppressing DMN activity (Menon and Uddin 2010; Sridharan et al. 2008).

The putative functions of these three modules as well as the functions resulting from interactions among them map well onto core aspects of depressive symptomatology, including rumination (DMN), emotional disinhibition (CEN), and responses to salient, emotional events (SN). Importantly, these observations have led to an integrative model of neural dysfunction in depression focused on the connectivity among these networks (Hamilton et al. 2013). Despite observing few differences between patients with major depressive disorder and healthy controls in global features of structural connectivity, Korgaonkar et al. (2014) observed lowered structural connectivity in two distinct brain modules. The first contained regions primarily of the DMN, while the second was comprised of regions in the frontal cortex, thalamus, and caudate regions – areas central to cognitive and emotional

processing. Increased levels of DMN dominance over CEN have been observed to be associated with higher levels of depressive rumination in participants with major depressive disorder (Hamilton et al. 2011). Findings also highlight a potential role for the SN in the balance of activity between DMN and CEN, with activity of right fronto-insular cortex (a core component of the SN) exhibiting increasing activation at the onset of increases in CEN activity and decreases in DMN activity, while the opposite pattern was observed in healthy controls.

In terms of the reward deficits observed in major depressive disorder, two studies have observed a role for disturbances in the functional connectivity of the salience network, default mode network, and a broader reward network (encompassing nodes such as the ventral striatum) with depression symptom severity (Satterthwaite et al. 2015; Sharma et al. 2017). Satterthwaite and colleagues observed that depression severity was associated with diminished activity in core nodes of the reward and reward salience systems during a monetary incentive task, as well as with reduced connectivity between the ventral striatum and other nodes of the reward system during rest. Sharma and colleagues focused on symptoms of anhedonia and observed that reward deficits were associated with hyperconnectivity within the DMN, diminished connectivity between the DMN and regions of a cingulo-opercular system involved in salience detection, as well as a decoupling of the nucleus accumbens from DMN system regions. Notably, these two studies included participants with diagnoses spanning a range of disorders, allowing for the identification of network features common to reward deficits across a range of disorders that included depression.

Dynamic connectivity studies indicate that time-varying network organization of DMN, CEN, and SAL systems deviates from organization observed in healthy controls in major depressive disorder. Increased connectivity variability has been observed between regions of the DMN in patients with major depressive disorder relative to controls, an association that was replicated in a second sample (Wise et al. 2017). In terms of connectivity across modules, patients with major depressive disorder exhibit decreased variability in the functional connectivity between nodes of the DMN and CEN relative to healthy controls at rest (Demirtaş et al. 2016). Increased variability between nodes of the DMN and SAL networks was observed in people with major depressive disorder relative to healthy controls, and, notably, higher levels of rumination were also associated with increased variability between DMN and SAL nodes (Kaiser et al. 2016).

In sum (see Table 2 for overview), large-scale organization features (e.g., small-world organization) seem less impacted in major depressive disorder relative to schizophrenia. An emerging finding is that major depressive disorder is characterized by dysconnectivity across both static and dynamic measures of connectivity among three functional modules that map onto core symptoms of the disorder. Efforts to classify major depressive disorder patients relative to controls based on these features of network organization show promising results (Demirtaş et al. 2016), indicating potential future clinical utility of network neuroscience findings in major depressive disorder.

Table 2 Summary of network neuroscience findings for major depressive disorder

Study	Modality	Sample	Main findings
Sacchet et al. (2014)	DWI	14 MD 18 HC	• Small-world properties similar in MD and HC
Korgaonkar et al. (2014)	DTI	95 MD 102 HC	• Small-world properties similar in MD and HC • Connectivity in two networks, one involving DMN regions and a second comprising frontal cortex, thalamus, and caudate regions, was reduced in MD relative to HC
Demirtaş et al. (2016)	rsfMRI	27 MD 27 HC	• Connectivity variability between DMN and FPN decreased in MD relative to HC
Hamilton et al. (2011)	rsfMRI	17 MD 17 HC	• DMN dominance over CEN similar in MD and HC • rFIC showed increased activity during initiation of rise in CEN activity in MD but not in HC • rFIC showed increased activity during initiation of rise in DMN activity in HC but not in MD • Greater DMN dominance over CEN associated with greater depressive rumination in MD
Jin et al. (2011)	rsfMRI	16 MD 16 HC	• Small-world properties reduced in MD relative to HC
Kaiser et al. (2016)	rsfMRI	100 MD 109 HC	• Connectivity variability among mPFC and DMN regions decreased in MD relative to HC • Connectivity variability among mPFC and a region of the right insula
Satterthwaite et al. (2015)	rsfMRI	27 BPD 25 UPD 37 HC	• Depression severity correlated with diminished reward network connectivity
Sharma et al. (2017)	rsfMRI	32 MD 32 BD 51 SZ 51 PR 39 HC	• Reward deficits were associated with decreased connectivity between NAcc and DMN and increased connectivity between NAcc and CON across all groups, including MD • Reward deficits were associated with DMN hyper-connectivity and diminished connectivity between DMN and CON
Wise et al. (2017)	rsfMRI	20 MD 19 HC	• Connectivity variability between mPFC and PCC (nodes of DMN) greater in MD relative to HC
Zhang et al. (2011)	rsfMRI	30 MD 63 HC	• Small-world properties reduced in MD relative to HC

DWI diffusion-weighted imaging, *DTI* diffusion tensor imaging, *rsfMRI* resting state fMRI, *MD* major depression, *HC* healthy controls, *BD* bipolar disorder, *SZ* schizophrenia, *PR* psychosis risk, *BPD* bipolar depression, *UPD* unipolar depression, *DMN* default mode network, *FPN* fronto-parietal network, *CEN* central executive network, *rFIC* right fronto-insular cortex, *mPFC* medial prefrontal cortex, *NAcc* nucleus accumbens, *CON* cingulo-opercular network, *PCC* posterior cingulate cortex

The review of imaging connectivity features in schizophrenia and major depressive disorder indicates that network neuroscience is providing insight into psychiatric disorders. Identifying differences across participants with psychiatric disorders compared to healthy controls may lead to the discovery of features that

aid in diagnosis – a key aim for biomarkers. Differences across patients and healthy controls in the organization of brain networks may also provide insight into the mechanisms underlying the disorders, as molecular correlates of network features are beginning to be uncovered. To provide a richer intuition for these relations, we next examine genetic, molecular, and environmental correlates of brain network features.

4 Genetic and Molecular Correlates

Imaging genetic approaches aim to identify genes that are associated with network features of interest. The identification of genes associated with features of network organization allows a greater understanding of how that feature is related to biological processes by considering the biological actions of the associated genes. A number of quantitative and molecular genetic approaches have been enlisted in network neuroscience efforts, from establishing that brain network features are under some degree of genetic control to examining the mechanisms of individual genes (see Thompson et al. 2013).

Foundational work has demonstrated that there is substantial heritability of brain network organization (Bohlken et al. 2014). Notably, the genetic factors involved in network organization have been observed to be independent of the genetic factors associated with gray matter density of nodes within particular regions (i.e., local features of the brain; Glahn et al. 2010). Global network features, such as cost-efficiency, path length, and the small-world organization of both structural and functional brain networks, have all been demonstrated to exhibit substantial heritability (Fornito et al. 2011b; Jahanshad et al. 2012; Schmitt et al. 2008).

Findings of heritability provide important initial evidence that brain network organization is under genetic control. However, heritability estimates provide little information concerning the specific genes that contribute to the observed heritability. Candidate gene approaches examine the influence of variations in genotypes, chosen based on biologically plausible mechanisms, to determine how specific genetic factors affect the organization of brain networks. These approaches are beginning to shed light on how genetic variation may influence risk for psychopathology through associations with network connectivity. A number of studies have examined associations between genetic variants and a number of indices of brain connectivity. These studies have implicated a number of genetic variants in between-person differences in connectivity across regions of the brain (see Fornito and Bullmore 2012 for a recent review).

Much less work has examined associations between genetic variants and large-scale network organization. We discuss two noteworthy exceptions. Li et al. (2013) examined how variation in the disrupted-in-schizophrenia 1 (DISC1) gene was related to the efficiency of structural brain networks in healthy participants. DISC1 is involved in a number of neurodevelopmental processes with implications for brain connectivity, including neurite outgrowth, myelination, and axon guidance (Chen

et al. 2011; Jaaro-Peled et al. 2009). A common missense variant, ser704Cys (rs821616), in the DISC1 gene has been associated with schizophrenia and also affective disorders (Arias et al. 2014; DeRosse et al. 2007; Qu et al. 2007). Li and colleagues observed that Cys-allele carriers, relative to Ser homozygotes, exhibited longer shortest path length and lower global efficiency of structural networks, suggesting a role for DISC1 in the topological properties of brain network features implicated in psychiatric disorders.

In another noteworthy study, Markett et al. (2016) examined associations between variation on the tryptophane hydroxylase 2 gene's promotor region (TPH2 rs4570625) and structural connectivity of rich-club pathways. The rich club is a collection of nodes that are particularly rich in connections, tend to connect to one another, and thereby play a prominent role in the brain's overall network organization (Van Den Heuvel and Sporns 2011). The focus on TPH2 was chosen due to its role as a regulatory enzyme involved in limiting the rate of serotonin biosynthesis in the brain (Zhang et al. 2004). Based on findings of decreased mRNA expression for TPH2 in TPH2-703 T-allele carriers relative to G/G carriers and resultant reductions in levels of TPH2 concentrations throughout serotonergic neurons (Scheuch et al. 2007), Markett and colleagues hypothesized that reduced serotonin biosynthesis would be present in the T-allele carriers. Given that serotonin inhibits axonal growth (Trakhtenberg and Goldberg 2012), increased structural connectivity was hypothesized in T-allele carriers relative to G/G carriers due to decreased inhibition of axonal growth. In line with this hypothesis, higher connectivity in the rich club was observed in carriers of the TPH2 T-variant relative to G/G carriers.

While the candidate gene approach has been popular in work to date, genome-wide association studies (GWAS) will be useful to identify novel genetic determinants of network features (Bush and Moore 2012). GWAS approaches involve genotyping markers spanning the genome and searching for *loci* that influence phenotypes (e.g., network features). These approaches are beginning to provide insight into the genetic variants that are associated with features of brain network organization that are disrupted in disorders. For example, using a GWAS approach, O'Donovan et al. (2008) identified a single nucleotide polymorphism, rs1344706, in ZNF804A that was associated with schizophrenia. A later study, by establishing an association between rs1344706 genotype variation and functional connectivity among regions of the dorsolateral prefrontal cortex and hippocampal formation, provided evidence that the genetic risk for schizophrenia associated with variation in rs1344706 may be conferred through impacting brain network organization (Esslinger et al. 2009).

Findings of associations between genetic variants and features of brain organization from either candidate gene or GWAS approaches are compelling, especially in the context of plausible biological mechanisms. However, experimental approaches that manipulate or observe the proposed mediating mechanisms linking genetic variation to network organization (e.g., neurotransmitter activity) are important for establishing the viability of the proposed mechanisms and also, in turn, for highlighting other potential candidate genes that may confer risk for

psychopathology. This will be especially important for major depressive disorder for which, in contrast to schizophrenia, it has been relatively difficult to identify associated genetic variants (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium 2013; but see also Hyde et al. 2016; Okbay et al. 2016).

Studies using a range of techniques have established roles for dopamine, glutamate, and norepinephrine in preserving efficient network organization. A key role for dopamine in modulating spontaneous oscillations in basal ganglia and the coherence of neuronal activity between components of cortico-striato-thalamic circuits has emerged from nonhuman animal studies (Dejean et al. 2008; Walters et al. 2000). Recent work is further establishing a role for dopamine in brain networks in humans. Carbonell et al. (2014) examined how alterations in the dopamine system related to resting state network modularity and overall patterns of connectivity. Participants were scanned twice, once following a balanced amino acid mixture and once following a mixture that was tyrosine and phenylalanine deficient. This acute tyrosine and phenylalanine depletion technique decreases dopamine synthesis and reduces baseline dopamine levels as well as dopamine release in response to stimulation (Montgomery et al. 2003). Blood samples drawn to measure plasma amino acid concentrations indicated that the amino acid precursors of dopamine were indeed reduced following the tyrosine and phenylalanine deficient mixture relative to the balanced mixture. In the lowered dopamine state, following the tyrosine and phenylalanine deficient mixture, a number of effects on brain network connectivity were observed. The global and local efficiency of brain networks, as well as the modularity of brain networks, were reduced following dopamine precursor depletion. Short-range connections within the frontal lobe were reduced in the lowered dopamine state, and reduced connectivity between the frontal lobe and posterior association areas was observed. Finally, connectivity between the default mode network and the task positive network was increased in the low dopamine state. This experimental manipulation and its associated results highlight a role for dopamine in maintaining the modularity and efficiency of resting state brain networks, as well as in maintaining segregation of the default mode and task positive networks. Reductions in functional network efficiency have also been observed following a dose of a dopamine receptor antagonist (Achard and Bullmore 2007).

In terms of the role of glutamate, alterations in the cellular excitation-inhibitory balance have been theorized to disturb the neural synchrony of large-scale cell ensembles, giving rise to dysconnectivity at the level of neural ensembles that has been observed in psychopathology (Krystal et al. 2003; Uhlhaas 2013; Yizhar et al. 2011). As neural excitation-inhibitory balance is dependent on glutamatergic *N*-methyl-D-aspartate (NMDA) receptor function (Carlén et al. 2012), the effects of NMDA receptor antagonists on brain network organization have been examined. Braun et al. (2016) tested the effects of a single dose of the NMDA antagonist

dextromethorphan (DXM) on functional network flexibility during a working memory task in healthy participants. Note that while DXM binds to NMDA receptors, it may also impact serotonin transporters and other targets, including sigma-1 receptors (Werling et al. 2007). Network flexibility was increased following application of DXM relative to a placebo condition. Notably, the association between DXM and network flexibility was observed as a brain-wide effect and was not driven by changes in the flexibility of a single system. Thus, the hypo-glutamatergic state induced a network hyper-flexibility consistent with differences in network flexibility observed across patients with schizophrenia and healthy individuals (Braun et al. 2016). Further evidence for a role for NMDA receptor function in the organization of functional networks has emerged in work showing that administration of ketamine (an NMDA receptor antagonist) disrupted the association between CEN and DMN neural systems in a way that correlated with working memory performance, as well as the expression of symptoms of schizophrenia (Anticevic et al. 2012).

Pharmacological intervention approaches are not always feasible for examining the neuromodulatory systems that underpin network features due to ethical issues but also due to the timescales on which certain network features change. An example of a non-pharmacological intervention approach comes from Shine et al. (2016) who examined coupled changes in functional connectivity and pupil diameter over the course of a resting state scan. Fluctuations in pupil diameter co-vary with locus coeruleus activation, an activation that is linked to norepinephrine release that results in coordinated neural activity patterns throughout many parts of the brain via modulation of neural gain (Aston-Jones and Cohen 2005; Eldar et al. 2013; Joshi et al. 2016). By classifying brain network organization into two states characterized by either integration or segregation and capturing both fluctuations in these two states and in pupil diameter across the resting state scan, Shine and colleagues observed that brain network integration correlated with increases in pupil diameter. These findings highlight a role for norepinephrine in the relatively fast fluctuations in network organization that underpin fast and accurate cognitive performance. Further support for a role for norepinephrine and fluctuations in network organization comes from a study by Betzel et al. (2017) that observed an association between level of arousal (a state known to be associated with norepinephrine; España et al. 2016) and the flexibility with which nodes changed communities across time.

In sum, a combination of candidate gene, GWAS, and experimental studies is providing insight into the extent to which brain organization is heritable, associated with certain genetic variants, and with differences in neurochemical functioning. The focus on genetic drivers of network features is in line with the high heritability estimates of psychiatric disorders. However, there are also environmental risk factors for psychopathology (Rutter 2000), and the effects of genotype on psychiatric disorders may be conditional on environmental experiences (Kendler et al. 1995). As such, we turn to a discussion of the role that environmental factors play in psychopathology and brain network organization.

5 Environmental Factors

In terms of environmental factors involved in schizophrenia, approximately 60% of patients with schizophrenia do not have an affected first-degree relative and heritability estimates range between 60–80% (Brown 2011; Sullivan et al. 2003). For major depressive disorder, heritability estimates are in the range of 31–42% indicating substantial environmental factors in the disorder (Lohoff 2010; Sullivan et al. 2000). The substantial variance in psychopathology associated with environmental experiences necessitates a consideration of factors beyond genetics. Emerging work is examining how environmental factors may lead to changes in network connectivity indices observed to be associated with psychopathology. We provide an overview of two environmental factors, socioeconomic status and social network structure, that exhibit associations with both psychopathology and brain network organization.

Socioeconomic status (SES) is a multidimensional construct that includes measures of economic resources and is typically assessed with income, education, occupation, as well as combinations of these indicators (Braveman et al. 2005; Krieger et al. 1997). Low SES is associated with greater risk for schizophrenia (Werner et al. 2007) and major depressive disorder (Lorant et al. 2003), as well as psychopathology more generally (Kohn et al. 1998). The mechanisms driving associations between low SES and psychopathology may be articulated as social causation and social selection hypotheses. Social causation hypotheses posit that people with low SES develop psychological problems in response to exposure to adverse life circumstances. Social selection hypotheses, in contrast, posit that people with psychopathology drift down the SES ladder due to an inability to fulfill role obligations resulting from their psychopathology or by inheriting risk through genetic pathways. Of course, either hypothesis alone is unlikely to capture the complex, reciprocal dynamics between selection and causation processes that may operate across development, leading to interactionist perspectives of SES that consider both processes (Conger and Donnellan 2007). Tests of interactionist models of the effects of SES require complex study designs over long periods of time (e.g., Capaldi et al. 2003) to disentangle the contributions of social selection, social causation, or a combination of the two to psychopathology and brain network organization.

The difficulties in establishing causality notwithstanding, network neuroscience is in a prime position to examine the structural and functional brain network features that are impacted by low SES experiences, which place individuals at risk for psychopathology. There is substantial evidence from nonhuman animal work that exposure to deprived environments modifies the brain (Mohammed et al. 2002; Van Praag et al. 2000). There is also human work showing local structural and functional brain differences across levels of SES (Gianaros et al. 2011; Kishiyama et al. 2009).

Much less work has examined large-scale network features but two noteworthy exceptions exist. Krishnadas et al. (2013) examined the modular architecture of brain network structure in men from the most deprived and least deprived neighborhoods

of Glasgow, Scotland. Using region-wise cortical thickness correlations, they observed differences in the modular structure of brain graphs. Structural networks of the least deprived group showed stronger modular organization relative to random graphs, while structural networks of the most deprived group showed the same number of modules relative to their corresponding random network. The most deprived group, then, exhibited a weakened modular structure, with more edges between modules relative to the least deprived group. The least deprived group also had greater indications, relative to the most deprived group, of brain network architecture that would facilitate efficient information transfer between modules. The results, as a whole, establish evidence of a relationship between socioeconomic status and network topology.

A second study examined the implications of SES for the development of functional networks during the first year of life (Gao et al. 2015). Longitudinal growth trajectories of nine functional modules (Smith et al. 2009) were examined in terms of their within-module connectivity, between-module connectivity, and overall similarity to adult references at five time points during the first year of life. At age 6 months, both higher income and higher maternal education were associated with greater similarity to adult references and higher within-module connectivity. Further, higher income was associated with lower between-module connectivity. Thus, indications of reduced modular structure across functional brain networks were associated with low SES – in line with findings of structural connectivity in adults. An important future direction for work on SES is to examine the mechanisms through which low SES "gets under the skin" to influence network connectivity. Candidate mechanisms for SES effects on the brain include exposure to stressful experiences, social support, toxins, and stimulating activities (Hackman et al. 2010).

An additional environmental factor that is beginning to receive attention for its impact on brain networks is one's social network structure. Social support is the emotional support, guidance, and tangible aid available to the individual through social ties to other individuals, groups, and the larger community (Lin et al. 1979; Wills 1991). Social support has long been known to have beneficial effects on mental and physical health (for review see Taylor 2011). In schizophrenia, positive relationships with the individuals in one's social network are associated with fewer symptoms and greater levels of functioning (Pahwa et al. 2016). Further, social networks of greater size, containing a greater number of individuals outside of the family and that provide greater levels of support, are associated with greater quality of life in people with schizophrenia (Cechnicki et al. 2008). Lower levels of social support may also act as a risk factor for major depressive disorder (Wade and Kendler 2000). Increased contact with one's social network is associated with fewer depressive symptoms (Sugisawa et al. 2002), and multiple aspects of social network characteristics are associated with depression recovery over a 2-year period (van den Brink et al. 2017).

In terms of etiology, frameworks for understanding the role of social network stress and support in impacting both schizophrenia and major depressive disorder have implicated the engagement of neural stress regulatory circuits that, with chronic stress, lead to long-term physiological and neurobiological changes that increase

the risk for pathological states (Akdeniz et al. 2014; Slavich and Irwin 2014). In these frameworks, social networks play stress-buffering roles, attenuating the physiological stress response (Cohen and Wills 1985; Seeman and McEwen 1996; Young et al. 2014).

The extent to which social network structure and function buffers experiences of stress to protect against brain network changes that increase vulnerability to developing psychopathology remains to be seen. However, a number of groundbreaking studies linking brain network function to social network structure have laid the foundations for future work in this area (see Falk and Bassett 2017 for review). Schmälzle et al. (2017), for example, examined the moderating role of social network structure on the effect of social exclusion on functional brain network architecture. The experience of social exclusion was simulated through the use of a well-validated game referred to as Cyberball (Williams et al. 2000) in neurotypical adolescent males. Functional connectivity within the mentalizing network – a group of regions involved in the process of inferring others' affective states encompassing the medial prefrontal cortex, precuneus/posterior cingulate cortex, and the temporoparietal junction (Frith and Frith 2006; Schnell et al. 2011) – increased during social exclusion. Notably, the strength of functional connectivity between two key nodes of the mentalizing network was related to social network structure as measured by ego-network density based on objective social media data. A dense ego-network indicates a close-knit social network in which participants' friends are also friends with one another (Hurlbert et al. 2000). Less dense ego-networks, in contrast, reflect social networks in which a participant's friends do not know each other. Participants with a less dense social network exhibited stronger coupling between key regions of the mentalizing network during social exclusion. The findings highlight differences in the brain network response to social exclusion, a potent source of stress, based on preexisting social network characteristics.

Important foundational work remains to be conducted to examine the extent to which network features, especially large-scale topological features, are associated with environmental factors such as SES and social network structure and function. Establishing the mechanisms through which environmental factors impact brain network structure and function will also be integral for a better understanding of the causes and consequences of psychopathology. In considering these mechanisms, it will be valuable for both our understanding of mechanisms and for intervention possibilities to work within frameworks that emphasize the bidirectional interplay among intra- and extra-organismic levels of analysis across development (Magnusson and Cairns 1996). Observed brain network features reflect, in part, experience-dependent organization (Sporns 2013). Indeed, patterns of structural and functional connectivity are thought to result from histories of co-activation of regions across cognitive processes and actions. The more frequently processes are used, the more entrenched they are thought to become in functional modules detectable during resting state analyses. This proposition stems from a network-level application of Hebbian theory in which "neurons that fire together, wire together" and is consistent with observations of training-induced changes in network organization (e.g., Bassett et al. 2015; Takeuchi et al. 2010; Taubert et al. 2010).

The experiences available across different environments also likely impact network features given that behavior can be conceived of as the leading edge of adaptation – with the individual's activity at the boundary of the individual and their environment capable of inducing change in the environment and the structures of the individual to facilitate adaptation (Gariepy 1996). As such, incorporating measures of everyday behaviors, using experience-sampling designs, for example (Bolger et al. 2003; Shiffman et al. 2008), into studies examining network organization changes in response to the environment will be key for establishing mechanisms underlying change.

6 Open Frontiers

Work to date suggests that network neuroscience has the potential to inform our understanding of psychiatric disorders. In this section, a number of open frontiers are described that will bring the field closer to its aims of providing tools that aid in the diagnosis, determination of prognosis, and prediction and monitoring of responses to intervention.

A key challenge for the study of network neuroscience in psychiatry is to adopt designs that move beyond an examination of associations between genetic variants and environmental factors and the presence or absence of psychopathology. Developmental psychopathology perspectives hold that psychiatric disorders constitute end points of interactions between genetic and environmental risk factors that impact normal brain development, leading researchers to highlight that adult imaging phenotypes represent systems resulting from a developmental process in which environmental stressors interact with genetic vulnerability to contribute to the emergence of psychopathology (Viding et al. 2006). From this perspective, an understanding of psychopathology and its causes will be bolstered by situating the study of network neuroscience and psychiatry within a developmental framework and making use of intensive longitudinal data to capture within-person change in brain network organization across typical and atypical development (Bergman and Magnusson 1997; Menon 2013).

Existing classification systems in psychiatry are descriptive, relying on identifying combinations of symptoms to reach a diagnosis of a disorder, and provide a challenge for network neuroscience applications. There is tremendous heterogeneity in brain network organization among individuals with the same diagnostic label (Fried and Nesse 2015; Galatzer-Levy and Bryant 2013). Work to date has relied on diagnostic categories to define patient versus healthy control groups and, as such, has inherited existing difficulties in identifying the mechanisms underlying heterogeneous disorders. Going forward, imaging connectivity approaches must be paired with innovations in theoretical frameworks that rely less on monothetic diagnostic criteria and more on approaches that recognize the dimensional nature of mental disorders. The theoretical components of such an approach are emerging in RDoC (Insel et al. 2010) and in network approaches to psychopathology (Borsboom 2017; Fried and Cramer 2016).

To date, imaging connectivity approaches have provided insight into the network features that distinguish healthy controls from participants with psychiatric diagnoses. Much less work has examined the ability for network features to contribute to differential diagnoses of psychiatric disorders. Given that the standard of care differs across diagnostic categories, this will be an important achievement to meet in order for imaging connectivity features to act as feasible biomarkers (Savitz et al. 2013). This aim will be met by including participants across diagnosis categories to identify common and dissociable aspects of connectivity (e.g., Satterthwaite et al. 2015).

There are a number of challenges associated with network neuroscience methodologies that place limitations on their capacity to act as biomarkers. Chiefly, there are many freely selectable parameters during the analysis of imaging data. Small variations in the implementation of connectivity analyses can impact resulting features to the extent that associations with genetic variation and network features, for example, can be observed for specific implementations of connectivity analyses but not others (Bedenbender et al. 2011). While this will be an ongoing challenge, work providing a better understanding of the effects of differing data processing pipelines is emerging that will aid in establishing guidelines for best practices in the analysis of brain networks (Ciric et al. 2017; Zhang et al. 2016).

7 Conclusion

Network neuroscience provides an array of tools and concepts capable of capturing the complex features of brain (dys-)organization that have been long-theorized to underpin psychiatric disorders. Applications of network analysis have revealed organizational principles of healthy brains that allow for efficient, flexible, and robust information processing. These mathematical tools and conceptual frameworks have allowed for fruitful research into how brain organization deviates from optimal organization in psychiatric disorders. There is promising potential for network neuroscience to highlight the mechanisms associated with psychopathology and to provide tools that aid in diagnosis and in evaluating treatment response. Further progress will be gained by incorporating network neuroscience techniques within developmental psychopathology frameworks that recognize the limitations of current clinical nosology.

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Cognitive Phenotypes for Biomarker Identification in Mental Illness: Forward and Reverse Translation

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Contents

Abstract Psychiatric illness has been acknowledged for as long as people were able to describe behavioral abnormalities in the general population. In modern times, these descriptions have been codified and continuously updated into manuals by which clinicians can diagnose patients. None of these diagnostic manuals have

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Curr Topics Behav Neurosci (2018) 40: 111–166
DOI 10.1007/7854_2018_50
Published Online: 30 May 2018

attempted to tie abnormalities to neural dysfunction however, nor do they necessitate the quantification of cognitive function despite common knowledge of its ties to functional outcome. In fact, in recent years the National Institute of Mental Health released a novel transdiagnostic classification, the Research Domain Criteria (RDoC), which utilizes quantifiable behavioral abnormalities linked to neurophysiological processes. This reclassification highlights the utility of RDoC constructs as potential cognitive biomarkers of disease state. In addition, with RDoC and cognitive biomarkers, the onus of researchers utilizing animal models no longer necessitates the recreation of an entire disease state, but distinct processes. Here, we describe the utilization of constructs from the RDoC initiative to forward animal research on these cognitive and behavioral processes, agnostic of disease. By linking neural processes to these constructs, identifying putative abnormalities in diseased patients, more targeted therapeutics can be developed.

Keywords Cognition · Cross-species · Phenotypes · RDoC · Translational biomarkers

1 Introduction

Psychiatric illness is assuredly a vexing aspect of the human condition as old as society itself. Systematic approaches to the classification and treatment of mental conditions might still be considered in its infancy. Noting the torturous conditions the individuals with abnormal behavior were often subjected to, early pioneers such as the American Physician Benjamin Rush (born 1746) and the German Physician Emil Kraepelin (born 1856) provided the first efforts at classifying mental illness and treating such afflictions as medical conditions (Bonkalo 1956; Carlson and Simpson 1964). Yet, formal classification systems did not emerge until the mid-twentieth century with the addition of mental health conditions to the 6th revision of the World Health Organization's International Statistical Classification of Disease (ICD-6) in 1949 and the introduction of the Diagnostic and Statistical Manual of Mental Disorders (DSM-1) in the USA in 1952. Both classification systems have undergone periodic revisions with the most recent versions being the ICD-10 (World Health Organization 1993) and the DSM-5 (American Psychiatric Association 2013).

Although each revision of these classification systems has been shaped to an extent by the societal norms and cultural values of their time, each iteration has sought to more rigorously define behavioral and biological phenotypes predictive of functioning and treatment response. A variety of mental health conditions outlined in these classification systems are defined in part by cognitive impairments or changes in cognitive functioning (e.g., schizophrenia, attention deficit hyperactivity disorder, and major neurocognitive disorder). While the DSM-5 provides an expanded and more detailed characterization of cognitive domains relevant to mental health conditions, the revision stops short of recommending specific neurocognitive tests or firm boundaries for meeting cognition-related criteria. This omission enables flexibility for incorporating new approaches and novel findings into the diagnosis of

conditions. Given the rapid pace of development in the field of cognitive testing, continuous review of biomarkers for cognitive dysfunction has taken on critical importance.

In recognition for the need to develop biomarkers and more concrete definitions of impacted cognition, and behaviors, the National Institute for Mental Health (NIMH) created the wide-reaching Research Domain Criteria (RDoC) initiative. RDoC project seeks to "develop new ways to classify mental disorders based on behavioral dimensions and neurobiological measures" in hopes of facilitating translational research by remaining agnostic toward diagnostic borders derived from clinical presentation in humans. Already, numerous lines of research have supported the premise that there is significant evidence of shared gene-expression perturbation across numerous psychiatric conditions (Glahn et al. 2016; Hess et al. 2016; Sumner et al. 2016). With shared genetic expression abnormalities, the next logical step is to determine whether shared neurobiological underpinnings underlie cognitive dysfunction between such psychiatric conditions.

Cognitive dysfunction based on these RDoC dimensions could therefore be considered as biomarkers of cognitive dysfunction agnostic to disease state (Fig. 1). Identifying techniques to quantify these domains (https://www.nimh.nih.gov/research-priorities/rdoc/constructs/cognitive-systems.shtml) remains critical for such biomarker assessment. Identifying such techniques that are available for use in human and preclinical models would enhance the opportunity to develop: (1) a greater understanding of the mechanism(s) underlying that behavior, (2) animal models of dysfunction relevant to disease states, and therefore (3) potential treatments for such dysfunction that can be tested similarly first in preclinical models

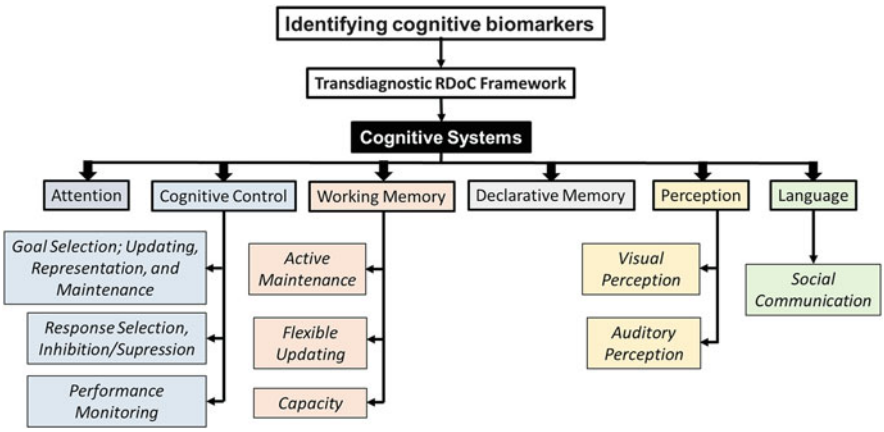

Fig. 1 Utilizing the transdiagnostic RDoC framework to identify cognitive biomarkers of disease. The National Institute for Mental Health (NIMH) Research Domains Criterion (RDoC) initiative identified a transdiagnostic framework with which to quantify behavioral abnormalities. With such specific quantified behavioral outcomes, rodent paradigms can be utilized to better understand the neural mechanisms underlying these behaviors. Hence, specific treatments can be developed that would target specific domains that are biomarkers of disease functioning

then in patient populations. Such forward- and reverse-translated cognitive biomarker techniques are detailed below, based on each of the cognitive constructs identified by the ever-evolving RDoC initiative.

2 Construct: Attention

In order to properly define attention as a cognitive construct unto itself, a key distinction must be made. An implicit concept in attention is that an individual's ability or capacity for awareness, perception, or motor action is finite and that these functions are often in competition with one another. For example, it can be very difficult to simultaneously compose a text message, listen to directions, precisely control a motor vehicle, and notice a child readying to leap onto the roadway to retrieve an adrift football. In RDoC, the construct of attention attempts to specifically quantify individuals' *implementation* of cognitive processing, that is to effectively enhance processing of task-relevant information while suppressing processing of task irrelevant information. This construct is distinct from appropriate *control* in selecting which tasks are relevant, which is a closely related subconstruct subsumed under goal selection, i.e., updating, representation, and maintenance (NIH 2011, 2016). Returning to our example, the domain of attention attempts to quantify proficiency in divided task performance, while goal selection – updating, representation, and maintenance – attempts to quantify one's ability to sustain concentration on driving safely and suppress the urge to look at their phone.

RDoC has classified three types of experimental paradigms that can be useful in the quantification of attention: bottom-up/top-down processing tasks (also known as overt/covert), capacity and interference control tasks, and vigilance tasks. Assays of bottom-up/top-down processing attempt to measure how cognitive representations of goals (top down) interact with incoming sensory information (bottom up) to determine how to allocate attention in order to guide goal-directed behavior. To date, three types of tasks have been identified by RDoC for clinical evaluation of this type of processing (NIH 2011, 2016). The first are the spatial and nonspatial cueing tasks (Petersen and Posner 2012; Posner and Petersen 1990), which monitor reaction time (RT) to respond to a target symbol following presentation of a cue. This cue can be exogenous (0.50 probability of coincidence with target) or endogenous (0.80 probability of coincidence with target). When cue presentation is *invalid*, meaning this cue was not coincident with subsequent presentation of the target stimulus; RTs will be slowed compared to *valid* trials where the cue and target coincide, facilitating RT. The costs and benefits associated with orienting cognitive resources toward a cue can be effectively measured using these paradigms (Petersen and Posner 2012; Posner and Petersen 1990). In schizophrenia, though reaction times are generally slowed across trial types compared to controls, the ability for enhanced processing for validly cued targets compared to invalidly cued targets is broadly conserved (Luck and Gold 2008).

Attentional network tasks (ANTs) have also been identified for the assessment of bottom-up/top-down processing. In these tasks, participants must indicate the direction of a centrally presented arrow. The target is flanked on either side by arrows pointing in the same (*congruent*) or opposite (*incongruent*) direction. In addition, this series of arrows can be presented above or below a fixation cue. Presentation of the arrows can be temporally (*alerting*) or spatially (*orienting*) cued, or uncued (Coull et al. 2001; Fan et al. 2002). This task was designed to selectively parse network functions involved with alerting, orienting, and *executive* functions. When target detection was preceded by an alerting cue, classic right hemisphere thalamo-fronto-parietal network activation was detected using fMRI. When the orienting cue preceded target detection, bilateral superior parietal lobe activation was witnessed. Finally, when executive function was engaged by the incongruent stimulus condition, bilateral activation was observed within anterior cingulate, and frontal cortices as well sparse activation distributed across a number of related areas (Fan et al. 2005). In the ANT, patients with schizophrenia-spectrum disorders, as with cuing tasks, exhibit RT slowing across all task conditions compared to controls but alerting and orienting features are largely spared (Gooding et al. 2006; Orellana et al. 2012). Although these tasks failed to identify a specific deficit that could serve as a biomarker, they could be useful in ruling out schizophrenia.

Deficits in top-down processing have been observed using various visual search tasks. In these experiments, participants are tasked with identifying a target stimulus among a set of distracter stimuli similar in shape, size, orientation, and color. Depending on the definition of the target and nature of the distracter stimuli, these tasks can parse top-down control from bottom-up input. For example, the target may always share one of the possible discriminative features with distracter stimuli. In other words, all distracters are equal in terms of bottom-up salience. In this case, target detection can be facilitated compared to random search by exercising top-down control to focus the search on one of the relevant discriminative dimensions. Bottom-up salience can be enhanced by eliminating features shared by distracter and target stimuli. Diminished performance compared to controls in either/both conditions could indicate deficits in top-down control or bottom-up processing (Treisman and Sato 1990; Wolfe 1994; Woodman and Luck 1999, 2003). In schizophrenia, patients exhibit RT slowing compared to controls on trial types requiring top-down control but do not differ from controls when salience of the target stimulus is enhanced (Gold et al. 2007). Hence, impaired top-down processing with intact bottom-up processing may have potential as a biomarker for this aspect of attention in schizophrenia, especially if performance of other processing tasks is preserved.

Capacity and interference control is the second class of attention paradigms identified by RDoC, which consist of attentional blink and dual-task paradigms (NIH 2011, 2016). These tasks attempt to measure the ability to implement attention to process rapidly presented visual stimuli, which is necessary to form accurate visual perception. The phenomenon of *attentional blink* was first discovered using *rapid serial visual presentation* (*RVSP*). In this task, participants were presented with a stream of rapidly (~100 ms) presented characters. Within that stream, they are

asked to identify two specific target stimuli. If presentation of the second target stimulus quickly follows the first, detection is highly accurate. Likewise, if the second target follows the first by >500 ms, target detection is equally accurate. However, if the second target is presented 100–500 ms after the first, a considerable decrement in detection is observed. This decrement is attentional blink, which is thought to represent the period of time required to integrate a salient visual stimulus into a percept. When presented closely together, both stimuli can fit into a single percept, or two separate percepts if separated by >500 ms. Additional percepts are difficult to form during the intervening period due to interference from processing the first target (Broadbent and Broadbent 1987; Dux and Marois 2009; Raymond et al. 1992). This is an attentional and non-perceptual process, as detection of the second target is facilitated if the participants are told to ignore the first target (Raymond et al. 1992). Patients with schizophrenia exhibit two deficits in these tasks. First, target detection in general is decreased, indicating a general impairment in processing visual stimuli. Second, attentional blink is more pronounced and slightly longer in patients compared to controls (Cheung et al. 2002; Mathis et al. 2011; Wynn et al. 2006), which has been associated with a lack of P300 event-related potentials (ERP) at the longer lag intervals where attentional blink attenuates in healthy controls (Mathis et al. 2011) indicating deficient integration of stimuli in visual perception. Moreover, both target 1 and target 2 ERPs are smaller in schizophrenia compared to controls (Verleger et al. 2013), which could account for general impairments in target detection.

Dual-task paradigms can also be used to assess capacity and interference control. Various types of psychometric tasks can be utilized in these types of assessments, provided the tasks induce *dual-task interference* by requiring participants to perform two tasks simultaneously. These tasks allow for in-depth analysis of behavior in order to distinguish the origins of attentional dysfunction (Nuechterlein et al. 2006). When participants are capable of effectively performing each individual task accurately but are impaired at performing the tasks simultaneously, these deficits are attributed to limited *processing resources* (Ninio and Kahneman 1974; Wickens and Kessel 1980; Wickens et al. 1984). On the other hand, when the same limited attentional resource is required to perform both tasks while others are independent, performance on one of the tasks will become delayed as processing occurs serially between tasks. This is known as a *structural processing bottleneck* (Pashler 1994a, b).

Dual-task interference is more severe in individuals with schizophrenia (Nuechterlein et al. 2006). Available research suggests that this deficit arises, at least in part, from reduction in processing resources. For instance, in one report deficits were only observed in patients performing dual-task version of a multiple-frame search task, while performance on the single task version was comparable between patients and controls (Granholm et al. 1996). This deficit is detectable early in the course of illness (Nuechterlein et al. 2011), possibly even prodromal (Bearden et al. 2000; Niendam et al. 2003), and is predictive of professional and social functioning later in life (Nuechterlein et al. 2011). Whether structural processing bottlenecks are more pronounced in schizophrenia remains an open question (Nuechterlein et al. 2006), although recent reports suggest that processing

bottlenecks are not a contributing factor to impairments in schizophrenia (Nieuwenstein and Wyble 2014).

The third and final class of paradigms identified for the assessment of attention attempt to quantify vigilance. In this context, vigilance refers to sensitivity to incoming stimuli. A loss of sensitivity is termed hypovigilance, whereas heightened sensitivity is termed hypervigilance. These states are critically mediated by limbic–amygdalar systems. In the RDoC domain of attention, vigilance explicitly does not refer to sustained attention, which is subsumed under goal maintenance in the domain of cognitive control, nor does it refer to willingness to work in the domain of effort valuation. In order to quantify vigilance, investigators seek to capture lapses in attention or periods in time in which the "mind wanders" off task (NIH 2011, 2016). Continuous signal detection tasks, or continuous performance test (CPTs), comprise the bulk of paradigms for assessing vigilance. In short, the goal of these tasks is to respond when a given signal is presented. These paradigms also incorporate "catch" trials that are used to quantify off-task behaviors, enabling the calculation of index variables that distinguish accurate and vigilant task performance (sensitivity) from more habitual (biased) responding, which could be interpreted as vigilant performance in the absence of competition (Gold et al. 2012; Nuechterlein et al. 2008). The identical pairs continuous performance test (CPT-IP), distracter-sustained attention task (dSAT), and the 5 choice continuous performance test (5C-CPT) have been previously identified by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) and Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) consensus panels for the assessment of vigilance. These tasks were chosen based on their potential for translation between patients and model laboratory animals given their particularly high construct validity (Cope et al. 2016; Lustig et al. 2013; Nuechterlein et al. 2008). Degraded stimulus CPTs (DS-CPT), dichotic listening tasks, and forced choice Span of Apprehension task are also frequently utilized to assess the effect of increasing perceptual load on vigilance (Barch et al. 2012; Nuechterlein et al. 2004, 2015).

Patients with schizophrenia exhibit poorer CPT performance compared to healthy controls, which is highly replicable (Nuechterlein et al. 2015), detectable in both psychotic and remitted states (Asarnow and MacCrimmon 1978; Nuechterlein et al. 1992; Wohlberg and Kornetsky 1973), and heritable in parents and siblings (Chen et al. 1998, 2004; Grove et al. 1991; Maier et al. 1992; Saoud et al. 2000). Although, many of these tasks do not effectively dissociate working memory to accurately pinpoint vigilance. One exception, the 5C-CPT, has been used to detect reduced vigilance and target responses in patients with schizophrenia (Bhakta and Young 2017; Young et al. 2013a). This task, which has shown great potential in preclinical translation, does not depend on intact working memory (nor does it correlate with working memory performance, Bismark et al. 2018), demonstrating that a fundamental deficit in vigilance is present in schizophrenia (Bhakta and Young 2017; Cope and Young 2017; Young et al. 2009a, 2013a). Animal studies using the 5C-CPT have identified numerous aspects consistent with human observations, such as the need for parietal cortices underlying performance (Young et al. 2018),

amphetamine-induced improvement in humans and mice (MacQueen et al. 2018), vigilance decrements (Young et al. 2009a), nicotine-induced improvement (Higa et al. 2017; Young et al. 2013b), and nicotine withdrawal-induced deficits (Higa et al. 2017; van Enkhuizen and Young 2016), similar to human CPTs (Levin et al. 1998), etc. More importantly, the 5C-CPT is now being used to determine neurobiological aspects that might underlie schizophrenia. For example, mice with reduced Sp4 receptor expression – as is observed in patients with schizophrenia – exhibit 5C-CPT deficits, which can be remediated by a glycine-1 transporter inhibitor (Young et al. 2015). This deficit may be linked to their reduced NMDAR1 expression levels given that subchronic treatment with an NMDA receptor antagonist impaired performance – an effect remediated by a dopamine D1 receptor agonist (Barnes et al. 2016). Reduced dopamine D4 receptor expression also impaired performance in mice (Young et al. 2011). Hence, tasks like the 5C-CPT could be useful in disentangling neural mechanisms underlying deficient performance, with treatments developed targeted at such mechanisms.

Recently, a new class of task has been incorporated into the study of vigilance in attention. *Mind wandering* is empirically defined as an individual's attention drifting from a current train of thought, such as ongoing performance of a task, to internally self-generated thoughts that are presumably driven by intrinsic-state changes rather than external cues (Smallwood and Schooler 2015). Neurologically, these *stimulus-independent thoughts* are thought to modulate activity of the *default mode network*, the regions of the brain that remain activated when the brain is minimally functioning, to facilitate arousal, planning, autobiographical memory, or divided attention (Mason et al. 2007; Seli et al. 2016). In schizophrenia, patients' minds wander more frequently compared to controls, which is correlated to PANSS positive symptom scores. In these patients specifically, mind wandering events were associated with decreases in connectivity between ventromedial prefrontal cortex (PFC) and the default mode network. This network is thought to represent a possible mechanism by which subjective experience becomes unlinked from introspective processing that could lead to the generation of positive symptoms (Shin et al. 2015). As these investigations are early on in their development, their validity has yet to be thoroughly demonstrated (Kane et al. 2016; Smallwood and Schooler 2015). Further, they have limited translatability due to inability to quantify introspection in nonhuman laboratory animals. Nonetheless, these tasks could provide a powerful biomarker to quantify probability to develop positive symptoms.

In summary, the utility of RDoC defined measures of attention as biomarkers in schizophrenia may be highly task dependant. Further, given the complexity of the human tasks described within this domain paired with the subjectivity of inferring a rodents' interoceptive state – a necessary variable for these analyses – many of these assessments are currently unable to translate from humans. For instance, most of the tasks described reliably detect general deficits in patients versus controls (slowed reaction time, avolition, etc.). These types of general deficits can easily be detected in rodents using operant tasks such as two-choice probabilistic reward/reversal learning (Amitai et al. 2014; Milienne-Petiot et al. 2017; Young et al. 2015), or progressive ratio breakpoint tasks (Cagniard et al. 2006; Schneider and Koch 2003).

Diagnostic classifications, however, only interact with task-modulated factors in specific instances: (1) Patients exhibit impairments in top-down processing in visual search (Gold et al. 2007). (2) Attentional blink in schizophrenia is slightly longer and more pronounced compared to controls and is associated with decreases in stimulus-related ERPs (Cheung et al. 2002; Mathis et al. 2011; Wynn et al. 2006). (3) Dual-task interference related to decreases in processing resources is detectable in schizophrenia, possibly even prodromally (Granholm et al. 1996; Nuechterlein et al. 2006). (4) Measures of vigilance in schizophrenia have been shown to interact across multiple task conditions, indicating that deficient vigilance may represent a fundamental cognitive deficit with biomarker potential for schizophrenia within the domain of attention (Bhakta and Young 2017; Nuechterlein et al. 2015). (5) Mind wandering is more prevalent and related to decreases in functional connectivity between vmPFC and the default mode network (Shin et al. 2015; Smallwood and Schooler 2015). It is worth noting again that deficient attention has long been characterized to be a hallmark feature in schizophrenia and other psychiatric disorders. This is certainly not in contrast to the results reported here. The preponderance of the literature substantiating that consensus has focused on the control of attention, however, rather than capacity or processing speed. These analyses will be discussed in detail in the domain of cognitive control.

3 Construct: Cognitive Control

Effective goal-directed behavior relies on dynamic and accurate tuning of cognitive and emotional systems when a more practiced, habitual, or prepotent response is not appropriate. The cognitive control domain refers to function of an *executive* system modulating this response. By design, the hierarchal nature of cognitive control results in overlap with all other behavioral domains to some degree. This executive control is not dictatorial but rather must outcompete more prepotent responses in order to override them. In other words, the behavioral response is the product of competing top-down inhibition pitted against bottom-up physiological drives and/or stimuli. Hence, tasks that attempt to assess cognitive control often model this competition behaviorally. The cognitive control domain is divided into three subdomains that parse select aspects of this competitive behavior (NIH 2011, 2016).

3.1 Subdomain: Goal Selection; Updating, Representation, and Maintenance

This subdomain is often referred to as preparatory cognitive control. In other words, this domain quantifies the determination of a behavioral objective and the efficacy of systems supporting ongoing performance or need for editing of that behavior. The

first component of this behavior, goal selection, depends on prioritization of the possible rules or goals. Modern theories refer to this as a *representational hierarchy*, positing that goals are ranked according to their level of abstraction (Badre 2008). In an elegant series of studies, two separate teams of researchers have demonstrated, using whole-brain fMRI, that this representational hierarchy is neurally mapped along a cortical rostro-caudal gradient, with the most abstract and temporally distal representations activating anterior frontopolar cortex moving caudally with more concrete and temporally immediate actions activating primary motor cortex. This architecture is said to be cascading, in that these regions exert behavioral control by eliciting downstream action plans according to past events, incoming stimuli, context perception, and temporal structure in that order (Badre 2008; Badre and D'Esposito 2007; Koechlin et al. 2003).

Disruptions in functional connectivity, particularly between prefrontal executive control regions, have been well characterized in schizophrenia (Fornito et al. 2012; Frith 1996; Ragland et al. 2007). Recent investigations have sought to characterize these misconnections in light of the hierarchical rostro-caudal organization detailed by Koechlin, Badre, and colleagues. This architectural hierarchy appears to be preserved in schizophrenia, but patients make more errors on a multidimensional stimulus/rule discrimination task when contextual and episodic signals were increased (Barbalat et al. 2009). Connectivity within this network is disrupted, which could indicate that the representations mediating different modes of processing and controlling goal selection become blurred in schizophrenia. This could contribute to the heterogeneity of aberrant thoughts and behaviors witnessed in schizophrenia (Baker et al. 2014).

It is essential that these goals remain appropriately flexible in order to adapt to changes within the environment. This leads to the second component of this subdomain, *goal maintenance*. Closely related tasks, the AX continuous performance test (AX-CPT) and dot pattern expectancy task (DPX) have been used extensively in the study of this subdomain. As with most CPTs, a string of characters (AX-CPT) or dot patterns (DPX) are presented to the participant. In the DPX, dot patterns are used instead of letters to avoid linguistic associations that produce strong practice effects, but the target behavior is the same. Participants are required to record a response when an X (or target dot pattern) is presented, but only if that X was preceded by an A (or the defined dot pattern cue). This design is intended to prime two specific prepotent responses based on *expectancy*. First, it biases participants to respond anytime an X is presented (AX or BX trials, where B can stand for any character). Second, it primes responding on any presentation following an A presentation (AY trials, where Y can stand for any character). Accurate performance on AX and BX trials with comission errors on AY trials would indicate good goal maintenance, whereas AX and BX errors with accurate AY performance would reflect poor goal maintenance (Lopez-Garcia et al. 2016; MacDonald 2008). Accurate performance of these tasks has been repeatedly associated with activation of dorsolateral PFC (dlPFC) (Barch et al. 1997, 2001; Holmes et al. 2005; Lesh et al. 2013; MacDonald et al. 2005; Paxton et al. 2008). Specifically in schizophrenia, impairments in goal maintenance have been demonstrated repeatedly, which has

been strongly associated with disruptions in dlPFC activity (Barch et al. 2001; Holmes et al. 2005; MacDonald et al. 2005). Behaviorally, this manifests as an overreliance on inefficient encoding and retrieval strategies (MacDonald et al. 2005), which could be interpreted either as a general impairment in cognition or a compensatory mechanism in response to dysfunction with the dlPFC goal maintenance circuitry.

Aspects of these types of behaviors and their functional circuitry have been quantified in experimental animals. The attention set shift task (ASST) can be performed in rats and mice, wherein a reward is buried in one of two wells filled with a type of material (bedding, paper, sawdust, etc.), that is baited with a specific scent (vanilla, anise, peppermint, etc.). Mice must learn to discriminate between different sensory stimuli to determine which stimuli will reliably lead them to the reward on every trial. When the animal is able to follow the rule consistently (goal selection and maintenance), the experimenter switches the discriminative sensory stimulus. For instance, if the rodent has learned to follow a particular scent to a reward, the experimenter will make the new discrimination dependant on a different dimension (e.g., medium). The rodent must update their goal in order to continue to gain rewards (Birrell and Brown 2000; Tait et al. 2007). Using this type of task, researchers have defined a critical role of noradrenergic innervation of the medial prefrontal cortex (mPFC) in mediating this behavior (McGaughy et al. 2008; Newman et al. 2008; Tait et al. 2007). Similar operant chamber and touchscreen based versions of these tasks have been developed to overcome the time and difficulty in performing the traditional ASST, although extradimensional shifts have not been readily demonstrated (Bussey et al. 2008, 2012; Darrah et al. 2008; Floresco et al. 2008). Using similar behavioral principles, it may be possible to develop a rodent version of the AX-CPT to further assess cognitive translational biomarkers within this subdomain.

3.2 *Subdomain: Response Selection and Inhibition/ Suppression*

This subdomain is comprised of two components: *response selection* and *inhibition/ suppression*. Response selection refers to configuration of neural systems to initiate a motor response that is most appropriate according to conditions of available stimuli and responses (known as a *task set*) to support consistently accurate performance of a behavior (Mayr and Keele 2000; Schumacher et al. 2003). The Eriksen and Eriksen (1974), Simon and Berbaum (1990), and switching Stroop tasks (Stroop 1935) are utilized to assess this behavior. All of these tasks are utilized to induce conflict, or competition between different stimuli or response options, in ways that elicit quantifiable neural substrates. In short, this is done by surrounding target stimuli with either response congruent (low-conflict) or incongruent (high-conflict) stimuli. The behavior of response selection is understood easily enough, though empirically

defining that behavior is challenging as it requires understanding of the introspective status of the participant. Hence, differences in response selection have been substantiated using ERPs, particularly the *lateralized readiness potential* (LRP), recorded by EEG during epochs of these tasks preceding the behavioral response (Luck et al. 2009; Magliero et al. 1984). The LRP is a negative potential generated above the cortex contralateral from where the behavioral response is generated, which begins before the response is initiated. This potential is larger compared to the ipsilateral cortex and therefore is thought to specifically encode preparatory action to make an intentional motor response (Coles 1989; Haggard and Eimer 1999). The magnitude of this potential is smaller when preparing to respond under high-conflict conditions, reflecting a high degree of competition between two responses that are, notably, performed with different hands. This relationship is also present on trials where the participant commits an error. In schizophrenia, however, the amplitude of this response is diminished and delayed compared to controls (Karayanidis et al. 2006; Kieffaber et al. 2007; Luck et al. 2009; Mathalon et al. 2002). This is thought to reflect impairment in selecting the correct response and subsequently anticipating the likely outcome. In other words, these patients may be impaired in accurately perceiving benefits and consequences of their own actions (Luck et al. 2011).

The selected response may, however, require dynamic editing if conflicting information intervenes between selection and response. In order for this to occur, one must *suppress/inhibit* the selected response to avoid inappropriate responding or to make a different, but perhaps, more appropriate response. These tasks commonly take on one of two forms, both of which have versions that have been validated in humans, nonhuman primates, and rodents. The first uses a semi-random probabilistic stimulus presentation (i.e., 80% target:20% nontarget) to engender a *prepotent* response to a target stimulus. On the rarely presented nontarget trials, participants must avoid making the prepotent response. These are *Go/NoGo* tasks, which measure the ability to suppress a prepotent response (Casey et al. 1997; Eagle et al. 2008). This domain is subserved by activity in pre-supplementary motor area (pre-SMA) (Simmonds et al. 2008).

Alternatively, the go stimulus may be presented on every trial, followed on a small number of trials by a cancellation stimulus of varying temporal presentation. When this cancellation signal is proximal to the go stimulus, inhibition of responding is considerably more accurate compared to a signal presented closer to the participants' average RT. These are the countermanding tasks or stop-signal reaction time tasks (SRTT) utilized for measuring *response inhibition* (Boucher et al. 2007; Eagle et al. 2008; Ito et al. 2003; Thakkar et al. 2011). This behavior is supported by activity in the right inferior frontal gyrus (rIFG) (Aron 2007; Aron et al. 2004; Thakkar et al. 2011).

Patients with schizophrenia exhibit diminished response suppression, evidenced by increased Go/NoGo errors of commission (responding on nogo trials) compared to healthy controls, which has been associated with decreased ERP (N275) negativity. This ERP negativity is completely absent when those patients were psychotic (Kiehl et al. 2000). Slowed reaction times (Fallgatter et al. 2003) and increases in Go

condition omissions (Ford et al. 2004) have also been measured in schizophrenia, which is associated with decreased cortical activation (Arce et al. 2006). Response inhibition is also impaired in schizophrenia as they require more proximal stop signals to accurately inhibit the behavioral response, although this effect may be dependent on symptom severity (Bellgrove et al. 2006; Thakkar et al. 2011). Hypoactivity within the rIFG has been associated with the slowed *stop-signal reaction time* (Hughes et al. 2012).

3.3 Subdomain: Performance Monitoring

The final subdomain of cognitive control refers to the ability to monitor efficacy of ongoing goal-directed behaviors (NIH 2011, 2016). The Eriksen Flanker (Eriksen and Eriksen 1974), Simon (Simon and Berbaum 1990), and switching Stroop tasks (Stroop 1935) are also frequently utilized to assess this behavior. Relevant to this domain are the behavioral adjustments following errors or high-conflict conditions. Healthy controls will typically slow their responding to improve accuracy on future trials (Botvinick et al. 2004; Larson et al. 2014). Distinct ERPs related to these behaviors have been characterized using these tasks. When an error is committed in these tasks, a strong error-related negative (ERN) potential is elicited (Yeung et al. 2004). Detection of conflict also elicits N200 (N2) and N450 ERPs, while N2 and slow potentials (SPs) are elicited by conflict adjustment and resolution (Larson et al. 2014). ERNs and N450 ERPs are believed to be elicited by activity in the anterior cingulate cortex (ACC) signaling conflict, whereas N2 and SPs are thought to reflect interactions between ACC and control circuitry within the dlPFC goal maintenance and control circuitry (Botvinick et al. 2004).

ERNs are among the best characterized biosignatures in schizophrenia. Specifically, ERNs are attenuated in schizophrenia (Alain et al. 2002; Morris et al. 2006; Olvet and Hajcak 2008). The reduced magnitude of ERNs in schizophrenia has also been associated with reductions in post-error or post-conflict slowing that would indicate effective detection of conflict and error in a healthy control, which was associated with decreases in relative activity of ACC (Barch and Dowd 2010; Kerns et al. 2005). ERN has also been detected in the nonhuman primates (Amiez et al. 2005; Emeric et al. 2008), and even in the rodent ACC. Using a simple feedback-related negativity task that provided positive or negative feedback following a operant response (e.g., PRL as described above), negative inflection of local field potentials immediately following a negative feedback signal has been characterized (Warren et al. 2015). Given the relatively simplistic task designs with which these signals can be assessed in rodents, ERN has been identified as a strong preclinical biomarker to target cognitive control deficits in schizophrenia (Javitt et al. 2008).

Taken together, these results suggest general impairment within the cognitive control domain in schizophrenia, which has led many researchers to characterize schizophrenia as a disorder of executive function that transcends throughout all other behavioral domains (Hutton et al. 1998; Minzenberg et al. 2009). Clearly, this

interpretation is too reductive for such a complex disorder. But, the consistency of effects within the domain of cognitive control is notable. Patients with schizophrenia exhibit: (1) disrupted frontoparietal connectivity associated with errors of goal selection (Fornito et al. 2012; Frith 1996; Ragland et al. 2007), (2) poor goal maintenance concurrent with disrupted dlPFC activity (Barch et al. 2001; Holmes et al. 2005; MacDonald et al. 2005), (3) poor perception of action and consequence related to diminished and delayed LRPs in assays of response selection (Karayanidis et al. 2006; Kieffaber et al. 2007; Luck et al. 2009; Mathalon et al. 2002), (4) poorer response suppression and inhibition related to hypoactivity in pre-SMA and rIFG, respectively (Aron 2007; Aron et al. 2004; Simmonds et al. 2008; Thakkar et al. 2011), and (5) loss of post-error and conflict slowing compared to controls associated with diminished ERN and disrupted ACC activity (Alain et al. 2002; Barch and Dowd 2010; Kerns et al. 2005; Morris et al. 2006; Olvet and Hajcak 2008).

4 Construct: Working Memory

Working memory describes the set of processes involved in actively maintaining and flexibly updating information relevant to successful completion of a task or goal. These processes are considered to have limited capacity and are susceptible to interference by the acquisition of new information. The term "working memory" was coined in the 1960s by Miller et al. (1960) and was based upon the observation that there appeared to be a consistent limit to the number of items of new information (seven, plus or minus two) that an individual could maintain in memory without reference (Miller 1956). The concept, however, predates the term, as this idea was described in the late nineteenth century by Hermann Ebbinghaus in his iconic list learning studies, and by American physician Oliver Holmes from observations with his patients (for a historic review, see Richardson 2007). The term "memory-span" was coined shortly thereafter and refers the *amount* of information (e.g., numbers, letters, or syllables) that one can reliably reproduce, in order, after a single learning trial. Thus, a "span task" involves presenting an individual with a sequence of stimuli which they are required to repeat verbatim or in a different sequence (e.g., reverse order). Increasing sequence lengths are presented to identify the point at which the individual can no longer reliably remember and reproduce the sequence. This point is inferred to reflect the individual's "span," i.e., the amount, or "capacity," of information that can be retained in working memory.

Span tasks have been included in intelligence tests for children, such as the Stanford-Binet, since the early twentieth century and are still used in contemporary testing. The modern Wechsler Adult Intelligence Scale (WAIS-IV; Wechsler et al. 2008), and the variant for children and adolescents (WISC-IV; Wechsler and Psychological Corp 2004), both include span tasks as measures of working memory. The digit span task is a core test of both the WAIS and WISC and is essentially an elaboration of the earliest span tests. In brief, an examiner reads a string of digits at a rate of 1 digit per second and subsequently the examinee is asked to repeat this string

of digits in order. As the task continues, the examinee is presented with increasingly longer strings of digits and is tested twice at each string length. In addition to this procedure, termed "digits forward," the modern digit span task (WAIS-IV) also includes a "digits backward" component where the string is to be reported by the examinee in reverse order, and a "digits sequencing" component in which the digits are to be reported in ascending order. These components require manipulation of the information being actively maintained in memory. In addition to digit span, the letter–number sequencing (LNS) and arithmetic subtests also contribute to the working memory component, though LNS is optional on the WAIS-IV and arithmetic is optional on the WISC-IV. The LNS test is structured like the digit span however, both letters and numbers are included in each string. The examinee is instructed to report all stimuli in the string but with the numbers first in ascending order followed by the letters in alphabetical order. In the arithmetic test, the examinee is told a short narrative ending with a question that requires basic mathematical operations to solve. Items are time-limited and the examinee cannot use a pen or pencil to arrive at the answer. Thus, the examinee must actively maintain and manipulate the information presented in story form. As such, the span from these separate components can be compared to evaluate the impact of manipulation on the amount of information that can be retained.

When completing span tasks which utilize letters, numbers, or words, individuals frequently report that they maintained the items in memory by repeating the list to themselves in their head, a process referred to as subvocal rehearsal. Notably, a word length effect is observed in which the number of list items that can be correctly recalled is reduced as the number of syllables in each word is increased. With increasing syllable length, more time is required to rehearse the list and it becomes more likely that early items will be forgotten by the time an iteration of subvocal rehearsal of the list has completed (Baddeley et al. 1975). This suggests that the maintenance of a list in working memory through the process of subvocal rehearsal is limited by time, rather than discrete number of items (e.g., words or numbers) included in the list. To avoid confounds related to the processing speed and the verbal capacity of individuals (children especially), alternative span tasks have been developed in which verbal processes are not used in the presentation or recall of items.

The Knox "cube imitation test" was an early span task developed to evaluate individuals who did not speak the language of the examiner. In this task, four cubes of different colors were placed in a line in front of the individual. The examiner would then touch these cubes in a particular order, which the examinee was expected to repeat. This procedure was subsequently adapted into the Corsi block-tapping task, which used nine blocks and follows a similar presentation format to that of the digit span. Though a spatial span task is not included in the Wechsler intelligence scales, the Wechsler Memory Scale (Wechsler et al. 2009) includes a 3-dimensional "Spatial Span Task" analogous to the Corsi block-tapping task.

With the advent of personal computers and mobile technologies, a variety of span tasks have been adapted for automated testing. One of the most popular computerized batteries for assessing cognition, the Cambridge Neuropsychological Test

Automated Battery (CANTAB; Cambridge Cognition 2017), includes a "Spatial Working Memory" test which functions as a 2-dimensional variant of the Corsi block-tapping task. Other working memory tasks benefit from the precision that can be achieved with computer-based testing. The "N-back task," for example (Kirchner 1958), has received considerable empirical attention. The task contains several components which vary in terms of working memory capacity requirements. In the initial component, the "0-back," examiners are presented with a sequence of stimuli (usually letters) on a computer screen and are instructed to press one button on a response box when the presented stimulus matches a pre-defined target stimulus (e.g., the letter "X"), and to press a separate button in response to all stimuli other than the target. During the "1-back" component, a target is defined as any stimulus that matches the last stimulus presented. Thus, to perform accurately the examinee must constantly keep in mind and update in memory the last stimulus which appeared. The capacity demands can be increased further in a "2-back" component in which the target is defined as any stimulus matching the stimulus which appeared two presentations prior. Additional components (e.g., 3-back) may be included and, as one might expect, performance deteriorates as capacity demands increase. The "AX" variant of the continuous performance test (AX-CPT; Servan-Schreiber et al. 1996) is another computerized task of working memory which functions similarly to the 1-back. In brief, examinees are presented with a sequence of letters and are instructed to respond to the letter "X," but only when this letter is preceded by the letter "A."

The concept of working memory originated from the observation that there is a limit to the amount of information that can be retained in memory over a short time period and accordingly, human testing has focused on capacity limits. For the purposes of testing in alternative species however, operational definitions of working memory have largely focused on the aspect of "single-trial learning"; stimuli are presented only during a single learning trial and are only relevant to controlling behavior during that same trial/session (Dudchenko 2004). One approach to assessing single-trial learning in animals is through the use of match to sample (MTS) and non-match to sample (NMTS) tasks. In an MTS task, an animal is given a learning trial in which it is exposed to a stimulus (the sample). Subsequently, in a retention trial the animal is given the choice to respond to two stimuli, the sample and a stimulus not presented during the learning trial. Accurate performance is trained by delivering a reward for selection of the sample, or a punishment for selection of the alternative. The stimulus serving as the sample and comparison can be alternated or changed between trials, ensuring that accurate performance can only be guided by memory of the stimulus presented during the last learning trial. The NMTS procedure is similarly structured with the exception that the animal is trained to respond to a comparison stimulus (non-sample) during the choice phase. Accuracy declines on these tasks with increasing delays between the presentation of the sample stimulus and the choice. As such, delayed MTS and NMTS designs (DMTS and DNMTS) have been used to demonstrate time-limited retention of information consistent with the short-term nature of working memory.

The radial-arm maze (RAM), developed by Olton and Samuelson (1976), is a more elaborate working memory paradigm designed to leverage the natural foraging behaviors of rodents. The maze is composed of a central platform from which a number of arms extend in all directions. The end of each arm can be baited with a food reward. A rodent, placed in the center of this maze, will eventually explore each arm, collecting the rewards. Across sessions of testing, rodents become quicker at collecting all of the rewards and learn to avoid revisiting arms which they have already entered in that session. Reentering an arm which has already been visited is considered an error of working memory. The frequency of such errors serves as the primary outcome measure of the task. By never baiting a subset of arms, working memory performance can be compared with long-term memory. For example, in an 8-arm maze the experimenter may choose to not to bait two of the arms throughout training and testing. Rodents learn that entering these arms never yields reward and begin to avoid them entirely. In contrast with working memory errors (avoiding previously visited arms), avoidance of unbaited arms requires enduring memory shaped over many sessions of training. Entries into these arms are often termed "reference memory errors" and can be compared with working memory errors to determine if a particular manipulation altered one or both processes. While sometimes cumbersome, capacity effects can be inspected by testing animals on the maze with fewer or a greater number of arms available.

Spatial working memory can also be tested in rodents using variants of the Morris water maze (MWM; Morris 1981). In traditional MWM testing, a small pool is filled with water and a platform is submerged below the water line in one quadrant of the pool. When a rodent is placed in the water, it will swim around the pool attempting to escape. While the rodent is unable to escape the pool, it is able to stand upon the submerged platform once it has been found. Across repeated trials, separated by hours or days, animals learn to navigate directly to the platform using the visual cues of the environment. Improvements in latency to reach the platform across trials demonstrate spatial learning. To adapt the procedure for assessment of working memory, animals are tested daily, receiving multiple trials in quick succession. Importantly, the location of the platform is moved on each day of testing (Vorhees and Williams 2006). Across sessions, rodents learn to search out the platform on the first trial of each day and remember where it is situated for subsequent trials. With repeated testing, the improvement of escape latencies between the first and subsequent trials becomes stable and is considered to represent spatial working memory ability. Further, subjects can also be tested with an exposed, visible platform. Latency to reach the hidden platform can be compared to latency with the exposed platform to evaluate the quality of spatial memory and navigation. While both the RAM and MWM can be used to assess spatial working memory, there are notable differences between the tasks. For example, performance on the RAM is developed through positive reinforcement (collection of rewards), the MWM relies on negative reinforcement (removal of an aversive stimuli; i.e., escape from water).

With the RAM and MWM paradigms, spatial positions serve as the items to be remembered, and it is presumed that these spatial positions are inferred primarily from visual cues. However, the extent to which visual cues guide learning can be

somewhat ambiguous. For example, above chance performance on the RAM has been observed even when extrinsic cues are unavailable, suggesting that intrinsic cues related to movement in part guide the performance (Brown and Moore 1997). Other designs, such as MTS or DMTS with objects, suffer similar stimulus control ambiguity, as it is often unclear which aspects of the sample object are being retained in memory and compared with the stimuli presented in the choice phase. This potential confound can be avoided by using stimuli which differ only in one attribute such as the wavelength of light, the frequency of a tone, or the composition of an odor.

Given the ethological relevance of olfaction to rodents in their natural environment, odors may be particularly useful for training the behaviors necessary to assess working memory. The odor span task (OST), developed by Dudchenko et al. (2000), uses odor presentation to evaluate working memory capacity in rodents. To deliver odors, household spices, such as rosemary or turmeric, are mixed with sand and presented to rodents in small plastic cups. Subjects are trained to dig in these cups to retrieve hidden food rewards. To begin the OST, the rodent is placed on a platform with a single odor cup situated on the periphery. The animal is removed from the platform after digging in the cup. After a brief interval, the animal is returned to the platform where two odor cups are situated in randomly chosen positions on the periphery. One of the odors is identical to the preceding trial while the second odor is novel. Only the novel odor cup, however, contains a food reward. On the third trial, three odors are presented. Again, only one of the three odor cups is novel and contains a food reward. The procedure continues in this fashion, typically until 12–24 odors have been presented. Across sessions of training, rodents learn to avoid digging in cups presenting odors they have sampled earlier in the session. Importantly, the same odors are used in each session, albeit in a different order. Thus, only the memory of which odors have been sampled during the ongoing session are relevant to guiding accurate performance. Digging in a cup which does not present a novel odor is considered an error of working memory. With repeated OST testing, performance becomes stable at high levels of accuracy.

The OST procedure can also be trained using spatial positions instead of odors. In this format, each successive trial includes a stimulus cup presented in a novel spatial position along with comparison cups placed in the positions which they occupied on previous trials. As with the OST, only the novel stimulus cup contains a food reward. In contrast to the OST, in which high levels of performance are maintained across the task, performance on the spatial variant deteriorates to chance levels in as few as 12 trials. Thus, the effect of capacity on working memory for odors and spatial positions can be dissociated in rodents. The OST is unique in that it provides a means of assessing working memory capacity in animals; however, it should be noted that OST performance is qualitatively different from patterns observed on human span tasks. In typical human span tasks, such as the digit span, accurate reproduction of the string of digits tends to fail entirely after a capacity threshold (the number of digits presented) is reached. On the OST, accuracy declines steadily as the memory load (the number of odors presented) increases. The OST has also been conducted with human participants and this same pattern emerges (MacQueen and Drobes

2017). Human OST performance corresponded to 0-back accuracy when tested in the same individuals but was not associated with accuracy on more conventional working memory tasks such as the digit span or LNS.

As with the testing conducted to evaluate other cognitive constructs such as attention, or executive function, operational definitions of working memory, and the facets emphasized in testing, have varied across research groups. To aid in the interpretation of data derived from different species, and from different levels of analysis (e.g., molecular, cellular, physiological, and behavioral), the RDoC framework provides a scaffolding for organizing data related to cognition across disciplines. The RDoC matrix of cognitive constructs defines working memory as "the active maintenance and flexible updating of goal-/task-relevant information that has limited capacity and resists interference." Within this framework, four subconstructs are identified: (1) Active Maintenance, (2) Flexible Updating, (3) Limited Capacity, and (4) Interference Control.

4.1 Subdomain: Active Maintenance

Active maintenance refers to an intentional effort to retain in mind the information that otherwise would have limited persistence. For example, if given a new phone number that will be needed later, one may repeat this number to themselves until they are able to dial or write the number down. Active maintenance is fundamental to working memory and is therefore required of all tasks assessing this construct. At a minimum, RDoC advises that tasks of working memory be constructed such that the information which is to be maintained in memory is not available in the external environment during retention and response phases.

The DMTS/DNMTS tasks provide a straightforward model for inspecting active maintenance and have been well validated for this purpose in multiple species. The PFC is critically involved in the active maintenance of information during these tasks (for a review, see van Schouwenburg et al. 2010). Performance is grossly impaired in humans and nonhuman primates with lesions of the PFC. This effect can be reduced by limiting extraneous stimuli, suggesting a role for the PFC in protection from distractibility. Electrophysiological recordings taken from the PFC of non-human primates during an MTS task also demonstrate activity coinciding with the presentation of the sample which is sustained through the delay period in the absence of the sample.

With modern imaging technologies, it is possible to evaluate neural signals occurring during the sample, retention, and choice phases of these tasks in humans as well. For example, using magnetoencephalography (MEG), Cashdollar et al. (2009) evaluated coupling between regions of the brain during visual MTS tasks in which the spatial relationship of items from a sample image (configural information) were either relevant or irrelevant to identifying the matching image in the choice phase. Consistent coordinated activity (theta wave oscillations) was observed during the delay phase. When configural information was relevant to the MTS task,

coupling was observed between frontal and temporal regions, while frontal–parietal coupling was observed with the non-configural task. Further, patients with bilateral hippocampal damage were not impaired on the non-configural task but showed both behavioral impairment and an absence of frontal–temporal coupling during the configural task. These findings demonstrate the use of MTS tasks for evaluating neural substrates of active maintenance and provide further evidence of frontal cortex activity and theta oscillations as substrates of working memory. In addition, it suggests that circuits supporting the maintenance of working memory are to some extent dissociable based upon aspects of the information to be retained. While the role of the PFC for supporting active maintenance is well acknowledged, Cashdollar et al. (2009) also demonstrate the involvement of hippocampus in configural working memory. This finding fits with a robust literature in rodents demonstrating necessity of the hippocampus for spatial working memory tasks such as the RAM (Myroshnychenko et al. 2017), MWM (Morris et al. 1986), and spatial span (Morris et al. 1986), but not nonspatial tasks such as the odor span (Dudchenko 2004).

As noted, all working memory tasks require the active maintenance of information over periods where this information is otherwise unavailable. This can include discrete stimuli, such as the digits of a phone number, or goal-/task-related rules that will need to be applied at a later point. The latter application of active maintenance has been termed "goal maintenance." Tasks suitable for assessing biomarkers of goal maintenance have been previously reviewed by the CNTRICS consortium which recommended the AX-CPT and DXP for this purpose (Barch et al. 2009). Recall that the AX-CPT requires the examinees to respond to the presentation of an "X" only when it was immediately preceded by an "A." Thus, performance on "A-X" (target) trials can be compared with performance on A-Y, B-X, and B-Y trials (all non-targets) to evaluate retention and application of the rule. When goal maintenance is strong, individuals will have the greatest difficulty with A-Y trials as the presentation of the A produces an expectation for a response which should be inhibited if a Y subsequently appears. In contrast, poor goal maintenance is indicated by relative difficulty with B-X trials (Barch et al. 2009). The DXP is analogous to the AX-CPT but uses configurations of dots, rather than letters, for stimulus presentation. Using these tasks in tandem with fMRI imaging activation of the DPFC, anterior cingulate and inferior parietal cortices have been implicated in the process of goal maintenance.

4.2 Subdomain: Flexible Updating

The second RDoC subconstruct of working memory, flexible updating, refers to the ability to enter or remove information from working memory in an ongoing fashion to meet task goals. This process is emphasized in the N-back task as examinees are required to update the list of items being retained on each trial, i.e., adding the most recently presented stimulus and discarding the most distal. One method to assess the updating abilities is to compare N-back performance across the 1-back and 2-back

conditions. Performance on the 1-back can potentially be sustained by familiarity of the last presented stimulus; however, accurate performance on the 2-back requires that the examinee update an item in memory every trial. Using this strategy, Colzato et al. (2013) demonstrated that oral administration of tyrosine (a precursor to dopamine) improved 2-back accuracy but had no effect on 1-back accuracy. The role of dopamine in the flexible updating of working memory has also been demonstrated with the AX-CPT. Moustafa et al. (2008) tested predictions regarding the role of dopamine by assessing Parkinson's disease patients both on and off dopaminergic medications. Patients completed three versions of the AX-CPT in sequence: (1) the standard version, (2) a variant in which numbers were presented as distractors in between presentations of relevant stimuli, and (3) a shift in the rules of the second variant such that numbers served as the relevant stimuli and letters were sometimes presented as distractors. It was hypothesized that by boosting dopamine signaling in the striatum (STR) medication would improve flexible updating of information but also increase susceptibility to distraction. As expected, patients were impaired on the AX-CPT relative to healthy controls. When medicated, accuracy on B-X trials improved across completion of the standard task, demonstrating better adoption/retention of the "only after A" rule. The introduction of distractors impaired performance both with and without medication. Importantly, when the working memory rule was shifted to numbers, medication resulted in performance deficits but only on trials in which letters were also presented as distractors. Further, the presence and salience of distractors increased across the three AX-CPT variants and, as hypothesized, performance deteriorated across these conditions when patients were medicated. Thus, while the flexible updating of working memory was improved by dopaminergic medications, these drugs resulted in greater difficulty ignoring cues that had become irrelevant.

4.3 Subdomain: Interference Control

To maintain information in working memory, it must be protected from interference by irrelevant stimuli (i.e., distractors). This notion forms the basis for the interference control subconstruct outlined in the RDoC framework. While related to active maintenance, interference control is differentiated by the effortful recruitment of resources to resist distraction and can be assessed through the introduction of competing stimuli and/or demands. The N-back and the Sternberg item recognition paradigm (SIRP) tasks are recommended for this purpose. In the standard SIRP task, examinees are presented with a stimulus set of 1–5 numbers that they are instructed to learn. They are then sequentially presented with numbers and instructed to indicate whether each number was, or was not, a part of the stimulus set. Thus, the examinee must maintain the digits in memory for an extended duration and protect against interference produced by the repeated presentation of alternative digits (foils). Capacity of WM can be assessed by altering the size of the initial stimulus set while interference can be manipulated by altering the frequency of foils, or their

similarity to items of the stimulus set. Both capacity and interference manipulations are associated with activation of ventrolateral PFC (vlPFC), dorsal PFC (dPFC), anterior insula, anterior cingulate, and parietal cortices as assessed by fMRI (Bunge et al. 2001). While the regions associated with both manipulations overlap, activations in the right middle frontal gyrus and left inferior frontal gyrus predict accuracy in response to increased interference but are not associated with increased capacity demands (Bunge et al. 2001).

The role of the inferior frontal cortex (iFC) in interference control has also been supported by research using alternative manipulations of the SIRP procedures. For example, in the "Recent-probes Task" (Jonides and Nee 2006; Monsell 1978) participants are given a stimulus set of four letters followed by a probe trial where a single letter is presented. After indicating whether this letter was included or not included in the stimulus set, the examinee is then presented with a new stimulus set (4 letters) followed by a probe. If the probe letter was included in both prior stimulus sets, it can be considered a "recent-positive trial." If the probe was in the earlier stimulus set, but not the current stimulus set, it is considered a "recent-negative trial." Response time and accuracy on these probe trials can be compared to probe trials in which the letter presented was not included in prior stimulus sets ("non-recent positive" or "non-recent negative"). In this format, examinees take longer to respond to, and are less accurate, on negative trials (non-match) if the probe had been included on a recent stimulus set, demonstrating interference with memory for the current stimulus set (Jonides and Nee 2006).

Using positron emission tomography (PET) imaging, Jonides et al. (1998) demonstrated left inferior frontal gyrus activity that discriminated recent and non-recent negative probe trials, an association that has been repeatedly replicated (see Jonides and Nee 2006). Using increased temporal precision of event-related fMRI, the activation of iFC has been isolated to the probe phases of recent-negative trials, providing additional support for the specific role of IFC in interference control. Patients with damage in this area are grossly impaired on interference trials (Thompson-Schill et al. 2002), and activation of this region is associated with performance metrics in healthy examinees (Jonides and Nee 2006). Inferior frontal regions have also been implicated in interference control from studies using the N-back task. In such studies, "lures" are intentionally programmed into the N-back procedure to provide contrasts relevant to interference control. For example, in a 3-back task, a lure trial can be defined as a trial in which the stimulus presented matches the stimulus 2, 4, or 5 positions back but does not match the 3-back position. Accuracy and response time suffer in lure trials compared with non-lure, non-match trials, thus demonstrating the effect of interference (Gray et al. 2003). Consistent with studies using SIRP variants (e.g., recent-probes task), iFC activation has been implicated in interference control on the N-back using the lure trial approach (Derrfuss et al. 2004).

Conceptually, flexible updating and interference control might be considered two sides of the same coin. Thus, it is unsurprising that the neural regions, circuits, and transmitters underlying these constructs overlap to a great degree. As Moustafa et al. (2008) study demonstrated, dopaminergic drugs which improved flexible updating

also result in increased susceptibility to distraction. From a circuit-based perspective, flexible updating may represent a process which initiates active maintenance of information and also modulates the degree to which maintenance can be disrupted by new information (i.e., interference control). A theory proposed by van Schouwenburg et al. (2010) suggests that dopaminergic activity in the basal ganglia serves to modulate active maintenance and interference control processes which are driven by the PFC. In this model, dopaminergic circuits in the PFC produce active maintenance by driving sustained activity in parietal and temporal regions associated with the form of information being retained (e.g., fusiform face area for faces and parahippocampal place area for locations). In tandem, dopaminergic activity in the basal ganglia serves to select information for maintenance by the PFC.

4.4 Subdomain: Capacity

The concept of working memory was born out of the observation that there is a strong limit on the number of items an individual can intentionally keep in memory and report back accurately. As reviewed earlier, working memory has classically been tested with span tasks, which remain popular for clinical assessment. Such tasks, however, are less than ideal for evaluating the neural substrates of capacity as mnemonic strategies (e.g., rehearsal and chunking) can be employed to improve WM capacity beyond typical limits. The RDoC framework recommends change-detection tasks, popularized by Luck and Vogel (1997), for this purpose. In a typical change-detection task, examinees are first presented with an array of objects which vary on a dimension (e.g., color or shape). After a delay, a second array is presented and subjects are asked to report if any items of the array differ from the original array. In some variants, examinees are presented with a cue, prior to the array, which indicates which item(s) may potentially change. Capacity effects can be evaluated by manipulating the number of objects in the array, or the number of items which the examinee is cued to remember. An advantage of the change-detection task is that stimuli can be used which are not easily verbalized, thus minimizing the contribution of mnemonic strategies to the capacity estimate.

Individual differences in capacity estimates derived from the change-detection task remain stable even with extensive training on the task, consistent with expectations regarding capacity limits. To evaluate the neural bases of capacity with the change-detection task, one approach is to cue participants to remember one side of the array (left or right) and evaluate hemispheric differences in brain activity with imaging and/or electrophysiology. Vogel and Machizawa (2004) have reported that the magnitude of ERP from lateral occipital and posterior parietal electrodes is greater in the hemisphere contralateral to the side of the array that examinees were cued to remember. Further, the magnitude of the ERP on the contralateral hemisphere was associated with the number of objects in the stimulus array, plateauing at the point in which behavioral performance begins to deteriorate (i.e., capacity limit). A similar effect has been observed with fMRI using a delayed discrimination task in

which examinees were sequentially presented with nonnatural shapes to remember and subsequently tested with probe trials (Linden et al. 2003). Activity in dlPFC monotonically increased with the number of items to be remembered.

The neural mechanisms subserving WM capacity remain poorly characterized, in part due to the dearth of capacity-relevant tasks available for cross-species testing. One notable exception is the OST (described earlier), which has been tested in rats, mice, and humans. In rats, OST accuracy is selectively disrupted by systemic administration of NMDA receptor antagonists such as MK-801 (Galizio et al. 2013; MacQueen et al. 2011), CPP (Davies et al. 2013a; MacQueen et al. 2016), ketamine (Galizio et al. 2016), or the GluN2B subtype antagonist, Ro 25-6981 (Davies et al. 2013a). Inactivation of mPFC with localized muscimol/baclofen infusion disrupts OST performance without affecting odor discrimination (Davies et al. 2013b). Performance on the OST is similarly disrupted by inactivation of STR, disconnection of mPFC from STR, or localized infusion of a GluN2B antagonist into STR (Davies et al. 2017). Conversely, transgenic mice bred to overexpress GluN2B show increased long-term potentiation in PFC (which is blocked by a GluN2B antagonist), and markedly improved performance on the OST (Cui et al. 2011). These studies implicate the PFC and STR as critical regions supporting WM performance, as has been observed in human WM. Additionally, these findings suggest that NMDA receptor dependent transmission within these circuits is essential for WM performance. The performance deficits produced by NMDA antagonists are magnified by increasing memory load suggesting that NMDA receptor subtypes may play a specific role in modulating WM capacity (Galizio et al. 2013; MacQueen et al. 2011, 2016), though a firm capacity limit has not yet been observed for OST performance (April et al. 2013; MacQueen and Drobes 2017).

Several theories have been proposed to describe the neural mechanisms which give rise to capacity limits. One suggests that the active maintenance of "items" in WM is sustained by synchronized oscillatory neural activity, presumably driven by the PFC, which decays across time. Thus, there may be a limit to the individual streams of maintenance that can be generated. It has been proposed that the neural representation of items in memory is sustained by high frequency gamma oscillations. Multiple items may be stored by keeping each representation out of phase with other. As such, capacity could be limited by the degree to which these higher frequency oscillations can be embedded within a lower frequency data cycle. Within these models, the extent to which capacity limits vary by the modality of the items to be remembered (i.e., sounds, shapes, faces, and odors) remains an open question.

4.5 Working Memory and Psychiatric Illness

Cognitive deficits are a hallmark characteristic of schizophrenia and these deficits predict global functioning, including the extent of social and occupational impairment (Cervellione et al. 2007; Heinrichs et al. 2008). In particular, deficits in WM have received considerable empirical attention. Estimates suggest that patients with

schizophrenia typically perform between 1.0 and 1.8 standard deviations below the mean on tasks of WM (Heaton et al. 2001) and a meta-analysis of studies comparing schizophrenia patients to healthy controls has established that deficits are present irrespective of the modality of stimuli or length of delay used in WM testing (Lee and Park 2005). These deficits are likely driven in part by genetic disposition as they are also observed in the otherwise healthy relatives of patients with schizophrenia (Thermenos et al. 2004). Heritability estimates of WM performance in schizophrenia probands are comparable to estimates in nonclinical samples, in the 30–50% range (Greenwood et al. 2007). Patients with schizophrenia and their first degree relatives both exhibit deficits in goal maintenance and patients are more susceptible to distractors, indicating poor interference control (for a review, see Barch and Smith 2008). Patients also demonstrate impairments in capacity. For example, dlPFC activity increases with memory load in patients as is observed in healthy controls; however, the peak of such activity occurs at smaller loads and subsequently declines, indicating that patients reach capacity limits earlier (Murray et al. 2017; Potkin et al. 2009).

The neurobiological perturbations present in schizophrenia overlap greatly with circuits supporting WM performance. For example, patients with schizophrenia exhibit decreased frontal cortex volume (Cannon et al. 1998), and reduced fronto-striatal connectivity (Lin et al. 2017). The role of dopamine in schizophrenia has long been recognized and dopamine D2 receptor antagonists are a first line treatment for the disorder. While these drugs are effective in controlling the positive symptoms of schizophrenia (e.g., hallucinations and delusions), they do not remediate the cognitive deficits of the disorder. As we have reviewed, dopaminergic manipulations can be expected to impact facets of WM differentially (e.g., active maintenance vs. flexible updating) depending on region of action and receptor selectivity.

The NMDA receptor has also been implicated in the etiology of schizophrenia. Multiple genes relevant to NMDA function are associated with schizophrenia and a variety of abnormalities in proteins modulating NMDA expression and function have been observed in postmortem tissue which are suggestive of hypofunction (Coyle 2012). Administration of ketamine, an NMDA antagonist, to healthy participants produces many of the characteristic features of schizophrenia, including impairment of memory (Newcomer et al. 1999). Cognitive testing in rodents supports the proposition that NMDA hypofunction plays a critical role in WM deficits as a variety of NMDA receptor antagonists reduce WM capacity on the OST (Davies et al. 2013a, 2017; Galizio et al. 2013, 2016; MacQueen et al. 2011, 2016). Given that the OST can be performed in both rodents and humans, this task may have particular utility for screening NMDA-related therapeutics intended to ameliorate the deficits present in schizophrenia.

While progressive deterioration of episodic memory is the hallmark cognitive symptom of Alzheimer's disease (AD), deficits in WM are present as well. Much of the research involving WM impairment in AD derives from neuropsychological testing and as such involves clinical measures of WM. Patients with early or mild AD are impaired relative to controls on the Corsi block-tapping task while digit and word span deficits do not emerge until a later stage of disease progression (Huntley

and Howard 2010). Hence, distinct aspects of WM are observed across different disorders, with each subdomain being a potentially useful biomarker for cognitive dysfunction.

5 Construct: Declarative Memory

Declarative memory refers to the conscious knowledge of facts and events which can be reported (i.e., declared). Within this domain, the memory for facts is referred to as semantic memory (Quillian 1967). For example, reporting that Christopher Columbus sailed across the Atlantic Ocean in 1492 would be a demonstration of semantic memory. Alternatively, episodic memory refers to reportable knowledge of an event (i.e., episode) that one has experienced (Tulving 1972), e.g., reporting that you ate ice cream cake in the kitchen, before opening presents with your mother in the living room, on your 16th birthday. Both semantic and episodic memories can be decomposed into more primary elements which relate to each other in a specific way. The semantic memory of Columbus' journey is composed of the person (Columbus), the action (sailing), the location (the Atlantic Ocean), and the date (1492). The episodic memory of your 16th birthday contains actions (eating ice cream and opening presents), locations (the kitchen and the living room), and individuals (you and your mother) which are ordered in a specific temporal sequence. Declarative memory refers to the processes involved in encoding, consolidation (storage), and retrieval of these individual elements and their respective relationships. Thus, declarative memory includes the processing of the relationship between items that allows for both semantic and episodic memory.

The concept of declarative memory, also referred to as "explicit memory" (Graf and Schacter 1985), derives in part from the observation that patients with amnesia, who are unable to report on events they have experienced, are still able to learn a variety of skills despite having no recollection of learning them. Initially, it was believed that preserved learning/memory skills in amnesia were restricted to motor abilities (Squire 2004), learning to serve a tennis ball, for example. However, it has been observed that amnesics are also able to learn a variety of non-motor skills. For example, individuals with amnesia produced by Korsakoff's syndrome, electroconvulsive therapy, or head trauma are able to learn to read words presented in mirror image at the same rate as unaffected individuals (Cohen and Squire 1980). Thus, declarative memory is contrasted with non-declarative memory, which includes classical conditioning, skill and habit learning, perceptual learning as well as reflexes (Squire 2004).

Declarative memory can be assessed in humans through a variety of paradigms including list and story learning, delayed recall, paired associate learning, or transitive inference. List learning paradigms have historically been the most commonly used tests of declarative memory. In a verbal learning task (VLT), an examinee is presented with a list of words to study and is later asked to report on the words contained on the study list. Memory for the items can be probed simply by asking the

participant to report the items (free recall) or they can be presented with the item and asked if the item was contained on the list (recognition). Both recall and recognition can also be tested after providing the examinee with a cue related to the item (cued recall/cued recognition). These tasks assess episodic memory as individuals are asked to report on memory for specific items presented on a discrete occasion.

Most VLT paradigms assess multiple aspects of episodic memory within the test. Take, for example, the California Verbal Learning Test (CVLT) which is administered by reading a list of 16 nouns to the examinee at the rate of one word per second. This list is presented five times with free recall assessed after each of these trials. Each word from the list is an exemplar from one of four categories (e.g., tools, fruit, clothing, or spices). After the fifth trial, a second list of equal length is presented. Words on this list are exemplars of two of the categories from the first list (e.g., fruits or spices) or exemplars of two new categories (fish or kitchen utensils). After presentation of the second list, free recall is tested immediately and after a 20-min delay (delayed recall). Cued recall is tested by asking the examinee to report all the words from the first list which fit within a particular category (i.e., fruit). Finally, recognition is assessed through 44 trials in which the examinee is presented with a word and asked to report if the word was contained on the repeatedly presented list.

The CVLT yields a variety of measures including total recall from the five learning trials of the first list, free recall of the second list, cued recall, delayed recall, recognition hits, as well as measures of intrusions (reporting items of the second list when queried about items from the first list), and clustering (degree to which recall is organized by category or the serial position of items). Factor analysis suggests that a single component composed of the total free recall, semantic clustering, free and cued recall, and recognition hit measures accounts for about 35–40% of total variance and is associated with other neuropsychological measures (for a review, see Elwood 1995). Other similarly structured VLTs are available; however, the CVLT in particular has received widespread use. The CVLT has been translated for multiple languages, alternative forms have been produced to facilitate repeated testing, and unique variants are available for assessing children (CVLT-C) and adults with dementia (CVLT-D). The CVLT can also be administered by computer, preventing experimenter effects and facilitating computation of certain measures such as clustering.

Another common method for evaluating episodic memory through auditory/verbal learning is the use of story learning paradigms such as the Logical Memory subtest of the Wechsler Memory Scale (WMS-IV; Wechsler et al. 2009). The logical memory subtest begins with instructions explaining that a brief story will be read and that the examinee should remember as many details as they can. After the examiner presents the story, the examinee is asked to describe everything they can remember about the story. Subsequently, the examiner provides the same instructions and presents a second brief story. After the examinee has described everything, they can remember about the second story, it is repeated with instructions to remember the story in detail, "as close to the same words as you can." Recall of the second story is again assessed. Following a delay, during which other tasks are typically completed (approximately 30 min), delayed recall of both of the stories is assessed. A

standardized scoring procedure is used to quantify the detail of the story descriptions reported by the examinee, yielding measures of immediate and delayed recall. The task then concludes with a recognition component in which the examinee is asked yes/no questions about both stories. Though the CVLT and Logical Memory tests differ in format (list vs. story learning), both are tests of verbal episodic memory and the measures derived from these tests show moderate to strong correlations (Delis et al. 1988; Psychological Corporation 2002).

In addition to Logical Memory, the WMS tests auditory declarative memory through the Paired Associates subtest. In this test, the examiner reads a list of eight word pairs that the examinee is instructed to remember. Subsequently, the examiner presents one of the words from each of the pairs and the examinee is expected to recall the other word of the pair. After each report, the examiner gives feedback either indicating that the examinee was correct or providing the correct answer. This process is repeated for four separate lists, with accuracy reflecting immediate verbal/auditory recall. After a delay, during which other memory tasks are administered, recall of the first list is again assessed (delayed recall). This delay is followed by a recognition component in which word pairs are presented with the examinee expected to indicate whether the pair did or did not appear on one of the four lists reviewed earlier. Recall performance on the Paired Associates and Logical Memory tests are moderately correlated (0.48), as are delayed recall scores on the same tests (0.46; Psychological Corporation 2002). It is worth noting, however, that much stronger correlations are observed between immediate and delayed recall measures within the same test (0.85 and 0.81 for Logical Memory and Paired Associates, respectively). Index measures derived by combining measures from these auditory/verbal memory subtest measures of the WMS correlate strongly with a variety of the measures derived from the CVLT (Delis et al. 1988).

Verbal paradigms, such as those described above, represent the most common form of episodic memory testing; however, similar procedures can be used for assessment in visual, or nonverbal auditory domains. For example, in the brief visuospatial memory task (BVMT-R; Benedict et al. 1996) an examinee is given 10 s to study a page with an array of six figures of moderate complexity. The examinee is then given a blank page and asked to draw all six figures in their appropriate location on the page. This procedure is repeated for three trials using the same six figures. After the final trial, the examinee is told to remember these figures and delayed recall is assessed after a 25-min delay. Subsequently, recognition is tested by presenting the examinee with a series of figures and asking whether each was a part of the original display. As with the Logical Memory subtest of the WMS, recall responses are evaluated through a standardized procedure in which responses are compared to examples which outline the credit to be given for various levels of detail. If the drawing abilities of the examinee are questionable, the examinee can be asked to directly copy the figures while viewing the array after mnemonic testing has been completed. A similar task titled “Visual Reproduction” is included on the WMS (Psychological Corporation 2002). In this paradigm, five displays of figures of increasing complexity are sequentially presented to the examinee. The examinee is given 10 s to study each display before being asked to replicate the design on a

blank page. Subsequently, delayed recall, recognition, and direct copy are also assessed. Moderate to strong correlations are observed between immediate and delayed recall scores of the Visual Reproduction subtest with the BVMT (0.66 and 0.80, respectively; Benedict et al. 1996).

The paradigms described are just a few example of the many tests available for assessing episodic memory in the auditory and visual domains. Evaluating semantic memory, however, presents some unique challenges. Because episodic memory refers to conscious knowledge of events an individual has experienced, tests of this domain rely on providing examinees with learning trials and subsequently testing the examinee's memory for these events. This approach is less feasible for evaluating semantic memory as it is conceptualized as knowledge for facts and events that is divorced from memory of the occasions during which the knowledge was obtained (which may have been distributed across time). Thus, one approach to assessing semantic memory is to evaluate an individual's general fund of knowledge for facts or events. Several subtests of the WAIS (Psychological Corporation 2002) accomplish this aim. In the Information subtest of the WAIS, examinees are presented with questions about factual information (e.g., historical figures, familiar objects, and physical principles) while the Vocabulary subtest of the WAIS tests the examinees ability to describe the meaning of words which are presented visually and read aloud. The Boston Naming Test is a similar paradigm which involves presenting a series of illustrations of objects and requesting that the examinee provide the name of the object. Strong performance on these tasks demonstrates a history of intact semantic memory encoding and present retrieval. Yet, when poor performance is observed it is difficult to disentangle the degree to which the individual's level of knowledge is indicative of semantic memory abilities or simply reflects how much exposure the individual has had to these objects or information.

Other tasks of semantic memory focus on the categorization of knowledge, or the relationships formed between objects and/or words. For example, in a test of verbal fluency an examinee is presented with a letter and is asked to report as many words beginning with this letter as they can within a fixed time frame (e.g., 1 min). A category fluency test functions similarly with the examinee instructed to report as many words as they can that fit into a category such as animals, or vegetables. In a typical fluency test, responses for several letters or categories are assessed. The WAIS also provides a test of categorization/relatedness. In the Similarities subtest, examinees are presented with two objects or concepts and are asked to report in what ways these objects/concepts are alike. As with the WMS tests of episodic memory, responses on the Similarities subtest of the WAIS are scored using a standardized rubric of potential responses. Other tasks use measures of response time to evaluate the categorization or relatedness of objects and concepts. In one form of a semantic priming task, examinees are presented with a priming word, such as "doctor." Subsequently, they are presented with a word (e.g., "nurse" or "guava"), or a nonword (e.g., "seunr" or "agavu"). The examinee is instructed to indicate whether the second exemplar is a word or nonword, and their response time is inferred to reflect relatedness. In healthy individuals, response times are typically faster when related words are presented (doctor–nurse) when compared with unrelated words

(doctor–guava). Importantly, each of these tests evaluates retrieval from, rather than encoding into, semantic memory.

5.1 Neural Substrates of Declarative Memory

As introduced earlier, the concept of declarative memory originated from the observation that individuals with amnesia are able to learn from experience to acquire new skills without the ability to report upon the events from which this learning stemmed. Thus, the delineation of declarative memory countered earlier notions of a unitary system of human memory (Tulving 1972). Much of our understanding of the neural processes supporting reportable memory has been ascertained from evaluating patients suffering from conventional amnesia. The case study of one patient in particular has shaped understanding of the brain regions involved. Henry Molaison, known in the scientific literature as "H.M." prior to his passing, suffered from intractable seizures as a child. In an attempt to ameliorate the debilitating effect of these seizures, he underwent an experimental procedure in which sections of his temporal lobes were removed bilaterally. This included large portions of his hippocampus, amygdala, and adjacent cortex. While the procedure was somewhat successful in reducing seizures, he was left with a profound inability to report on experiences occurring after his surgery. The subsequent study of Mr. Molaison's condition provided the first insight into the structures involved in declarative memory.

Since the effects of Mr. Molaison's surgery were observed, case studies of amnesics have repeatedly implicated the medial temporal lobes (MTLs), and the hippocampus in particular, as a critical region for declarative memory (Squire and Zola-Morgan 1988). Due to the organization of brain vasculature, this region is particularly susceptible to damage during ischemic events (Di Paola et al. 2008) which can result in significant anterograde amnesia (the inability to encode new declarative memories after the event). Retrograde amnesia (declarative memory for facts and events prior to the event) may also be present but generally follows a recency gradient (Squire and Zola-Morgan 1988). That is, forgetting is more likely for recent events. That distant memories remain intact suggests that MTL structures do not likely serve as a final storage site for declarative memory. The encoding of both episodic and semantic memory appears to be negatively impacted by MTL insult. This can be difficult to test however, given that most tests of semantic memory assess retrieval rather than encoding, per se. In this regard, case studies of patients who developed amnesia at a young age are particularly informative.

Vargha-Khadem et al. (1997) reported on three individuals who suffered from bilateral damage to the hippocampus in childhood (insults ranged from birth to 9 years of age). While all three demonstrated pronounced deficits in episodic (autobiographical or "autonoetic") memory, each was able to develop reasonable language abilities, progress through schooling, and achieve low-average to average scores on standardized IQ tests. These observations further substantiated the role of

the hippocampus in episodic memory but also suggested that acquisition of semantic memory may not be dependent on the hippocampus proper. Reflecting on these case studies, Tulving and Markowitsch (1998) postulated that the encoding of episodic memory is dependent on the hippocampus and occurs downstream of semantic processing, which is supported by adjacent regions of the MTL (e.g., perirhinal and entorhinal cortex). Additionally, it was suggested that retrieval from either system could occur independently. Thus, semantic encoding deficits would necessarily produce deficits in episodic coding, but impairments of retrieval from either system could occur in isolation. Others have contended that these case studies do not refute the notion of a unitary system for declarative memory (Squire and Zola 1998). This view suggests that insults to the system will produce proportionally equivalent effects on episodic and semantic memory. It is reasoned that while semantic measures of these patients were generally within normal range it cannot be established that such performance does not represent a deficit from otherwise intact function for the specific individual.

A variety of designs have also been employed with modern imaging techniques to evaluate the neural substrates of declarative memory. Several fMRI studies of episodic memory retrieval in healthy subjects have suggested that hippocampal activation is greatest for tasks which require relational/contextual processing, or in which greater amounts of information are recollected (Giovanello et al. 2004; Rugg et al. 2012; Yonelinas et al. 2001). The notion that the hippocampus is particularly engaged during contextual/relational retrieval is further supported by observations from magnetoencephalogram (MEG) imaging in patients and controls in which the extent of hippocampal loss was associated with contextual but not item retrieval performance (Horner et al. 2012). Another case-control fMRI study suggests that hippocampal activation is particularly strong for the retrieval of autobiographical memories as well, which tend to have richer contextual detail (Maguire et al. 2001). Contrasting episodic encoding with retrieval, Gabrieli et al. (1997) reported that retrieval was associated with activation of the subiculum (a component of the hippocampal region), while the encoding of novel items associated with parahippocampal activation, a region providing neocortical input to the hippocampal formation. Declarative memory declines with age and this has been evaluated with fMRI as well. Age was associated with decreased hippocampal activation (observed with recollection) and increases in activity in rhinal cortex (observed with familiarity; Daselaar et al. 2006).

The neurobiology of semantic memory has also been explored with imaging. Given the challenges associated with assessing the encoding of semantic memory, most of these studies have evaluated brain activation during retrieval. A wide variety of tasks have been employed for this purpose and vary substantially with regard to the perceptual attributes of presented stimuli (e.g., visual, auditory, and olfactory) and the modality and complexity of semantic constructs (e.g., visual, auditory, object, action, and emotion). As might be expected, the regional activation patterns observed in these studies have been quite diverse. Following a meta-analysis of imaging studies of semantic memory (Binder et al. 2009), Binder and Desai suggest an "embodied abstraction" theory of semantic networks "in which conceptual

representation is embodied in multiple levels of abstraction from sensory, motor, and affective input" (Binder and Desai 2011). In their neuroanatomical model, dorsomedial and inferior prefrontal cortices are involved in activating task-relevant concepts from multimodal (i.e., "supramodal") convergence zones in temporoparietal cortex. These multimodal convergence zones integrate modality-specific representations from unimodal cortical areas. To the extent that a semantic task requires deeper processing of a concept, unimodal areas supporting sensory, motor, or affective systems may be recruited. Within this model, posterior cingulate cortex and precuneus regions serve to coordinate conceptual information with episodic encoding through the hippocampal formation.

While human imaging studies have significantly advanced our understanding of the neural processes involved in declarative memory, there are limits to what can be learned from the exclusive use of human participants. With standard assessment paradigms, it is feasible to test episodic memory immediately after a learning experience and after modest delays; however, the testing of distant memories presents challenges. If a human participant is asked to report on an experience that occurred long ago, corroborating evidence may not be available to check the accuracy of the report. Further, it is typically not feasible to provide human participants with a structured learning experience and conduct follow-up testing months or years afterward. Thus, the level of experimental control available in research with nonhuman animals provides some critical advantages in the study of memory. Additionally, animal models allow for the application of neuroscience methods which can provide data on the genetic, cellular, and circuit level processes involved in the encoding, storage, and retrieval of memory.

5.2 Animal Paradigms Assessing Declarative Memory

Conscious awareness has traditionally been considered a defining characteristic of declarative memory (Tulving 1972). As such, the modeling of declarative memory in nonhuman animals remains quite controversial. Verbal report has provided the foundation for the assessment of both episodic and semantic memory in humans. Thus, the most commonly used assessments of declarative memory cannot be directly translated for use in nonverbal species. Yet, a variety of procedures have been developed for nonhuman animals which model the critical aspects of declarative memory as outlined by Tulving and others (Tulving and Markowitsch 1998). For example, testing procedures described as assessments of "episodic-like" memory focus on behaviors which demonstrate recall of an event or occurrence (what) at a discrete time (when) and the context in which it occurred (where; Clayton et al. 2001). Paradigms of episodic-like "what–where–when" (WWW) memory have been developed for a variety of species including nonhuman primates, birds, and rodents. In the present review, we will focus on tasks developed for rodents as they represent the most frequently used and readily available animal model.

A commonly used approach to evaluating learning and memory in rodents involves characterizing the amount of time a subject spends inspecting objects that are either familiar or novel. In a typical novel object recognition test (NORT), a subject is first exposed to an object that serves as a sample. Subsequently, the subject is presented with both the sample and a novel object. In this situation, rodents tend to preferentially inspect the novel object, demonstrating memory of prior experience with the sample. Object recognition procedures have long been used to model amnesia in rodents and deficits are observed both in aged animals and following damage to MTL structures (Antunes and Biala 2012; Baxter 2010). In a basic NORT task however, preference for the novel object can be supported by familiarity with the sample. That is, the subject may *know* that it has previously encountered the sample without explicitly *remembering* the occasion. The distinction of knowing, versus remembering, is associated with differential performance of human recall and recognition tasks; recognition can be supported by familiarity alone (knowing) while recall requires remembering (Gardiner et al. 1998).

The basic NORT procedure can be elaborated upon to demonstrate the retrieval of contextual details which suggest episodic-like remembering. For example, Eacott et al. (2005) used E-shaped mazes for an object memory procedure that could not be supported by familiarity alone. Subjects received training in which they were entered into an E-maze at the end of the middle arm and allowed to explore. Two different objects were placed at opposite ends of the long section of the E-maze such that they were visible when the subject exited the middle arm. Subjects were also given the opportunity to explore a second E-maze which had a different flooring material. In this maze, the location of the same two objects was reversed. On each day of training, subjects were given multiple opportunities to explore both of the mazes to demonstrate that the position of objects remained consistent in each of the mazes. Though, new objects were used on each day of training. On the testing day, subjects are again given the opportunity to explore both mazes. Subsequently, each subject was returned to their home cage along with one of the two maze objects. After a period of time, subjects are then reintroduced into one of the mazes. In this maze, the objects had been moved to the end of the opposing arms such that the subject could no longer see the objects upon exiting the middle arm of the E-maze. In this scenario, subjects still preferentially turned toward the arm containing the object that had not been placed in their home cage during the holding period. This behavior demonstrates that the subject identified which maze it was in (presumably by the difference in flooring material) and remembered which side the two objects were located for that particular maze (recall that the position of objects was reversed in the two different E-mazes). Because the two objects were placed out of sight in the test phase, the subject could not simply navigate to the less familiar object, memory of the object locations in that particular context was required. Eacott and colleagues suggest that this form of what, where, and which (context) memory is more akin to human recall of episodic memory.

Though what–where and what–when memory have been demonstrated in rodents through a variety of procedures, it has been suggested that such testing may reflect semantic rather than episodic memory (Hampton and Schwartz 2004). To model

episodic-like memory, Dere et al. (2005) combined three adaptations of the NORT procedure to demonstrate WWW memory. The basic variant of the NORT establishes memory for "what" by demonstrating that the animal can distinguish between a novel and a familiar object. To demonstrate "where" memory in the NORT, subjects are presented with a sample phase in which two identical objects are present in discrete locations of the environment. In the subsequent test phase, both identical objects are again present however, one object is placed in the same location where it was situated in the sample phase while the other object occupies a new, novel location (Ennaceur et al. 1997). Temporal order ("when") can be evaluated with a procedure described by Mitchell and Laiacona (1998). In this NORT variant, the subject is exposed to two sample trials. In the first sample trial, the subject is presented with two identical objects. An hour later, a second sample trial is presented with two new identical objects. In the test phase, the subject is again presented with two objects, one drawn from the first sample trial and the other drawn from the second sample trial. Though both objects are familiar, rats preferentially attend to the object which was presented in the first sample trial, which was less recently encountered. This demonstrates that subjects can discriminate between objects which were encountered an hour apart.

The WWW paradigm combines the aforementioned variants of the NORT into a procedure with two sample trials and a test trial. In the first sample trial, a mouse is entered into a square open field containing four identical objects. The field can be divided into a 3×3 grid containing nine sections. In the first trial, the four objects are arranged such that three of the objects are placed in each of the sections along one wall and the fourth object is placed in the middle section of the opposing wall. The subject is free to explore the maze for 10 min before being returned to the home cage. Fifty minutes later, a second sample trial is conducted. In this trial, four new identical objects are placed in the four corner sections of the square field. Again, the subject is given 10 min to explore freely before being returned to the home cage for another 50 min. Subsequently, a test trial is run with four objects placed in each of the corner sections. All four of the objects in this trial are familiar with two of the objects drawn from sample trial 1 ("old familiar") and two of the objects drawn from sample trial 2 ("recent familiar"). Given the placement of all four objects in corner sections, both of the recent familiar objects are presented in locations in which they appeared in the sample trial. For the old familiar objects however, one is presented in a location consistent with the sample trial while the other is presented in a location which is novel for that object. Subjects are free to explore for 10 min and the time spent inspecting each object is quantified from video recordings. In this test, mice spend significantly more time with the old familiar objects, consistent with prior findings for temporal novelty effects (Dere et al. 2005; Mitchell and Laiacona 1998). This behavior demonstrates memory for what and when. Additionally, mice also spend more time with the old familiar object which has been placed in a novel position for that object relative to the other old familiar object and the mean of the recent familiar objects. This demonstrates memory for what–where in combination with the what–when effect (Dere et al. 2005; Mitchell and Laiacona 1998).

Several aspects of the WWW NORT procedure bolster its use as a test of episodic-like memory. By leveraging the natural novelty preference of rodents, the assessment does not require training or provide extrinsic reward for behavior. Trained behaviors can be problematic for a test of declarative memory because it suggests that acquisition and subsequent performance of the task will be to some extent dependent on perceptual and rule learning abilities of the subject (non-declarative learning processes). Further, encoding of memory in NORT designs is passive; that is, there is no indication that the WWW memory acquired from sample trials will be useful for a future task or goal. This is consistent with human episodic memory which is most often encoded without intentional effort. For example, one can report on what they had for dinner last night without having any expectation that they would be asked to provide such a report at the time the meal was consumed. The WWW information required to perform this NORT procedure is also obtained from unique discrete experiences, consistent with the encoding of events in human episodic memory. Again, information obtained from repeated experiences may depend in part or in whole on semantic encoding or other forms of non-declarative learning. Notably, the WWW procedures described by Dere et al. (2005) can also be extended to other ethologically relevant behaviors. For example, Fellini and Morellini (2013) report on a WWW procedure in which male mice are exposed to time-, location-, and context-fixed exposures to female and dominant male conspecifics. Subsequently, memory of encounters with female conspecifics was demonstrated by context, and time-specific spatial preference for the prior location of the female as well as by measures of urine marking. Memory for dominant male positions was evidenced by the production of submissive posturing behavior. Notably, the preferential production of these behaviors was blocked by hippocampal injections of the DNA/protein synthesis inhibiting drug, anisomycin.

In a procedure which does require rewarded training, WWW memory for odors has also been demonstrated in rodents (Ergorul and Eichenbaum 2004). The procedure involves training rats to dig in small cups filled with a mixture of sand and an odorant (household spices) for a food reward (Dudchenko et al. 2000) and builds upon a previously described paradigm for demonstrating temporal memory in rats with odors (Fortin et al. 2002). In brief, each trial involves a series of four sample phases in which the subject is sequentially exposed to a unique odor cup in a distinct spatial position of an open-field platform. In a subsequent test phase, subjects are presented with two of the odor cups from the earlier sample phases located in the same position in which they had appeared. Across trials, all two odor/location combinations are tested, but in each trial only responses to the odor which appeared earliest in the sample phase progression are rewarded.

Rats perform above chance on all odor/location combinations of the WWW odor task (Ergorul and Eichenbaum 2004) demonstrating temporal memory as previously reported (Fortin et al. 2002). The use of spatial ("where") and odor ("what") memory for guiding the temporal choice has been evaluated through the introduction of probe trials and assessment of approach behavior within the task. Though control tests demonstrated that the rats could discriminate between odors only within 3 cm of the odor cup, rats tend to preferentially navigate to the position of the odor cup which

had appeared earlier in the sample phases. This suggests that spatial memory guides initial approach. Trial accuracy, however, is greater than the percentage of trials on which the subject navigates directly to the correct odor cup. Thus, odor ("what") memory is also involved in accurate performance of the WWW odor task. The use of odor memory is further confirmed by above chance performance in probe trials in which the odor combinations are presented next to each other rather than in their original spatial position. Notably, lesions of the hippocampus reduce WWW odor task performance to chance but spare performance on the aforementioned odor probe trials. As a result, Ergorul and Eichenbaum (2004) hypothesize that the hippocampus is required for the contribution of episodic-like (WWW) memory to task performance. One potential criticism of the WWW odor task is that it is a motivated task and human episodic encoding is considered a passive process. It should be noted, however, that some level of motivation is presumably involved in typical assays of human episodic memory in which event encoding occurs experimentally.

While the most common tasks of declarative memory for humans cannot be directly translated for use in rodents due to the use of verbal stimuli or extensive verbal instruction, transitive inference tasks represent a notable exception. In a transitive inference task, animals receive training on the relationship between pairs of stimuli. For example, with a set of five stimuli (A, B, C, D, and E) training would consist of presenting the pairs A/B, B/C, C/D, and D/E. A hierarchy is applied to the stimuli such that when presented with these pairs the examinee (human or nonhuman) receives reward or positive feedback for responding to the stimulus that would be considered the "greater" of the pair (e.g., A+/B−, B+/C−, C+/D−, and D+/E−). After receiving equal training with each of the pairs, the examinee may come to understand the hierarchical nature of the stimuli (i.e., A > B > C > D > E). This can be tested by presenting novel combinations. If the examinee is presented with A/E, one would expect A to be selected because selection of A has always been rewarded, and the selection of E has never been rewarded, during training. If B/D is presented however, the correct choice is ambiguous because the selection of B or D were rewarded equally often during training. In this case, the choice of B would suggest that the examinee has inferred a hierarchical relationship between the stimuli.

Transitive inference emerges in childhood which can be demonstrated in most young adults using stimulus sets of five or six unfamiliar stimuli, such as foreign characters (Smith and Squire 2005). It has also been demonstrated in both rats and mice using olfactory stimuli (Andre et al. 2012; Davis 1992; Devito et al. 2010; Dusek and Eichenbaum 1997; Fijal and Popik 2011) and with visual stimuli in mice (Silverman et al. 2015; Van der Jeugd et al. 2009). While these tasks have generally been considered to require declarative memory, the procedures lack an important feature of episodic memory tasks in that the primary associations necessary for transitive inference are learned through the repetition of identical discrimination trials rather than a discrete trial-unique experience. Further, the critical tests of transitive inference (e.g., B/D trials in a five stimulus set) represent the emergence of an untrained relationship between stimuli rather than recall of connected aspects of an event. As such, these tasks may tap into semantic memory processes similar to

those necessary for human tasks such as letter/category fluency, or the similarities test of the WAIS. Performance in both rats and mice is grossly impaired by destruction, disconnection, or inactivation of the hippocampus (Devito et al. 2010; Dusek and Eichenbaum 1997; Van der Jeugd et al. 2009), though discrepancies exist in the extent to which the hippocampus is preferentially involved in learning of the stimulus pairs versus successful responding to the novel test pairs. Human patients with damage limited to the hippocampal area are grossly impaired on transitive inference tasks both in the learning of stimulus associations and during the novel pairs tests (Smith and Squire 2005). In an fMRI investigation of transitive inference performance in healthy subjects, inference was associated with anterior right hippocampal activation while trained stimulus associations produced more general activation temporal lobe regions centered around the anterior parahippocampal gyrus. These findings are consistent with the interpretation of transitive inference performance as a measure of declarative memory and further support is provided by the observation that successful inference is most frequently observed when individuals report conscious awareness of the hierarchy inferred from training with the stimulus pairs (Smith and Squire 2005). Transitive inference tasks are particularly useful for studying the neurobiology of semantic memory because they can be conducted in multiple species and in contrast with alternative human measures the procedure allows for assessment of the encoding phase.

5.2.1 Declarative Memory and Psychiatric Illness

As previously described, the concept of declarative memory emerged in large part from observation of amnesics who are unable to report on events they have experienced. Often, such conditions occur as a result of damage to the MTLs from anoxic events or head trauma however, deficits in declarative memory are also present in a variety of psychiatric illnesses as well. For example, progressive deficits in declarative memory are a hallmark symptom of Alzheimer's disease. The diagnosis of Alzheimer's can be difficult to separate from age-related cognitive decline and other conditions in its early stages and a true diagnosis can only be confirmed upon autopsy. Yet, several tests of declarative memory have been shown to predict subsequent cognitive deterioration and diagnosis of Alzheimer's, including the paired associates test of episodic memory and a variety of semantic memory measures such as category fluency, and semantic naming (Blackwell et al. 2004). Some of the earliest neuropathological changes associated with Alzheimer's disease present early within the hippocampal region (Webster et al. 2014). Thus, the observed deficits in episodic and semantic memory concur well with the neural networks associated with declarative memory. In a transgenic mouse model of Alzheimer's disease (i.e., 3×TgAD mice), episodic-like memory deficits have been observed on where–what–which tasks (Davis et al. 2013a, b). As a whole, cognitive deficits in domains such as working memory have been more thoroughly explored with mouse models of Alzheimer's disease as a result of the dearth of well-validated rodent measures of declarative memory. However, the recent advancement of cross-species procedures for transitive inference may speed translation in this area.

Huntington's disease, a genetic condition causing progressive deterioration of motor and cognitive function, also presents with deficits in declarative memory. Cognitive deficits including impaired verbal fluency are apparent before the onset of motor symptoms in Huntington's disease. On tests of episodic memory, Huntington's patients present with deficits in both recall and recognition, though impairment of recognition memory may be less severe or mostly preserved in some individuals (Montoya et al. 2006). Though both Alzheimer's disease and Huntington's disease present with declarative memory deficits, these conditions can be dissociated by task performance. During the early to moderate phases of the diseases, patients with Alzheimer's demonstrate greater forgetting across a delay than do Huntington's patients which can be demonstrated with the Logical Memory and Visual Reproduction subtests of the WMS (Troster et al. 1993). Transgenic and knock-in mouse models of Huntington's disease have been developed and several of these lines (e.g., R6/1, R6/2, and $HdhQ^{7/Q111}$ mice) have been shown to demonstrate impaired performance on novel object and spatial recognition tasks (Giralt et al. 2012). Hippocampal abnormalities such as reduced neurogenesis (Gil et al. 2005) and plasticity (Kolodziejczyk et al. 2014) have also been observed in multiple lines and may play a role in the observed deficits on tasks of declarative memory (Giralt et al. 2012).

Profound declarative memory deficits are also the defining characteristic Korsakoff's syndrome, a condition that can result from Wernicke's encephalopathy produced by a thiamine deficiency (vitamin B1). The affliction produces severe anterograde episodic amnesia with limited retrograde and semantic deficits while non-declarative memory processes are largely spared (Fama et al. 2012). The episodic deficits can be clearly observed with list learning paradigms with performance deteriorating rapidly with delay (Talland 1960). Unlike Alzheimer's disease which typically emerges gradually, the onset of amnesia in Korsakoff's syndrome can be abrupt and maybe accompanied by marked confusion both of which may indicate a poorer prognosis (Lennane 1986). Korsakoff's syndrome can be treated with delivery of high-dose thiamine, but recovery is not guaranteed. The immediacy of treatment is critical to outcome and the extent of residual symptoms will typically be apparent within approximately a week after treatment. Patients with the syndrome who undergo live imaging exhibit decreased volumes in multiple areas of the brain including the ventricles, temporal lobe gray matter, temporal horns, the mammillary bodies, and hippocampus. These reduced volumes are comparable to those observed with AD and the extent of the volume loss in the hippocampus and mammillary bodies are correlated with the magnitude of memory impairment (Sullivan and Marsh 2003). Comparisons with post-mortem brain studies have not always been consistent but have generally implicated hippocampal-thalamic circuits in the mnemonic effects of the disorder (Vetreno et al. 2012). Korsakoff's syndrome can be modeled in rodents either by providing food deficient in thiamine or by direct administration of a thiamine pyrophosphokinase inhibitor. These approaches produce disruption of both reinforced and non-reinforced hippocampus-dependent spatial tasks of declarative memory (Vetreno et al. 2012).

6 Construct: Perception

In order for decisions to be made about a subjects' complex environment – including during cognitive testing – sensory data require transformation and computation to make predictions and guide action. Alterations in sensory processing (perception) have long thought to underlie fundamental deficits in cognitive and behavioral functioning. For example, processing a prestimulus prior to a startling stimulus reduces the effect of the startling stimulus, referred to as prepulse inhibition (PPI). It was postulated that sensory processing deficits as observed in PPI may underlie global processing deficits in patients with schizophrenia (Braff et al. 1978). The fact that impaired PPI is observed across many psychiatric disorders (including schizophrenia, bipolar disorder, Tourette's syndrome, etc.) contributed toward the premise that such cognitive deficits could represent a biomarker of disease state. Numerous reviews on research in PPI have been presented (Swerdlow et al. 1992; Geyer et al. 2001; Powell et al. 2012; Swerdlow et al. 1994, 1999) and so will not be covered here. Focus is placed on the aspects of perception that directly relate to those tests used in human studies.

6.1 Subconstruct: Visual Perception

Human visual perception studies utilize a variety of visual perception paradigms. These paradigms include Face Identification, Contrast Sensitivity, and Coherent Motion. Although many rodent tasks use visual stimuli to train rodents between choices to be made, assessing visual perception requires more complex visual stimuli and their movement. The specifics of each of these tasks vary, but they all focus on a subject's ability to determine the differences between complex visual stimuli. Naturally, utilizing facial stimuli has not been attempted in rodent studies but numerous other studies utilize contrasting feature sensitivity or coherent motion differences.

Although visual processing studies in primates are numerous (Kumano and Uka 2013; Pelisson et al. 2010), rodent paradigms have only recently being conducted. Mice can perform a Coherent Motion visual perception task using touchscreens. Stimuli are presented that move in a random (0% coherence), semi-random (50% coherence), or consistent (100% coherence) manner (Stirman et al. 2016). Although training sessions require multiple days as opposed to single sessions in human studies, this procedure presents an opportunity to assay visual perception in mice in a manner consistent with humans. This task was utilized to demonstrate that mice with a null mutation of the dyslexia candidate gene Dcdc2 exhibit motion perception deficits (Rendall et al. 2017). Although not yet assessed in mouse models of psychiatric conditions, the opportunity exists to conduct parallel studies of visual perception in rodent models as is conducted in human populations.

In another area of visual perception research however, it was observed that rats do not exhibit a Configural Superiority Effect – that is they perform better at visual discrimination with simple images as opposed to whole constructs (Talpos et al. 2016). These results could indicate that in terms of visual perception, rodents may not be the laboratory species of choice.

Other simplified visual tasks exist, such as training and/or stimulus duration challenges in the 5-choice attentional tasks (Carli et al. 1983; Robbins 2002; Young et al. 2004, 2010, 2011, 2013b). They can be useful in terms of identifying brain regions subserving visual stimulus processing. For example, the posterior parietal cortex guides decision-making by rats trained to discriminate between the number of visual flashes and a standard, but it was not needed for auditory processing of the number of clicks (Licata et al. 2017). Such findings are similar to human observations of parietal cortical underpinnings of visual decision-making (Rosenberg and Angelaki 2014). Hence, while the complex visual perception tasks used in human testing may not be replicable in rodent studies exactly, simplified tasks exist that could be useful.

6.2 Subconstruct: Auditory Perception

Auditory processing has been assessed in rodents for decades similarly to humans using startle and PPI paradigms (described above). Many other auditory discrimination tasks exist in rodents. These tasks are often simple in terms of tone discrimination, numbers of clicks, etc. In fact, auditory processing in rodents is likely as keenly understood as any other nonhuman species (Willott 2007). King et al. (2015) describe a thorough review on auditory processing studies in rodents. Here, we focus on RDoC-relevant auditory perception tasks. Although many are human-only (e.g., detection of speech in noise), others are available such as novelty/oddball detection (AKA mismatch negativity) and tone matching.

In an ERP oddball task, rats and humans respond to an infrequent stimulus. Both rats and humans exhibit a higher amplitude to the oddball vs. standard stimuli, although they occurred 1.82 times earlier in rats vs. humans (Sambeth et al. 2003; Shinba 1999). Similar observations were made in mice, wherein NMDA receptor blockade impairs such auditory-evoked potentials (Siegel et al. 2003). Other auditory perceptual learning tasks also exist such as a reward processing task wherein rats have to distinguish between two durations of a 5 kHz tone, 0.5 or 2 s (Der-Avakian et al. 2013). Gradually, rats were rewarded more often for one stimulus over another, resulting in a response bias. This task mimics a human version that actually utilizes visual cues (Pergadia et al. 2014). Hence, while the task may be useful for auditory perceptual learning, it is currently primarily being used to understand reward processing.

An alternative set of tasks to model/understand is an auditory training paradigm used to improve verbal learning and memory in patient populations (Subramaniam and Vinogradov 2013). By training people to distinguish between sweep durations,

challenging them with shorter and shorter durations as performance improved, large effects size improvements have been observed in patient populations. Importantly, performance can be improved in this training task by treatments such as amphetamine even in healthy subjects (Swerdlow et al. 2017). Being able to recreate this task in rodents would enable the neural underpinnings of the task to be discovered, with opportunities to synergistically enhance such training (Swerdlow 2011). Certainly, these auditory perceptual processing tasks would be useful as a biomarker for cognitive dysfunction and a target for synergistic enhancement in psychiatric populations.

7 Construct: Language

Language is a complex construct most recently defined by RDoC as "a system of shared symbolic representations of the world, the self, and abstract concepts that supports thought and communication" [RDoC]. Alterations in language function are identified in numerous psychiatric conditions, such as reduced speech production (e.g., alogia in schizophrenia and autism), and rapidity of communication (e.g., pressured speech in bipolar disorder). Some tics from Tourette's syndrome take the form of verbal outbursts. Language is an important aspect of cognitive functioning, related to social interactions and identifying a person's place in the world. To date, the framework for understanding language in humans across psychiatric conditions has not been well established and requires further work (Elvevag et al. 2016). This difficulty and the complexity of the language construct limits opportunities for translational research.

Initial discussion on the potential for investigating verbal learning and memory in rodents suggested that since the primary mode of communication in rodents is olfaction, such studies could utilize olfactory discrimination testing (Young et al. 2009b). Such discrimination training and testing was covered above however, in Perception. Ultimately, language includes communication and opportunities to measure communication in rodents exist and are explored below.

RDoC has a specific construct on social communication that may prove useful toward understanding translational studies in language. Social communication is defined as a dynamic process that includes both receptive and productive aspects to exchange socially relevant information. The use of these processes is separated into the reception and production of facial and non-facial communication. Naturally, the opportunity for assessing social communication in rodent species is incredibly challenging, as discussed previously with reference to verbal learning and memory (Young et al. 2009b). Although the prospect of facial communication in rodents may not seem likely, advancements in this area have occurred. Differences in the facial shape of the tameness and aggressiveness of rats bred for such behaviors demonstrate significant differences in facial morphology, although these changes may not be consistent across species (Singh et al. 2017). Interestingly, facial indicators of positive emotions have also been reported in rats. These qualitative measurements

utilized high-speed photography and analysis of ear angle, head angle, eyebrow height, etc. (Finlayson et al. 2016). Additional evidence that these results reproduced aspects of positive emotions was that they correlated with ultrasonic vocalizations (USVs), associated with the tickling response (Finlayson et al. 2016). Therefore, perhaps more readily adaptable for use in modeling social communication in humans would be the use of such USV assessment.

The emission of USVs is utilized by mice and rats as a major means of communication. Specific calls have been identified for rats which include 22, 40, and 50-kHz (Simola 2015). The 22- and 50-kHz calls have been associated with negative and positive emissions in young adults respectively, while 40-kHz calls normally arise from young rats. The 50-kHz calls arise from rough-and-tumble juvenile play and being "tickled" by human hands. Interestingly, when 50-kHz sounds are recorded and replayed, rats will engage exploratory activity searching for the emitter of the sound, while avoiding the location of 22-kHz calls. Hence, in terms of social communication it seems that 22- and 50-kHz emission may represent the reception and production of non-facial social processes (Wohr et al. 2017).

Interestingly, drugs often used to model and/or treat psychiatric conditions include acute administration of the dopaminergic agent amphetamine which increased 50-kHz calls as well as approach behavior to such calls (Engelhardt et al. 2017; Rippberger et al. 2015). Interestingly however, other stimulants including cocaine, MDMA, nicotine, and caffeine modulate 50-kHz calls (Simola 2015). This 50-kHz call may simply reflect a heightened motivational or activated state, with larger increases with direct dopaminergic effects. Treatment with NMDA antagonists reduced tickle-induced 50-kHz calls, possibly reflecting a reduced affective state in patients with schizophrenia (Boulay et al. 2013). Buspirone reversed these deficits however, which do not treat negative symptoms in schizophrenia, hence the validity of the model remains to be determined. Rats with null mutation of the SHANK3 gene linked to autism exhibit reduced 50-kHz calls compared to their wild-type littermates (Berg et al. 2018), perhaps mimicking the impaired social communication of children with autism. This work, although in its infancy, could prove to be a novel approach for future research in social communication in rodents.

8 Conclusion

With the advent of the RDoC framework providing quantifiable biomarkers of cognitive dysfunction across diagnoses, there is great opportunity for cross-species research toward delineating neural networks underlying diseased states. By focusing on specific domains of function, understanding the overlap between domains and using that information to identify discrete biomarkers, a procedural framework has been developed to move preclinical researchers beyond attempting to recapitulate categorical distinctions derived purely from clinical observation. For the cognitive constructs of attention, cognitive control, working memory, and declarative memory, there are numerous cross-species paradigms and plenty of rich opportunities for

development and refinement. Although visual and auditory perception capabilities can be assessed similarly across species, there have been fewer studies completed with explicit cross-species consistency. Finally, while interesting paradigms exist for the concept of language – at least communication, there is little evidence for cross-species consistency to date.

Acknowledgements The authors would like to thank Prof. Mark Geyer for the wonderful example he has provided as a translational researcher in the field of psychiatric research. We also thank Ms. Mahalah Buell and Mr. Richard Sharp for their assistance throughout our work. We acknowledge the funding support from NIMH grant R01MH104344-03, UH2MG109168, T32MH018399-30 as well as by the Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center. The authors report no conflict of interest.

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Translational Shifts in Preclinical Models of Depression: Implications for Biomarkers for Improved Treatments

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Contents

Abstract Understanding the neurobiology of major depressive disorder (MDD) remains one of the major challenges in neuroscience. The disease is heterogeneous in nature, and patients present with a varied symptom profile. Studies seeking to identify biomarkers for MDD diagnosis and treatment have not yet found any one candidate which achieves sufficient sensitivity and specificity. In this article, we consider whether neuropsychological impairments, specifically affective biases, could provide a behavioural biomarker. Affective biases are observed when emotional states influence cognitive function. These biases have been shown to influence a number of different cognitive domains with some specific deficits observed in MDD. It has also been possible to use these neuropsychological tests to inform the

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Curr Topics Behav Neurosci (2018) 40: 169–194
DOI 10.1007/7854_2018_44
Published Online: 24 May 2018

development of translational tasks for non-human species. The results from studies in rodents suggest that quantification of affective biases is feasible and may provide a reliable method to predict antidepressant efficacy as well as pro-depressant risk. Animal studies suggest that affective state-induced biases in learning and memory operate over a different time course to biases influencing decision-making. The implications for these differences in terms of task validity and future ideas relating to affective biases and MDD are discussed. We also describe our most recent studies which have shown that depression-like phenotypes share a common deficit in reward-related learning and memory which we refer to as a reward-induced positive bias. This deficit is dissociable from more typical measures of hedonic behaviour and motivation for reward and may represent an important and distinct form of reward deficit linked to MDD.

Keywords Affective bias · Animal model · Antidepressant · Predictive validity · Pro-depressant · Reward

1 Introduction

Major depressive disorder (MDD) has a lifetime prevalence of approximately 10–15% with the incidence of the disorder increasing in modern society (Kessler et al. 2003; Lépine and Briley 2011). As current predictions suggest that this debilitating psychiatric disorder will soon become the leading cause of disability-adjusted life years (Wittchen et al. 2011), strategies which can improve diagnosis and treatment are needed. In psychiatry, diagnosis is almost always made based on the classification of symptoms reported by the patients. The diagnostic statistical manual (DSM-V 2013) provides details of how to interpret the different symptom clusters to categorise and inform diagnosis and treatment. For a diagnosis of MDD, a patient must present with one of the core symptoms, low mood and/or loss of pleasure in daily activities, and at least four other symptoms during the same 2-week period (DSM-V 2013). These can include additional psychological symptoms and changes in homeostatic processes including sleep, appetite and body weight. Most of the other measures used in the assessment of mood disorders (e.g. ICD-10, Hamilton Depression Rating Scale, Hamilton Anxiety Rating Scale, Beck Depression Inventory (Hamilton 1959; Hamilton 1960; World Health Organization 1992; Beck et al. 1996)) are based on similar methods where the patients self-reported experience of their symptoms is used to assess type of illness, severity of illness and response to treatment. This approach poses some major challenges to the field. These subjective self-report measures lack the precision and dynamics of an objective biological marker, and identification of robust biomarkers could greatly improve diagnosis and treatment. A summary of the nature of these challenges in the field of MDD is presented in Table 1.

A biomarker is defined as ‘a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention’ (Biomarkers Definitions

Table 1 Potential benefits of developing valid biomarkers for MDD research

Research area	Problem	Benefits of a biomarker
Diagnosis	Limited capacity to categorise subtypes of disease and link to the best treatment	Improved diagnosis of distinct patient populations which may be linked to treatment options
Response to treatment	Delayed onset of action can mean patients require 4–6 weeks of treatment before changing to an alternative medication	Early detection of response to anti-depressant medication
Aetiology	Lack of consistent findings across populations and animal studies	Early diagnosis, identification of at-risk populations
Animal models	Limited translational validity as current clinical methods cannot be translated to animal studies and vice versa	Improved cross species validation
Drug development	Limited knowledge of disease aetiology	Identification of novel drug targets
Clinical trials	High placebo responses and limited efficacy leading to phase II and II failures Loss of investment from pharmaceutical companies	Improved early detection of efficacy Less likely to be influenced by placebo response

Working Group 2001). An ideal disease biomarker would be sufficiently specific to enable a differential diagnosis and sensitive enough to provide a measure of treatment response (Jentsch et al. 2015). The heterogeneous nature of MDD and its presentation as a cluster of varied emotional and physical symptoms means that the current symptom-based evaluation is limited. Most treatments have a delayed onset of action when assessed using these subjective methods, requiring several weeks of treatment before any decision about efficacy can be made. It is clear that the symptom-based, subjective measures used clinically to diagnose and monitor treatment for MDD are insufficient, and therefore alternative, more objective methods are necessary.

There have been ongoing efforts to try to address the need for biomarkers for MDD research. To date, these attempts have not been able to identify any one method which delivers both scientific and diagnostic merit (Jentsch et al. 2015; Strawbridge et al. 2017). An ideal biomarker would be something which could be detected through a simple blood test. Most biomarker-based research has concentrated on areas linked to the different hypotheses about the cause of MDD (Jentsch et al. 2015; Strawbridge et al. 2017). The monoamine hypothesis is perhaps the most widely researched and has developed from early psychopharmacology studies which linked the monoamine transmitters with regulation of mood (Schildkraut 1965; Hirschfeld 2000; Ruhé et al. 2007). Further support for a key role for serotonin came from the development of the serotonin-specific reuptake inhibitors which were first licenced in the 1990s (Stahl 1998). Despite good preclinical data and some clinical evidence, studies have generally failed to find a consistent deficit in terms of a genetic risk factor or peripheral markers related to serotonin, e.g. the major

metabolite of 5-HT and peripheral 5-HT receptors, in blood, urine or CSF. This may be due to the limitations of peripheral markers in terms of their relationship to the central nervous system, but even measures of cerebral spinal fluid (CSF) have not provided a consistent picture (Uddin 2014; Jentsch et al. 2015; Strawbridge et al. 2017). More recently, other hypotheses relating to the aetiology of MDD have gained traction. These include dysregulation of the stress response and altered feedback via the hypothalamic-pituitary-adrenal (HPA) axis, neurotrophic deficits associated with neuronal, dendritic and/or synapse loss within key regions such as the hippocampus and prefrontal cortex and a role of neuro-inflammatory processes (see Strawbridge et al. 2017 for recent review). Although discussed as separate theories, these hypotheses may not be mutually exclusive, and there are obvious links between these different systems. Both clinical and preclinical studies have identified potential molecular biomarkers associated with the stress and neurotrophic hypotheses, e.g. brain-derived neurotrophic factor (BDNF) and cortisol/corticosterone. Like the monoaminergic markers, consistent deficits which can provide the level of sensitivity and reliability needed for a biomarker have not been forthcoming.

In their recent review, Jentsch et al. (2015) carried out a systematic evaluation of the different proposed biomarkers for depression considering studies based on imaging methods, molecular markers measured in blood, urine or CSF, genetic markers and animal models. This work considered evidence relating to a wide variety of markers related to genetic risk factors, the monoamine transmitters, neurotrophic factors and pro-inflammatory processes. They also reviewed the evidence relating to functional brain imaging studies and activity changes within key neural circuits, e.g. increased amygdala and subgenual cingulate. In their conclusions, they propose that no measure evaluated to date has met the criteria for a suitable biomarker for MDD and further investment is needed to try to address this area of unmet clinical need (Strawbridge et al. 2017). In this article, we consider the questions about biomarkers for MDD from the perspective of our recent animal research and consider whether new research, developing from translational methods to study neuropsychological deficits in MDD, supports the idea of a behavioural biomarker for MDD. It should be noted that the idea of mapping behavioural deficits in psychiatric disorders is not new and endophenotypes in psychiatry (Hasler et al. 2004) and recently the Research Domain of Criteria (RDoC) project (Insel et al. 2010; Cuthbert and Insel 2013; Insel 2014; Nusslock and Alloy 2017) have shown how it is possible to breakdown these complex psychiatric disorders into more objective symptom clusters.

2 Limitations Associated with Conventional Animal Models of Depression

Studies in animals can be particularly useful for research into the brain because they can provide a more readily manipulated system in which to test arising hypotheses. Animal research is less constrained by practical and ethical considerations than

studies in patients and has the benefit of being able to look at both normal and disease models before, during and after a manipulation. Despite the obvious benefits of using an animal model, studies investigating psychiatric disorders in non-human species are particularly challenging. The major problem researchers have faced is being able to recapitulate in an animal a disorder which is ultimately defined clinically by its subjective, self-report symptoms. The limitations of animal models for psychiatry research, particularly for the study of emotional disorders, have been reviewed elsewhere by ourselves and others (Willner and Mitchell 2002; Willner 2005; Cryan and Slattery 2007; Nestler and Hyman 2010; Neumann et al. 2011; Berton et al. 2012; Hales et al. 2014; Commons et al. 2017; Slattery and Cryan 2017) and are therefore only considered briefly here. Additionally, this section only considers methods used to detect a depression-related behavioural deficit and has not considered methods used to induce a disease model or their associated validity. Review articles relating to disease models include (Willner 1995; Willner and Mitchell 2002; Nestler and Hyman 2010; Berton et al. 2012; Slattery and Cryan 2014).

Following the serendipitous discovery of the first antidepressant drugs, researchers sought to develop methods which they could use in animals to predict whether a novel compound may have similar clinical efficacy. Porsolt et al. (1977, 1978) first published work showing that the antidepressant drug, imipramine, could change the behaviour of a rodent when exposed to an inescapable stressor. The animal is first given a pretest where they experience the stressor (e.g. placement in a container of water from which they cannot escape), followed 24 h later by re-exposure to the same apparatus. Pretreatment with an antidepressant before the retest resulted in the animal exhibiting a reduction in immobility time, i.e. they showed increased escape behaviours. The effect was relatively selective for antidepressant drugs although psychomotor stimulants were reported to yield false positive results. The forced swim test (FST) (Porsolt et al. 1977, 1978, 1979) and a subsequent modification of this model for mice, the tail suspension test (TST) (Steru et al. 1985), have been shown to be sensitive to monoaminergic antidepressant drugs. They have provided a valuable tool for the development of the second-generation antidepressants, but concerns about exactly what the FST/TST is measuring and its validity as a model of depression have been raised (for discussion about animal model validity (Geyer 1995; Cryan and Slattery 2007; Der-Avakian et al. 2016)). Specifically, these models have some predictive validity, but this may be limited to monoaminergic drugs. They also have some face validity as the immobility time is thought to be analogous to behavioural despair, comparable to the hopelessness exhibited by depressed patients. However, recent work suggests that the behaviour of animals in this task may be more relevant to stress-coping mechanisms than to MDD (de Kloet and Molendijk 2016; Commons et al. 2017). There are several examples of novel agents which have been shown to have an antidepressant effect in these assays but that have subsequently failed in the clinic or, in the case of rimonabant, were found to increase negative mood and induce suicidal ideation and behaviour in some patients (Stuart et al. 2014). An additional criticism of the FST/TST is their failure to predict the time course of effects of antidepressant drugs, with both conventional antidepressants and ketamine having similar rapid

onsets of action in this model, which is not seen clinically. The relevance of these assays, in terms of construct validity, is also difficult to interpret as the underlying cause(s) of MDD in people are not understood.

An alternative approach to testing for depression-related behaviour in animals has been to study reward processing using tests of anhedonia (Willner et al. 1987; Zacharko and Anisman 1991; Der-Avakian et al. 2016; Slattery and Cryan 2017). Anhedonia is defined as a 'loss of interest in pleasurable activities' and is one of the core symptoms of MDD (Der-Avakian et al. 2016). The most commonly used method is the sucrose preference test (SPT) although other methods including intracerebral self-stimulation thresholds and operant methods to look at reward motivation and effort or learning have also been used (Slattery et al. 2007; Der-Avakian et al. 2013, 2016; Slattery and Cryan 2017). In the SPT, animals are given access to a low concentration sucrose or saccharin solution, and their intake of this versus water is recorded. Normal animals show a preference for the sweet solution, but this is reduced in stress models of depression. In the first studies using the SPT, animals exposed to chronic mild stress were shown to exhibit an impairment in their ability to detect and respond to a low concentration of sucrose (Willner et al. 1987). This deficit was reversed by chronic but not acute treatment with antidepressants suggesting that the assay could better predict clinical outcomes over a timescale which more closely reflected the clinical effects. The problem for the SPT and other measures of reward sensitivity has been that patients with depression do not show similar deficits when tested using methods which measure 'in the moment' pleasure (Amsterdam et al. 1987; Berlin et al. 1998; Scinska et al. 2004; Swiecicki et al. 2009; Dichter et al. 2010). Instead, they show an impaired ability to cognitively value reward in questionnaire measures of prospective, retrospective or hypothetical experiences (McFarland and Klein 2009; Watson and Naragon-Gainey 2010; Strauss and Gold 2012). Reward learning, quantified using methods such as the probabilistic learning task (PRL), seems to be more sensitive to deficits in MDD and patient's ability to use both positive and negative feedback to adapt their behaviour and learn about reward (Pizzagalli et al. 2005, 2008; Pechtel et al. 2013; Vrieze et al. 2013). PRL tasks have been developed and tested for rodents, including a version involving serial reversals, but only a small number of studies have been published to date, and therefore there is limited evidence as to how well the human and animal versions of the tasks compare (Bari et al. 2010; Der-Avakian et al. 2013, 2017). The PRL tasks require animals to learn which response is associated with the better outcomes overall, but they must learn this whilst also receiving false feedback, i.e. the 'rich' stimulus is rewarded 80% of the time and the 'lean' stimulus 20%. In both human and animal versions of the task, the subject must learn to ignore false feedback and continue to respond to the stimulus which gives the better outcome overall. A detailed analysis of the behavioural responses to positive or negative feedback and how the subject adapts their subsequent choice behaviour can also be investigated.

The lack of valid animal models with which to evaluate depression-related behaviour has further limited our understanding of the aetiology of MDD. As neither the tests for behavioural despair nor anhedonia directly map onto clinical measures in people or, in the case of the SPT, an analogous task failed to find a deficit in

patients with MDD, translational studies are similarly restricted. This has led to a situation where the progress made in the preclinical field has failed to translate to clinical benefits. Additionally, it has not been possible to evaluate, in an objective way, hypotheses about the aetiology of MDD and potential biomarkers identified from clinical studies.

3 Why Neuropsychological Deficits in MDD Could Provide a Translational Biomarker?

To try to address the issues relating to a lack of translation between clinical and preclinical research into MDD, our group has sought to develop and validate novel approaches to study depression-related behaviour in non-human species. Alongside the more traditional use of subjective self-report measures to quantify the symptoms of MDD, researchers are also developing neuropsychological tests which could measure behavioural deficits objectively. This work builds on the ideas that negative schema could contribute to the development and perpetuation of mood disorders as first discussed by Beck in the 1960s (Beck 1967). The types of tasks and neuropsychological assessments used are discussed in Roiser et al. (2012a) and Robinson and Roiser (2016). The primary objective has been to try to understand the impact of MDD on behaviours which can be measured using an objective method and, most commonly, a computer-based task which tests a specific aspect of emotional or cognitive behaviour. These approaches therefore present new opportunities for translational studies (Paul et al. 2005). As computer-based assessment methods have developed, researchers have been able to show that patients with depression exhibit deficits in a range of cognitive domains as well as changes in the way they interpret emotional information (Gur et al. 1992; Surguladze et al. 2004; Mathews and MacLeod 2005; Leppänen 2006; Ressler and Mayberg 2007; Williams et al. 2007; Gotlib and Joormann 2010; Elliott et al. 2011; Roiser et al. 2012a). These deficits have been categorised as 'hot' = emotional and 'cold' = non-emotional deficits in cognition (Roiser et al. 2012a; Robinson and Roiser 2016; Fig. 1). MDD has been shown to be associated with deficits in both hot and cold cognition, and there is now increasing evidence to suggest that these different cognitive domains can be modulated by the emotional state of the individual. The terms 'affective biases' or 'cognitive affective biases' have been used to describe these modulatory effects of emotion, and both positive and negative biases can be observed dependent on the state of the individual. Recent clinical work in this field has focused on affective biases in relation to tasks where emotional stimuli are used (e.g. faces, words and pictures), however, for the purposes of our translational work, we have interpreted the term affective biases more broadly and suggest that this term can usefully be applied to describe any cognitive behaviour where a specific effect of the individuals emotional or affective state can be observed, human or animal (Fig. 1).

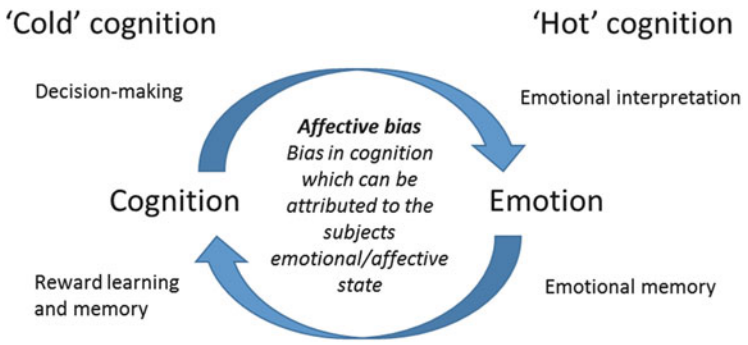

Fig. 1 The term 'affective bias' has been used to describe the biases in emotional processing observed in patients with affective disorders such as anxiety or major depressive disorder. In our attempts to develop a translational approach to studying these neuropsychological mechanisms in non-human species, we have expanded our definition of an affective bias as shown above. We propose that affective biases are not limited to domains of emotional or 'hot' cognition but are also relevant to other cognitive domains sometimes referred to as 'cold' cognition

One of the most commonly reported deficits in MDD is a change in the way emotional information is processed. Specifically, there is evidence that both depressed individuals and at-risk populations exhibit increased negative affective biases compared to healthy individuals (Clark et al. 2009; McCabe et al. 2012; Roiser et al. 2012b). Affective biases can occur at different stages of information processing, for example, in attention, interpretation, memory or decision-making (Mathews and MacLeod 2005). Depression has mainly been linked to explicit memory biases and interpretation biases (Mathews and MacLeod 2005; Mogg and Bradley 2005; Gotlib and Joormann 2010). Memory biases have been reported in both recent (e.g. word recall) and remote (e.g. autobiographical memory) recall tasks (Matt et al. 1992; Mathews and MacLeod 2005; Williams et al. 2007). For example, individuals with MDD preferentially recall negative compared to positive material, remembering ~10% more negative words than positive words (Matt et al. 1992; Gotlib and Joormann 2010). Additionally, patients with depression exhibit enhanced memory for negative compared to positive autobiographical material (Williams and Scott 1988; Brittlebank et al. 1993). There is also evidence of biases in the interpretation of ambiguous information, with depressed individuals being more likely to interpret neutral or ambiguous expressions as negative (Bourke et al. 2010). Attentional biases in depression are less robust, but when they have been reported, individuals with depression may display difficulties disengaging attention from negative material (Mogg and Bradley 2005; Rinck and Becker 2005; Caseras et al. 2007). This suggests that individuals with MDD display negative biases in emotion processing, particularly in explicit memory and interpretation.

To assess whether affective biases could provide a useful biomarker for MDD, it is important to consider (1) specificity of these biases to the disorder and (2) their sensitivity to treatment. Affective biases reported in MDD may be dissociable from those reported in other disorders such as generalised anxiety disorder (GAD).

Specifically, memory biases but not attentional biases have consistently been reported in depression, whereas the converse is found in GAD (Coles and Heimberg 2002; Mogg and Bradley 2005; Marchetti et al. 2018). Unlike MDD, individuals with GAD display a bias towards threat stimuli (Mogg and Bradley 2005). This suggests that whilst affective biases have been reported across affective disorders, the nature of these biases may differ (Gotlib and Joormann 2010). Future research investigating multiple affective biases, and the interplay between them, in both anxiety and depression is needed to gain a better understanding of the similarities and differences in affective biases across disorders and to determine whether a clear dissociation can be found (Klein et al. 2017; Salem et al. 2017). To the best of our knowledge, the field lacks a comprehensive systematic review that synthesises prior work on affective biases across different disorders. Affective biases may also be sensitive to treatment response. For example, acute administration of an antidepressant drug can reverse objective negative affective biases, without changes in subjective mood (Harmer et al. 2009b; Pringle et al. 2011). Interestingly, one study in depressed individuals reported that early changes in affective biases predicted later clinical outcome (Tranter et al. 2009). This is important as it may provide an earlier prediction of the antidepressant efficacy or pro-depressant risk associated with novel treatments. Overall, there is accumulating evidence to suggest that affective biases may provide a useful behavioural biomarker for MDD.

4 Development and Validation of Translational Tasks to Study Affective Biases in Non-human Species

Studies in animals cannot be directly based on the aforementioned emotional processing methods as they use stimuli which cannot be readily translated to use in non-human species. The underlying principles of affective bias are however translatable if we consider that the ability of positive or negative emotions to bias cognitive processes extends beyond only those associated with innately emotional stimuli. The approach used for the animal work has been to look at how affective biases may modulate cognitive behaviours where animals have been trained to associate specific, previously neutral cues, with positively or negatively valenced outcomes, e.g. reward or punishment (Hales et al. 2014). Behavioural studies, using learnt association between a novel stimuli and prediction of either a reward or punishment, are commonly used in animal research. Models such as fear conditioning have been used to study emotional learning and memory, whilst reward-based tasks involving operant responses can be used to study a wide range of cognitive processes including anticipation of reward and reward motivation. Combining these two areas of research, two different types of affective bias task have been developed in the non-human literature. These methods specifically look at decision-making behaviour in response to ambiguity and reward learning and memory.

5 Affective Biases and Decision-Making

The first example of a cognitive affective bias in a non-human species was published by Mendl and colleagues in 2004 (Harding et al. 2004). In a decision-making task, animals were trained to associate specific tone cues with positively or negatively valenced outcomes. Once the animals had learnt these associations, they were presented with intermediate, ambiguous cues, and their response selection was recorded. Animals in a putative negative affective state made less responses in anticipation of reward suggesting a negative bias or 'pessimism'. Several groups including our own have now replicated and extended this work, and affective biases have been reported across a wide range of species from insects to humans (Hales et al. 2014). The underlying principle of the judgement bias task (JBT, also sometimes referred to as an ambiguous cue interpretation task) is that an animal's affective state biases its decision-making behaviour when they are presented with an ambiguous cue, intermediate between the two reference cues they have learnt to associate with positive or negative/less positive outcomes. Different research groups have tested versions of this task including methods using spatial cues, textures, tones and visual stimuli (for review see Hales et al. 2014). The most commonly reported method utilises an operant chamber and tone cues which predict the response required to either obtain reward or avoid punishment or an adaptation to this task where the reference cues predict high or low reward.

There have been a number of different studies now published where animals in a putative negative affective state have been trained and tested in the JBT (Hales et al. 2014; see Table 2 for summary). These studies have found a broadly consistent picture where the depression-like state of the animal is associated with an increase in pessimistic choices. Similar to the findings of Mendl and colleagues, Enkel et al. (2009) reported that a genetic model of depression, the learned helplessness rat, exhibited a negative bias in this task. They could also replicate the effect by treating normal animals with a pharmacological stressor. Studies from Rygula's group have reported similar negative biases in rats exposed to chronic stress (Rygula et al. 2005; Papciak et al. 2013). In the high versus low reward version of the task, we have also observed that exposure to a chronic mild stress manipulation induced a negative bias (Hales et al. 2016). These findings suggest that a depression-like phenotype results in an increase in anticipation of negative events and/or a reduction in anticipation of positive events which compares favourably with the clinical scenario. There have also now been two publications where healthy human participants have been tested using a similar task (Anderson et al. 2012; Aylward et al. 2017). Although the effects observed in these studies appear to relate more closely to anxiety than MDD, studies in clinical populations have yet to be undertaken.

Animal studies provide a useful model for evaluating novel drug targets but require a high degree of predictive validity. Using a range of different pharmacological manipulations, we have tested whether decision-making behaviour in the JBT is sensitive to changes in affective state following acute or chronic administration (Anderson et al. 2013; Hales et al. 2016, 2017). The results for acute treatments

Table 2 Summary of results obtained from the judgement bias task illustrates the effects of acute treatments on decision-making behaviour in this task

Judgement bias task		
Treatment	Dose	Effects
Amphetamine	0.1 mg/kg	Neu (Rygula et al. 2014a, b; Hales et al. 2017)
	0.3 mg/kg	Pos (Hales et al. 2017)
	0.5 mg/kg	Neu (Rygula et al. 2014a, b)
	1.0 mg/kg	Pos (Rygula et al. 2014a, b)
AM251[a]	1.0 mg/kg	Neu (Kregiel et al. 2016)
AM630[b]	1.0 mg/kg	Neu (Kregiel et al. 2016)
Citalopram	1.0 mg/kg	Neu (Rygula et al. 2014a, b)
	5.0 mg/kg	Neu (Rygula et al. 2014a, b)
	10.0 mg/kg	Neu (Rygula et al. 2014a, b)
Cocaine	1.0 mg/kg	Neu (Rygula et al. 2014a, b; Hales et al. 2017)
	2.0 mg/kg	Neu (Rygula et al. 2014a, b; Hales et al. 2017)
	5.0 mg/kg	Neu (Rygula et al. 2014a, b; Hales et al. 2017)
Diazepam	0.3 mg/kg	Neu (Anderson et al. 2015)
	1.0 mg/kg	Neu (Anderson et al. 2015)
Desipramine	1.0 mg/kg	Neu (Rygula et al. 2014a, b)
	2.0 mg/kg	Neu (Rygula et al. 2014a, b)
	5.0 mg/kg	Neu (Rygula et al. 2014a, b)
FG7142	3.0 mg/kg	Neg (Hales et al. 2016)
	5.0 mg/kg	Neg (Hales et al. 2016)
Fluoxetine	0.1 mg/kg	Neu (Anderson et al. 2015)
	0.3 mg/kg	Neu (Anderson et al. 2015; Hales et al. 2017)
	1.0 mg/kg	Neu (Anderson et al. 2015; Hales et al. 2017)
Ketamine	0.3 mg/kg	Neu (Hales et al. 2017)
	1.0 mg/kg	Pos (Hales et al. 2017)
	3.0 mg/kg	Neu (Hales et al. 2017)
Lithium	10.0 mg/kg	Neu (Rygula 2015)
	50.0 mg/kg	Pos (Rygula 2015)
	100.0 mg/kg	Neu (Rygula 2015)
Mazindol	0.5 mg/kg	Neg (Rygula et al. 2014a, b)
	1.0 mg/kg	Neg (Rygula et al. 2014a, b)
	2.0 mg/kg	Neg (Rygula et al. 2014a, b)
Phencyclidine	0.3 mg/kg	Neu (Hales et al. 2017)
	1.0 mg/kg	Neu (Hales et al. 2017)
	3.0 mg/kg	Neu (Hales et al. 2017)
Reboxetine	0.3 mg/kg	Neu (Anderson et al. 2015; Hales et al. 2017)
	1.0 mg/kg	Neu (Anderson et al. 2015; Hales et al. 2017)
	3.0 mg/kg	Neu (Anderson et al. 2015)
URB597[c]	0.1 mg/kg	Neu (Kregiel et al. 2016)
	0.3 mg/kg	Neu (Kregiel et al. 2016)
	1.0 mg/kg	Pos (Kregiel et al. 2016)

(continued)

Table 2 (continued)

Judgement bias task		
Treatment	Dose	Effects
Valproic acid	100.0 mg/kg	Neu (Rygula 2015)
	200.0 mg/kg	Neu (Rygula 2015)
	400.0 mg/kg	Neu (Rygula 2015)
Venlafaxine	1.0 mg/kg	Neu (Hales et al. 2017)
	3.0 mg/kg	Neu (Hales et al. 2017)
Manipulation		Effects
Restraint stress and social isolation		Neu (Hales et al. 2016)
Tickling		Pos (Rygula et al. 2012)

Neu neutral, *Neg* negative, *Pos* positive
[a]Cannabinoid receptor type 1 (CB1) inverse agonist
[b]Cannabinoid receptor type 2 (CB2) inverse agonist
[c]Irreversible anandamide hydrolysis inhibitor

with conventional antidepressants suggest that they have no initial impact on decision-making (Hales et al. 2017). We have observed positive biases with acute amphetamine, whereas induction of an anxiety-like state with FG7142 induces a negative bias (Hales et al. 2016, 2017). The only other publications where antidepressant treatments have been tested acutely have also failed to observe a consistent positive bias with antidepressants but did observe similar, positive biases with amphetamine (Rygula et al. 2014a, b). In contrast, we have observed that chronic antidepressant treatment does induce a positive bias, and this develops over time (Hales et al. 2016). Furthermore, we have recently reported that the rapid-onset antidepressant, ketamine, induces a positive bias following acute administration (Hales et al. 2017). Taken together, these findings suggest that affective biases linked to decision-making behaviour in the JBT may provide an animal model which can predict the efficacy and rate of onset of an antidepressant. This may be useful for drug development, but it is more difficult to know if this task achieves construct validity. Studies in humans have suggested that interpretation biases are modulated acutely by conventional antidepressants (Harmer et al. 2009b; Pringle et al. 2011) suggesting the rat JBT likely involves different cognitive mechanisms. This may arise from the different types of stimuli being used for the animal versus human work and back translation of this rodent task and further studies in patients may help explain these differences.

6 Affective Biases and Reward Learning and Memory

The method developed by our research group, the affective bias test (ABT), has specifically focused on reward learning using a bowl-digging task where animals are required to learn the association between a specific cue (a digging substrate) and a rewarding outcome (a food reward). The task is designed to test how reward associated with two independently learnt experiences is valued by the animal and

whether this can be modulated by the animal's affective state at the time of learning (see Fig. 2). Unlike the JBT, the ABT assay requires a within-subject design where the animal receives the two independent rewarding experiences of equal value but with one learnt during an affective state manipulation and the other learnt under control conditions. To test for any bias, animals are tested in a final session where both previously rewarded substrates are presented at the same time and the animals choices are recorded over a series of randomly reinforced trials. Because the absolute value of the rewards is the same, the animals should not exhibit any preference; however, if the manipulation has induced a bias in terms of the memory of that experience, this would be apparent during the choice test. Consistent with our prediction, induction of a negative affective state using either acute stress or pharmacological treatments resulted in animals biasing their choices away from the treatment-paired substrate (Stuart et al. 2013, 2017; Refsgaard et al. 2016; Hinchcliffe et al. 2017). We have now evaluated a large number of treatments including drugs from different pharmacological classes which have been identified as increasing the risk of depression in patients. In contrast, pretreatment with conventional antidepressant drugs or social enrichment induced a positive bias in the ABT. The biases induced in this assay appear to be specific to manipulations which change affective state, as drugs of abuse and other control compounds failed to induce any effects. Table 3 provides a summary of the findings in the ABT to date. Comparing the effects of acute treatments with these manipulations in the ABT and results for emotional processing biases in humans, the results are remarkably consistent (Pringle et al. 2011). These data also suggest that the ABT can predict the longer-term effects on mood in patients based on the outcome of an acute treatment in this assay.

To improve our understanding of whether the ABT is also able to quantify relevant neuropsychological substrates involved in MDD, we have undertaken more extensive mechanistic studies. Our initial hypothesis behind developing the ABT was the observation that patients with MDD attribute reduced value to what should be rewarding experiences. For example, they are less motivated to re-engage in rewarding activities and become increasingly withdrawn from social and environmental interactions which

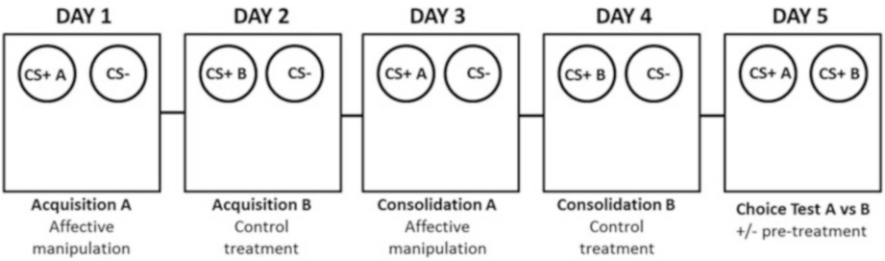

Fig. 2 Schematic representation of the affective bias test protocol illustrating the 5-day procedure which would be used for a single animal. The animals learn to associate substrates CS+ A or CS+ B with finding a food reward. The CS− is unrewarded. Following a counter-balanced design where one substrate is paired during treatment and the other is paired during control conditions, the animals are presented with both previously rewarded substrates and their choices recorded. Animals can also receive a treatment before the choice test to determine if the previously acquired biases can be subsequently modulated

Table 3 Summary of results obtained for the affective bias test illustrating the effects of different acute treatments on reward learning and memory

Affective bias test		
Treatment	Dose	Effects
Agomelatine	0.1 mg/kg	Neu (Stuart et al. 2013)
	0.3 mg/kg	Neu (Stuart et al. 2013)
	1.0 mg/kg	Pos (Stuart et al. 2013)
Amphetamine	0.3 mg/kg	Neu (Stuart et al. 2013)
Aprepitant	0.1 mg/kg	Neu (Stuart et al. 2013)
	0.3 mg/kg	Neu (Stuart et al. 2013)
	1.0 mg/kg	Neu (Stuart et al. 2013)
	10.0 mg/kg	Neu (Stuart et al. 2013)
	30.0 mg/kg	Neu (Stuart et al. 2013)
Carbamazepine	3.0 mg/kg	Neu (Stuart et al. 2017)
	10.0 mg/kg	Neu (Stuart et al. 2017)
	30.0 mg/kg	Neu (Stuart et al. 2017)
Citalopram	0.1 mg/kg	Neu (Stuart et al. 2013)
	0.3 mg/kg	Neu (Stuart et al. 2013)
	1.0 mg/kg	Pos (Stuart et al. 2013)
	3.0 mg/kg	Neu (Stuart et al. 2013)
Clomipramine	1.0 mg/kg	Pos (Stuart et al. 2013)
Cocaine	3.0 mg/kg	Neu (Stuart et al. 2013)
Corticosterone	0.1 mg/kg	Neu (Hinchcliffe et al. 2017)
	1.0 mg/kg	Neg (Hinchcliffe et al. 2017)
	10.0 mg/kg	Neg (Refsgaard et al. 2016; Stuart et al. 2017)
	30.0 mg/kg	Neg (Stuart et al. 2017)
Diazepam	0.3 mg/kg	Neu (Stuart et al. 2013)
	1.0 mg/kg	Neu (Stuart et al. 2013)
	3.0 mg/kg	Neu (Stuart et al. 2013)
Duloxetine	1.0 mg/kg	Pos (Refsgaard et al. 2016)
	3.0 mg/kg	Pos (Refsgaard et al. 2016)
	10.0 mg/kg	Pos (Refsgaard et al. 2016)
Ethanol	800.0 mg/kg	Neu (Stuart et al. 2013)
FG7142	1.0 mg/kg	Neu (Stuart et al. 2013)
	3.0 mg/kg	Neg (Stuart et al. 2013; Hinchcliffe et al. 2017)
	5.0 mg/kg	Neg (Stuart et al. 2013)
	6.0 mg/kg	Neg (Hinchcliffe et al. 2017)
Fluoxetine	0.3 mg/kg	Pos (Stuart et al. 2013)
	1.0 mg/kg	Pos (Stuart et al. 2013)
	3.0 mg/kg	Pos (Stuart et al. 2013)
Idazoxan	1.0 mg/kg	Neu (Refsgaard et al. 2016)
	3.0 mg/kg	Pos (Refsgaard et al. 2016)
	10.0 mg/kg	Neu (Refsgaard et al. 2016)
Interferon alpha	10.0 U/kg	Neu (Stuart et al. 2017)
	100.0 U/kg	Neg (Stuart et al. 2017)
LPS	0.01 mg/kg	Neu (Stuart et al. 2017)

(continued)

Table 3 (continued)

Affective bias test		
Treatment	Dose	Effects
	0.1 mg/kg	Neu (Stuart et al. 2017)
	1.0 mg/kg	Neg (Stuart et al. 2017)
	10.0 mg/kg	Neg (Stuart et al. 2017)
Mirtazapine	0.3 mg/kg	Pos (Stuart et al. 2013)
Montelukast	1.0 mg/kg	Neu (Stuart et al. 2017)
	3.0 mg/kg	Neu (Stuart et al. 2017)
Morphine	5.0 mg/kg	Neu (Stuart et al. 2013)
Nicotine	0.06 mg/kg	Neu (Stuart et al. 2013)
Reboxetine	0.1 mg/kg	Neu (Stuart et al. 2013)
	0.3 mg/kg	Pos (Stuart et al. 2013)
	1.0 mg/kg	Pos (Stuart et al. 2013)
Retinoic acid	1.0 mg/kg	Neu (Stuart et al. 2013)
	3.0 mg/kg	Neg (Stuart et al. 2013)
	10.0 mg/kg	Neg (Stuart et al. 2013)
Rimonabant	1.0 mg/kg	Neu (Stuart et al. 2013)
	3.0 mg/kg	Pos (Stuart et al. 2013)
	10.0 mg/kg	Pos (Stuart et al. 2013)
Sertraline	1.0 mg/kg	Pos (Hinchcliffe et al. 2017)
	3.0 mg/kg	Pos (Hinchcliffe et al. 2017)
	10.0 mg/kg	Pos (Hinchcliffe et al. 2017)
Tetrabenazine	0.1 mg/kg	Neu (Stuart et al. 2017)
	0.3 mg/kg	Neu (Stuart et al. 2017)
	1.0 mg/kg	Neg (Stuart et al. 2017)
Varenicline	0.03 mg/kg	Neu (Stuart et al. 2017)
	0.1 mg/kg	Neu (Stuart et al. 2017)
	0.3 mg/kg	Neu (Stuart et al. 2017)
	1.0 mg/kg	Neu (Stuart et al. 2017)
Venlafaxine	1.0 mg/kg	Pos (Stuart et al. 2013)
	3.0 mg/kg	Pos (Stuart et al. 2013; Hinchcliffe et al. 2017)
	10.0 mg/kg	Pos (Stuart et al. 2013; Hinchcliffe et al. 2017)
Vortioxetine	1.0 mg/kg	Neu (Refsgaard et al. 2016)
	3.0 mg/kg	Pos (Refsgaard et al. 2016)
	10.0 mg/kg	Pos (Refsgaard et al. 2016)
Manipulation		Effects
High value reward		Pos (Stuart et al. 2013; Hinchcliffe et al. 2017)
Social play		Pos (Stuart et al. 2013; Hinchcliffe et al. 2017)
Restraint stress and social isolation		Neg (Stuart et al. 2013; Hinchcliffe et al. 2017)

Neu neutral, *Neg* negative, *Pos* positive

should have positive outcomes. If affective biases in humans can modulate reward learning in the way we have observed in our ABT, this could contribute to the development of these symptoms. We were interested to see if the ABT could be used

to test a theory about the role neuropsychological mechanisms play in the delayed onset of antidepressant drugs first proposed by Harmer et al. (2009a, 2017). In their model, antidepressant drugs act acutely to remediate negative affective biases linked to emotional processing, particularly in relation to the interpretation and memory associated with social cues. These effects reveal that acute and short-term changes in relevant neuropsychological processes can be detected if the assessment method used does not depend on a self-report measure. In fact, Harmer and colleagues have been able to demonstrate that the same individual can show changes in emotional processing but not any subjective experience of a change in mood. The subjective effects are delayed because time and new learning are required to overcome the effects of the previously learnt and negatively biased experiences. If a similar model of affective bias applies to reward learning and memory in the ABT, then this could potentially be studied in our animal task. The first studies we undertook were to compare delayed versus rapid-onset antidepressants to determine if they would differentially interact with the learning aspect of the task versus the memory of the experience, i.e. have effects on recall (Stuart et al. 2015). We found that systemic treatment with ketamine blocked the negative bias induced by stress or a pharmacologically induced negative state. However, ketamine did not bias new learning. In contrast, the monoaminergic antidepressant, venlafaxine, did not attenuate the negative biases once it had been learnt but could modify new learning. We therefore proposed that this dissociation in effects on learning versus memory could explain the differences in the rate of onset of clinical benefit. This theory is further supported by evidence linking these different neuropsychological mechanisms to different brain areas. The effects of venlafaxine could be blocked by lesions to the amygdala, whilst ketamine's effects were localised to the medial prefrontal cortex, and interestingly, they could be replicated by an infusion of the $GABA_A$ agonist, muscimol, into the same area. Although more detailed studies are needed, the ABT seems to involve brain regions strongly linked to emotional processing in humans (Ressler and Mayberg 2007; Murphy et al. 2009). The temporal differences between conventional antidepressants and ketamine could be explained by their differential effects on the neuropsychological mechanisms which are involved in this task. Perhaps the most important observation from the perspective of a behavioural biomarker is the sensitivity of the assay in predicting clinical outcomes. Sensitivity has been observed in terms of antidepressant efficacy, time course of effects and pro-depressant risk.

7 Reward-Learning Deficits in a Modified ABT

One obvious challenge with the ABT is that it uses a within-subject study design and does not readily lend itself to studies in disease models. The assay requires the animal to make a relative assessment of the value it attributes to two different experiences during the recall test, and this can only be achieved if the animal receives both treatments in a counter-balanced design. However, we have routinely used a modified version of the ABT during training to check that each new cohort of rats is able to perform the task before we progress to testing novel manipulations. In the modified ABT, animals receive two rewards during one pairing session and one

reward in the other session (Stuart et al. 2017). This means that each substrate becomes associated with a different absolute value of reward and the animal should bias its choices during the preference test towards the higher value reward-paired substrate. We have called this a reward-induced positive bias. As part of our interests in how affective states influence different aspects of cognition, we have now also tested whether reward learning in this modified ABT is impaired in animals in putative negative affective states. We hypothesised that the acute negative biases associated with the pro-depressant manipulations may, if experienced chronically, induce a more generalised impairment in reward learning and memory which could be measured using this modified assay. We compared how animals performed in the modified ABT with more typical measures of anhedonia by also carrying out an SPT in the same cohort. We have now used this assessment in animals in putative negative affective states, induced by chronic treatment with interferon alpha and retinoic acid, and found that these treatments result in an attenuated reward-induced positive bias without affecting consummatory anhedonia in the SPT (Stuart et al. 2017). We have tested animals with chronic neuropathic pain, exposure to early life adversity, or chronic stress (unpublished observations) and observed similar results. All the manipulations attenuated the reward-induced positive bias in the ABT, but only chronic stress also affected sucrose preference. Studies in animals exposed to sub-chronic phencyclidine (PCP) to induce a model of schizophrenia have also observed a similar deficit in reward learning (Sahin et al. 2016). Previous studies using the PCP model have also generally failed to observe any changes in performance in the SPT, although there are some exceptions (Vardigan et al. 2010; Neill et al. 2014). This raises the possibility that the deficit we observe in reward learning is independent of changes in consummatory anhedonia. It also suggests that this is a form of affective bias, where the deficit is related to the ability of the animal to appropriately learn about the reward-cue association and then use this information to guide subsequent behaviour and choices when re-presented with those cues. If we reconsider the biases observed in the JBT, they may concur with this observation as the reduced anticipation of reward seen in this task occurred over a delayed time course, possibly due to a mechanism involving learning.

Impaired ability to learn about reward and use this information to guide behaviour has been discussed in relation to the clinical literature (Treadway and Zald 2011, 2013; Romer Thomsen et al. 2015; Thomsen 2015; Whitton et al. 2015) but not specifically considered from the perspective of an affective bias. However, if the deficits are related to learning and memory for reward rather than the perception of reward, as our animal data suggests, then this would mean that the anhedonia in MDD is potentially more about cognitive processes related to reward rather than hedonic mechanisms. The ability to quantify anhedonia in animals using tests which measure perception of reward (SPT) or motivation for reward (progressive ratio tasks) has made these appealing models for depression research. As discussed earlier however, the SPT seems to be particularly sensitive to impairments induced by chronic stress but is not always observed for other models of depression. Our findings suggest that the modified ABT is sensitive to impairments in reward learning and memory resulting from different biological or psychological substrates.

8 Summary of Preclinical Affective Bias Studies and Their Relationship to Clinical Findings

The field of affective biases in non-human subjects has developed over the last 13 years following Mendl et al. first publication in 2004. The studies in the JBT suggest that decision-making behaviour in a wide range of species is biased by the animal's affective state. The response to antidepressants is not yet fully understood as studies are limited, but they appear to mirror the clinical time course of subjective effects on mood rather than the more immediate effects seen in the clinic when using emotionally valenced stimuli. This may be a consequence of the differences in methodology as the animal studies use cues paired with positive or negatively valenced outcomes rather than emotional cues. Results in the ABT have shown that reward learning is biased by acute changes in both positive and negative affective states. The task can predict potential antidepressant efficacy and pro-depressant risk. We have also identified a novel deficit in reward processing using a modification to the ABT and suggest that learning about the value of reward and using this information to guide subsequent behaviour are impaired. This effect is distinct from consummatory or motivational forms of anhedonia more traditionally measured in animals, but how this relates to measures of anhedonia in patients requires further investigation. There have also been advances in clinical research into affective biases suggesting that these may provide an objective and dynamic method to categorise disease and detect early responses to antidepressants. These early responses may also predict longer-term outcomes. Taken together, these data suggest that studies investigating the impact of affective state on different cognitive domains may be a useful approach for translational studies into MDD. Although the tasks used in animals are not identical to those used in patients, because of species differences, they have shown that cognitive processes in non-human species are sensitive to the animals' affective state. The data for phenotypic models and pharmacological studies support improved validity over conventional models such as the FST/TST.

9 Conclusions: Affective Biases as a Behavioural Biomarker for Translational Studies into MDD

9.1 Specificity to Disease State

Evidence to date suggests that certain cognitive processes are modulated by affective states in both human and non-human species. It is not yet possible to fully evaluate how specific these deficits are to MDD as most studies only look at a limited number of tasks and in a single disease state. However, more detailed analyses involving assessment of biases across different cognitive domains and in different psychiatric disorders could reveal a pattern of deficits which provides a biomarker. For example, a meta-analytic study comparing anxiety versus MDD suggests that attentional

biases are associated with anxiety, whereas emotional memory is negatively biased in MDD (Marchetti et al. 2018). In their analysis they found that memory biases were reliably and strongly related to depression, whereas attentional biases were not. They also found no overlap between attentional biases and memory biases. In contrast Mogg and Bradley (2005) reported attentional biases in generalised anxiety disorder but did not find similar deficits in MDD. Our animal studies have also shown that biases in reward learning are observed in animals where the depression-like phenotype has been induced using a variety of different methods including social and pharmacological. Further developments in both fields, and particularly studies where the methods used are more closely aligned, will help determine the validity of this approach.

9.2 Sensitivity to Treatment

Acute effects of antidepressants in emotional processing tasks and the rodent ABT suggest that early positive biases in these tasks may predict longer-term clinical outcomes. Of the pharmacological agents tested to date, evidence strongly supports specificity to antidepressant and pro-depressant effects. The effects of antidepressants in the JBT do not directly mirror the interpretation biases observed when acute antidepressants are tested in people; however, there are potential benefits from a preclinical drug development perspective. If the preliminary data obtained to date are replicated with other antidepressants, then this assay may provide a valuable tool for predicting the rate of onset of antidepressants. The current findings suggest that rapid-onset antidepressants such as ketamine induce an immediate effect on decision-making behaviour, whereas the effects of conventional antidepressants develop more slowly following chronic treatment.

10 Future Directions

The similarities between the methods used to study affective biases in humans and animals have enabled researchers to more closely align the two fields and have also revealed the potential value of these behavioural deficits as a biomarker. Future validation of this approach would benefit from a wider evaluation of the cognitive domains which are modified by affective biases and how these differ between affective disorders such as anxiety and MDD and other disorders such as schizophrenia. As non-human studies are limited in terms of using emotional stimuli, studies focusing more on tasks involving cognitive processes linked to associative learning between novel cues and positive or negatively valenced outcomes would be a priority. The complementarity between these animal and human methodologies will also make studies to investigate the relationship between affective biases and the aetiology of MDD feasible.

Acknowledgments ESJR currently has research funding from the MRC, BBSRC, Wellcome Trust and academic research grants from Boehringer Ingelheim and Eli Lilly. She has also previously received research funding from MSD, Pfizer and GSK. Previous support which has contributed to the development of this work includes funding from RCUK and the British Pharmacological Society Integrative Pharmacology Fund. CS is funded by a Wellcome Trust doctoral training studentship. JKH is funded by a University of Bristol PhD Studentship.

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Reappraising Preclinical Models of Separation Anxiety Disorder, Panic Disorder, and CO_2 Sensitivity: Implications for Methodology and Translation into New Treatments

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Curr Topics Behav Neurosci (2018) 40: 195–218
DOI 10.1007/7854_2018_42
Published Online: 24 April 2018

Abstract Separation anxiety applies to multiple forms of distress responses seen in mammals during postnatal development, including separation from a caregiver. Childhood separation anxiety disorder is an important risk factor for developing panic disorder in early adulthood, and both conditions display an increased sensitivity to elevated CO_2 concentrations inhaled from the air. By interfacing epidemiological, genetic, and physiological knowledge with preclinical animal research models, it is possible to decipher the mechanisms that are central to separation anxiety and panic disorders while also suggesting possible therapies. Preclinical research models allow for environmentally controlled studies of early interferences with parental care. These models have shown that different forms of early maternal separation in mice and rats induce elevated CO_2 respiratory sensitivity, an important biomarker of separation anxiety and panic disorders. In mice, this is likely due to gene-environment interactions that affect multiple behavioural and physical phenotypes after exposure to this early adversity. Although several questions regarding the causal mechanism of separation anxiety and panic disorder remain unanswered, the identification and improved understanding of biomarkers that link these mental health conditions under the guise of preclinical research models in conjunction with human longitudinal cohort studies can help resolve these issues.

Keywords Acid sensing ion channels · CO_2 sensitivity · Epigenetics · Gene-environment interaction · Genetics · Pain · Panic disorder

1 An Epistemological Preamble

In order to comprehend and appreciate the role of biomarkers in psychiatry, it is beneficial to address two fundamental matters regarding the nature of psychiatric disorders. First, what are psychiatric disorders? Second, how are biomarkers integrated in the current epistemology of psychiatric disorders? Our attention will then be directed to panic disorder (PD) and separation anxiety disorder (SAD), CO_2 hypersensitivity as their common biomarker, and their connection with preclinical animal models involving early life interference with parental care. To conclude this chapter, potential mechanisms and therapeutic applications will be discussed.

1.1 Revisiting the Classification of Psychiatric Disorders

Psychiatric disorders are multifactorial in nature and depend on human classification for their formulation. This means that social and interpersonal factors are integral in the aetiology of psychiatric disorders and societal, historical, and philosophical trends influence classification systems. As a consequence, psychiatric disorders do not have the same degree of consistency as other objects that are independent of human notions and activities, such as π, or the elements from the periodic table which exist whether we recognise them or not. Yet, psychiatric disorders cannot be reduced to purely social constructs developed by cultures and societies who

categorise them as specific kinds (Kendler et al. 2011). On the contrary, we know that psychiatric disorders (as essentially every human behaviour) are at least partially influenced by genetic factors (Martin et al. 1997). This speaks against psychiatric disorders as pure culturally generated phenomena as seen in the constructivist's vision (Kendler 2016). One such example that refutes constructivism is that of dyslexia, which is moderately heritable and based on similar dysfunctional brain connections regardless of an individual's language and culture (Astrom et al. 2007; Paulesu et al. 2001; Eicher and Gruen 2013). While the biological basis of reading disability/dyslexia appears to be shared globally by those with this condition, its incidence and prevalence vary across populations (Paulesu et al. 2001). For example, the lowest frequency occurs in cultures with transparent languages (e.g., languages with a 1:1 unambiguous and univocal phoneme-grapheme correspondence, such as the Italian language), while the highest prevalence occurs in cultures whose languages have multiple options of phoneme-grapheme correspondence (Lindgren et al. 1985). This means that a child with a moderate biological predisposition to struggle with written language would not likely meet the criteria for dyslexia if raised in Italy, which has an environment with a transparent language. Conversely, the same individual would likely be diagnosed with dyslexia in an English-speaking country where phoneme-grapheme correspondence is often not univocal.

Added to the cultural-geographical dependency of psychiatric disorders is an evolutionary-temporal dimension. This dimension is relevant to the topic of biomarkers and brings about the reflection that the very same biologically rooted processes/aetiological factors may act as neutral/adaptive or be labelled as maladaptive, depending on temporal and cultural factors. Continuing with the example of dyslexia, it could be argued that the frequency of common genetic polymorphisms that underlie this reading disability have likely remained unchanged over the course of millennia and without being selected against. However, the detrimental effect of dyslexia has only surfaced in recent human history and within the context of specific cultural phenomena involving the emergence of written language along with societal pressures for efficient reading of written language. From a complementary yet opposite vantage point, the DRD4–7 repeat allele polymorphism associated with ADHD underwent positive selection since its appearance as a mutation some 40,000 years ago, suggesting positive effects on adaptation and better Darwinian fitness (Ding et al. 2002). In the present day, the same genetic variant is associated with maladaptation deriving from ADHD in the industrialised nations.

These examples illustrate that while a biological basis can be identifiable in psychiatric disorders, societal norms can influence whether individual variations are classified as a dysfunction and hence associated with a diagnosis. Thus, as mental health disorders appear to be inextricably rooted in our biological, cultural, and evolutionary history, neither the essential nor the social constructs are fit to answer the question of their nature satisfactorily.

A third approach to understanding psychiatric disorders is the so-called pragmatist/instrumentalist model (Zachar 2002; Zachar and Kendler 2007). This model is based on the concept that the classification of a condition should be made on the basis of categories that best help achieve pragmatic objectives, such as a reliable

diagnosis, prognosis, or treatment (Kendler 2016). According to this method, usefulness is the primary element that informs psychiatric classification. While the pragmatic approach has several appealing aspects, including removing itself from metaphysics and bringing psychiatry closer to the domain of life sciences, it is criticised for its relative insensitivity to the dimension of suffering inherent in psychiatric disorders (Kendler 2016). This approach also fails to emphasise issues such as the mind-body relationship or the reality of psychiatric disorders (Kendler 2016).

The practical kinds model is consistent with the non-theoretical-descriptive approach that became widely adapted following the advent of the DSM-III. Among the several advantages of this approach is the reduction of inferential reasoning and better replicability and reliability of diagnoses. As non-theoretical and operational classifications of mental health disorders became the standard for research, grant writing, teaching, and clinical practice, another problem arose from the reification of diagnostic categories (Kendler 2016; Hyman 2010). Reification is literally the act of mistaking a proxy for the object that it was originally meant to identify. In psychiatry, it is the conceptual mistake of assuming that mental health disorders are actually just the DSM criteria (Kendler 2016). Reification is deceptive for at least two reasons: Firstly, it acts as a form of implicit reasoning that can emerge from routinely applying operational criteria. Secondly, there is concern that highly operationalised psychiatry and the ensuing reification of psychiatric diagnoses may have contributed to the impoverishment of psychopathology, research, clinical work, and teaching (Jablensky 1999).

1.2 What Is the Role of Biomarkers in Understanding Psychiatric Disorders?

The current and future status of biomarkers must be harmonised with the questions we, and many others before us, briefly addressed so far. Just as a diagnosis of a psychiatric disorder does not necessarily coincide with the mental health condition it seeks to identify (rather, it creates reliable proxies to map its construct), biomarkers do not constitute a 'more true' entity than the psychiatric diagnoses they aim to characterise. Since biomarkers are replicable indicators of one or more mechanisms that fall under the framework of a condition, they can facilitate the process of moving psychiatry towards the causally based classification common to the rest of internal medicine. As such, biomarkers fit well into a model of psychiatric illnesses as objects that are not defined in terms of essences but rather as complex mutually reinforcing networks of causal mechanisms, the so-called homeostatic or mechanistic property cluster (MPC) kinds (Kendler et al. 2011; Boyd 1991, 1999; Battaglia et al. 2012).

Among the attractive features of MPC is their multidimensional matrix structure, whereby the matrix items (in psychiatry, the brain/mind states) are clustered into vicinities based on similar developmental, evolutionary, and physiological causal

mechanisms and constraints (Kendler et al. 2011). While MPC supports the expectation that underlying most psychiatric disorders there are robust explanatory structures to be discovered, it is flexible enough to tolerate that such explanatory structures can be 'messy' and hard to identify and need some level of abstraction. What matters most in the MPC perspective are the patterns of complex interaction between behaviour and physiology that have arisen through development, evolution, and interactions with the environment (Kendler et al. 2011). The MPC framework thus appears as a reasonable epistemological foundation to receive and integrate the information yielded by biomarkers.

1.3 Identification of Biomarkers for Psychiatric Disorders via a Biomedical Approach: The Case of Panic Disorder, Separation Anxiety Disorder, and CO_2 Sensitivity

While obstacles towards an aetiologically based mental health classification system are intrinsic, there are examples of successful diagnostic separation in psychiatry based on typical biomedical methods. These examples provide useful methodological guidance towards the development of translational biomarkers and new treatments. One such example is D. F. Klein's pharmacological dissection of the 'anxiety neuroses' (Klein and Fink 1962). By differentiating between anxious patients who responded to tricyclic antidepressants versus those who responded to benzodiazepines, Klein split the 'anxiety neuroses' and paved the way for the definition of panic disorder (PD) and its differentiation from generalised anxiety disorder (GAD). Subsequent research showed that PD and GAD have at least partially different aetiologies, different responses to treatments, and different developmental antecedents (Hettema et al. 2001; Schenberg 2016a). The successive step to support PD as a valid diagnostic category was to show its developmental continuity with childhood separation anxiety disorder (SAD), which in turn responds to imipramine (Gittelman and Klein 1984; Klein 1995; Kossowsky et al. 2013; Klein et al. 1992).

Another fundamental element was to demonstrate that both PD and SAD are characterised by emotional and respiratory hypersensitivity to CO_2-enriched air mixtures (Klein 1993; Pine et al. 2000). This finding led Klein to hypothesise the presence of a latent biological trait relating to an unbalanced suffocation alarm system. Our successive work was to develop a translational and interspecies (human and murine) model that rests on CO_2 hypersensitivity as an important biomarker of SAD and PD. The rationale for this pursuit is that translational research is not bench-to-bedside but rather a two-way process (Battaglia et al. 2014; Rutter 2016). We also speculated that by taking both genetics and environmental factors into account, one can gauge the biological embedding of experiences and develop a systems approach that is relevant to both early identification and prevention of a condition (Rutter 2016; Battaglia 2013).

Herein we review the accomplishments of this model, its recent developments, and possible therapeutic applications. We believe that three constituents of this work go beyond the specific field of anxiety disorders and can be singled out as valuable elements of inspiration for the broader realm of biomarkers and their application in psychiatry. The first is the identification of behavioural traits that are both relevant to a disorder being studied and have an unambiguous equivalent in other species. In our model this is represented by separation anxiety, which is present in all mammals, and its elevated and persistent form is relevant to SAD and PD (Battaglia et al. 2015, 2017). The second is the identification of a biomarker (CO_2 sensitivity) that can be linked to physiological mechanisms shared by humans and other species and that in its extreme expression (CO_2 hypersensitivity) manifests into the disorder(s) under investigation (SAD and PD). The third component is represented by developmental relevance and longitudinal cross-correlations. The latter designates variables that are recognisable and measurable both in longitudinal trajectories and reciprocal covariation. In our case, this component has multiple correspondences: it refers to the longitudinal continuity of SAD into PD, the time stability of the subjacent trait of CO_2 sensitivity, and their longitudinal reciprocity. In the following section, we will expand on this model and highlight its broader implications.

2 Separation Anxiety and Panic Disorders: Preclinical Models of Research

2.1 Importance of Preclinical Animal Models for Hypothesis Testing

Preclinical animal models to study human health and disease have limitations that are inherent in the very definition of the term ‘model’, a simulation of a real system that is under examination. However, the benefits of using animal models for preclinical research more than compensate for their approximation (Chesselet and Carmichael 2012; Ericsson et al. 2013). For example, cause-and-effect relationships are often difficult to prove in human studies but can be tested in a more directed manner in animal models. This includes a more effective control of the intricate gene-environment interdependence that affects all human studies of health and illness (Rutter 2007; Battaglia 2012). Moreover, results from animal studies often provide initial information on the safety and efficacy of therapeutic compounds. Furthermore, animal models are less limited in their ability to replicate complex interactions between a living organism and its environment when compared with in vitro models (Chesselet and Carmichael 2012; Ericsson et al. 2013). In essence, animal models serve as an effective preclinical hypothesis testing that lay between experimentally controllable in vitro models and complex clinical human studies.

2.2 *Understanding the Link Between Separation, Anxiety, and CO_2 Sensitivity via Preclinical Animal Models*

Parental care plays a vital role in the survival and well-being of new-born mammals (Battaglia et al. 2014). For example, the olfactory, tactile, and auditory stimuli that a mother provides her offspring are essential for their brain development. Conversely, an inadequacy of parental care can result in death or stress to the progeny. Nevertheless, it is difficult to assess the impact of parental care in child health and development as only natural experiments are possible in humans. Although studies using animal models are limited by species-specific differences and difficulties in assessing cognitive and emotional outcomes, they provide greater control over the environment and genetic background of both parents and offspring (Battaglia et al. 2014). While the relationship between early life adversity, separation anxiety disorder (SAD), and panic disorder (PD) remains unclear, human observational studies and animal research models have allowed for a greater understanding of the mechanisms that link these factors (Battaglia 2015). Needless to say, modelling adversities in animals need to be ecological, by being respectful of naturally occurring obstacles. As far as parental separation is concerned, we postulate that it occurs commonly in free-roaming rodents and that lactating female mice readily 'adopt' motherless pups as well as feed and groom them.

2.3 *Early Life Adversities on Offspring Development: Neonatal Maternal Separation Protocol in a Rat Preclinical Animal Model*

Early life adverse events and the impact they have on an offspring's overall health depend on the duration and intensity of the adversity, the developmental stage of the offspring when exposed to the adversity, and the presence or absence of alleviating factors that reduce or diminish the effects of the adversity on development. Unlike in human studies, these factors are easily controlled in animal research models, and many experimental protocols have been developed to simulate an interference or a deficiency in maternal care (Battaglia et al. 2014).

One protocol often used to mimic neonatal maternal separation (NMS) involves removing a rat pup from its mother for 3 h per day. This is done over the course of 10 consecutive days, postnatal days 3–12. During this separation procedure, each pup is physically isolated to prevent any tactile stimulation and interaction and is placed in a temperature- and humidity-regulated incubator (Genest et al. 2004; Kinkead et al. 2005a, b). After each NMS event, the pup is returned to its mother. At the end of the 10-day NMS protocol, the pup is allowed to develop under standard animal care procedures until experimental outcome measurements are performed. Results of these experiments are then compared to pups that do not receive any intervention (control group) during early life (Genest et al. 2004; Kinkead et al. 2005a, b).

The mechanism in which NMS affects the development of a rat pup is not fully understood, but several hypotheses have been made. A lack of maternal stimulation, change in maternal behaviour involving a reduction of care upon the pup's return to the nest, and the transmission of a stress hormone from the mother to the pup via maternal milk have all been suggested. Regardless of the exact mechanism, it is known that NMS increases circulating corticosterone levels in both male and female pups 24 h after the last separation procedure (Gulemetova and Kinkead 2011). This rise in stress hormones occurs at a critical period of development and is significant as these hormone levels are often low in pups during the neonatal period (Vazquez 1998). With its ability to bind to nuclear receptors and modulate gene expression, corticosterone can have a significant impact on a developing brain and result in abnormal physiology and behaviour that can manifest at various stages of life. The NMS protocol also disrupts the development of the Hypothalamic-Pituitary-Adrenal (HPA) axis, which results in elevated circulating levels of adrenocorticotropic hormone (ACTH) and corticosterone in adult male rats only (Genest et al. 2004). Similarly to being in a state of chronic stress, increased HPA activity is a significant risk factor for various disease states (McEwen and Gianaros 2011; Shonkoff et al. 2009).

2.4 Impact of Neonatal Maternal Separation on Respiratory Regulation

Respiratory regulation plays a crucial role in the pathophysiology of PD (Abelson et al. 2010; Nardi et al. 2009). Research using both animal and human models show that exposure to adversity during early life alters the development of the central nervous system (CNS) with negative effects on cognition and behaviour (Buitelaar et al. 2003; Charil et al. 2010; Fumagalli et al. 2007; Graham et al. 1999). Neonatal maternal separation in rats promotes anxiety-like behaviours, causes adults to exhibit a higher degree of variability in respiratory tidal volume, and causes a sex-specific alteration in the respiratory response to carbon dioxide (CO_2) inhalation (Genest et al. 2004; Kalinichev et al. 2002; Kinkead et al. 2009). More specifically, the hypercapnic respiratory response of NMS female rats is 63% greater when compared to controls. On the contrary, NMS male rats exhibit a 47% reduction in their hypercapnic respiratory response. From these findings it is evident that NMS in rats share several features consistent with PD in humans including parental separation/loss as a risk factor and a sex-specific effect on respiratory control (Battaglia et al. 2009, 2014; Donner and Lowry 2013; Moreira et al. 2013). This makes NMS a promising protocol when exploring the pathophysiology of PD and its high prevalence in women (Battaglia et al. 2014).

2.5 Possible Neural Mechanisms for the Effect of NMS on CO_2 Response

Using anaesthetised rat preparations, experiments investigating the neural mechanism of NMS on CO_2 response have found that abnormal integration of baro-, chemo-, and pulmonary receptor afferents contributes to this outcome (Dumont and Kinkead 2010, 2011). More specifically, the effect of NMS on respiratory control occurs by activating the central amygdala in female rats (Dumont and Kinkead 2010, 2011). However, there is no clear evidence that central CO_2 sensitivity is altered in males who have undergone NMS (Kinkead et al. 2014). Regardless of this sex difference, electrophysiological studies of the carotid body function provide no evidence that the NMS protocol affects CO_2 response via peripheral chemoreceptors (Soliz et al. 2016). Regarding the role of peripheral contributors (such as the vagus nerve) in rats exposed to early NMS, it is complicated by the fact that this protocol augments the ventilatory response only in females and is slightly blunted in males. Moreover, the NMS procedure potentiates the baroreflex in anaesthetised males, but their ventilatory response to CO_2 is blunted after NMS (Dumont and Kinkead 2010). Finally, with regard to pulmonary afferents, Kinkead's lab has shown that the NMS procedure decreases the Hering-Breuer reflex in males only (Dumont and Kinkead 2011). As the oestrogen cycle did not significantly differ between intervention and control groups, it has been postulated that anaesthesia-sensitive neural structures involved in cognition and/or emotional sensitivity to a CO_2 stimulus are necessary for the stress related to CO_2 response (Dumont and Kinkead 2011). These findings are relevant as they highlight neural structures and/or neurotransmitters that may cause the sex-specific differences seen in respiratory irregularities associated with PD. The amygdala is a neural structure thought to be of importance in the increased respiratory response to CO_2 in NMS females (Battaglia et al. 2014). However, individuals with rare bilateral amygdala damage have been found to panic after exposure to a 35% CO_2 challenge (Feinstein et al. 2013). This, thus, reduces support for the hypothesis that the amygdala plays a major role in the CO_2 response in NMS female rats and suggests that other CNS structures may be involved in triggering panic reactions to a CO_2 challenge. These CNS structures include the medulla oblongata, the brainstem, and the periaqueductal grey matter (Schenberg 2016a; Battaglia et al. 2001). Interestingly, there is an alternative interpretation of the lesion data that suggests the connectivity between the amygdala and other structures, rather than the amygdala itself, could play an important role in triggering panic reactions. At the moment, however, more information is needed to shed light on this notion.

2.6 Cross-Fostering Protocol in Mice

Although the NMS protocol has been used in rats, inconsistencies in behavioural and hormonal long-term effects were observed (Macri and Wurbel 2006; Millstein and

Holmes 2007). Methodological differences in the NMS protocol have also emerged, and species can vary in sensitivity, developmental timing, and maternal behaviour. These factors make it difficult to have a consistent understanding of the long-term effects of NMS (Battaglia et al. 2014).

Another protocol that interferes with maternal care in mice is cross-fostering. In this protocol, new-born pups are cross-fostered from their biological mother to another lactating female within 24–48 h of birth (Buxbaum et al. 2011). During the first days of life, mouse pups develop an attachment bond that includes associating maternal stimuli such as odour with food, warmth, and protection (Landers and Sullivan 2012). Thus, changes in maternal cues (most notably odour) due to cross-fostering could impair the physiological development of an attachment bond between a pup and its mother. While several studies have examined the developmental effects of cross-fostering, body weight and emotional behaviour may be affected in the offspring depending on the mouse strain. Metabolic and cardiovascular strain-dependent dysfunction have also been observed in cross-fostered pups, even when the adoptive mother provide essential care and nutrition (Bartolomucci et al. 2004; Leussis and Heinrichs 2009; Lu et al. 2009; Matthews et al. 2011).

2.7 *Repeated Cross-Fostering Protocol in Mice and Behavioural Phenotypes in Response to Elevated CO_2 Concentration*

A novel procedure called repeated cross-fostering (RCF) was developed by our group. This approach creates interference with the early maternal environment, induces early life adversities, stimulates separation anxiety without inducing major behavioural or metabolic adverse results, and does not use maternal neglect/maltreatment (D'Amato et al. 2011a). In this protocol, outbred mouse pups are repeatedly cross-fostered to adoptive mothers during the first 4 days of life. More specifically, after spending the first postnatal day (PND0) with their biological mother, culled litters are assigned to a RCF intervention or control group on PND1 (Battaglia et al. 2014). Pups in the RCF group have their caregiver changed every 24 h for 4 days during the PND1–PND4 interval. The RCF procedure involves first removing the mother from her cage and then her entire litter. The litter is then immediately introduced into a different mother's cage whose pups have also just been removed. The RCF pups are then semi-covered with the cage bedding of the adoptive mother who is then reintroduced into her cage and left with the new litter for the next 24 h. The pups are ultimately left with the adoptive mother on the fourth day (PND4) to PND28. It is important to mention that adoptive mothers are also lactating females with pups of the same age as the fostered litters. In order to control for the effect of being manipulated seen in the RCF intervention group, control litters are picked up daily, reintroduced into their home cage, semi-covered with home cage bedding, and have their biological mothers returned within 30 s. Similarly, this

control procedure occurs over a 4-day interval from PND1 to PND4 (Battaglia et al. 2014).

When examining the behavioural phenotype of RCF mice on PND8, changes in response to isolation are observed. More specifically, RCF mice display more heightened ultrasonic calls (the murine equivalent of separation calls) when compared to control group pups. Furthermore, RCF mice display exaggerated hyperventilation during 6% CO_2 breathing that is present from childhood into adulthood (D'Amato et al. 2011a). Similar to humans at the onset of PD, RCF animals do not show evidence of corticosterone increase or altered expression of hippocampal glucocorticoid, or mineral-corticoid receptors (Luchetti et al. 2015). The abnormal CO_2 sensitivity induced by RCF is also associated with a reduced response to natural rewards and increased susceptibility to adverse events in late adulthood (Ventura et al. 2013). It is important to mention that these findings related to RCF pups are not due to altered maternal care as the amount of nursing and licking/grooming behaviour received between them and control pups is indistinguishable (D'Amato et al. 2011a). When measuring the different respiratory responses between genetically related cross-fostered (siblings and half-siblings), unrelated cross-fostered, and control mice, a significantly greater genetic variance and almost twofold increase in heritability for the response to 6% CO_2-enriched air in RCF pups are seen (D'Amato et al. 2011a). This finding suggests the presence of gene-by-environment interaction effects provoked by an early life adversity and interference with the mother-pup bond similarly to what has been reported in human studies of CO_2 sensitivity (Spatola et al. 2011).

Table 1 provides a comparison of the NMS and RCF protocols and their corresponding physiological, behavioural, and neuroendocrine results.

2.8 Extending RCF-Associated Biometric Gene-Environment Interaction into Molecular Readouts: Epigenetic Findings

Among the possible molecular mechanisms explaining biometric gene-environment interactions are histone protein modifications and DNA methylation, which affect post-natal programming relating to health and disease (Feil and Fraga 2012). Histone proteins can undergo modifications in response to early life adversities. These protein changes can affect gene expression, and hence development, and adaptation to environmental pressure (Xie et al. 2013; Zhou et al. 2011). Based on this notion, it was hypothesised that CO_2 hypersensitivity associated with early life adversities (as modelled in the RCF paradigm) can be explained by epigenetic mechanisms (Cittaro et al. 2016). As a first test of this hypothesis, a genome-wide investigation of altered histone marks in the medulla oblongata (a major site of CO_2 chemoreception and respiratory control in the brain of animals and humans) of mice exposed to the RCF procedure compared to normally reared control mice was conducted (Xie et al.

Table 1 Comparison of the neonatal maternal separation (NMS) and repeated cross-fostering (RCF) protocols and their corresponding physiological, behavioural, and neuroendocrine results

Characteristic	NMS and RCF models – shared features	Neonatal maternal separation (NMS) model – unique features	Repeated cross-fostering (RCF) model – unique features
Experiment protocol			
Strain	Outbred animals	Rat	Mouse
Intervention	Early interference with mother-pup interaction	Pups are removed from their mother for 3 h each day over the course of 10 consecutive days (PND3–PND12)	Pups have their mother changed every 24 h between PND1 and PND4 and are left with their adoptive mother on PND4
Behavioural change in offspring due to intervention	Elicit respiratory CO_2 hypersensitivity, a key biomarker of human PD and SAD	NMS promotes anxiety-like behaviours, such as PD	RCF mice display more heightened ultrasonic calls than control pups
Neuroendocrine change in offspring due to intervention		Disruption in the HPA axis leads to elevated circulating levels of ACTH and corticosterone in adult male rats only	No corticosterone increase or altered expression of hippocampal glucocorticoid and mineral-corticoid receptors
Respiratory change in offspring due to intervention		Hypercapnic respiratory response of NMS females is 63% greater than controls, but NMS males have a 47% reduction	RCF mice display exaggerated hyperventilation during 6% CO_2 breathing that is similar to humans at the onset of PD

PND postnatal day, *PD* panic disorder, *SAD* separation anxiety disorder, *HPA* hypothalamic-pituitary-adrenal

2013; Zhou et al. 2011; Cittaro et al. 2016; Goossens et al. 2014). In order to identify both gene activation and silencing, three reliable histone marks were used: H3 acetylation of lysine 9 and 14 (H3Ac), trimethylation of H3 lysine 4 (H3K4me3), and trimethylation of lysine 27 (H3K27me3). The first two histone marks are associated with gene activation, while H3K27me3 is involved in gene silencing (Barski et al. 2007).

From this study, an association between the RCF procedure and multiple histone marks in the medulla oblongata was discovered. Functional enrichment and RNA transcript analyses revealed genes affecting chemoreception, nociception, and neurodevelopment, including the acid-sensing ion channel 1 (asic1) gene (Cittaro et al. 2016). The enrichment of Asic1 has been observed in the presence of an abnormal response to injury (abnormal acid-base homeostasis), irregular touch or nociception, and atypical m-TOR signalling. Interestingly, these are among the top phenotypes associated with the RCF procedure. These three phenotypes are also

linked to acidosis, a state detected by ASIC channels and underlying the variation in both CO_2 sensitivity and nociception (Cittaro et al. 2016; Wemmie et al. 2013).

In keeping with the main hypothesis, RCF mice showed both exaggerated respiratory response to hypercapnia and altered nociception compared to control animals. Widely expressed in the brain, including the medulla oblongata, are ASIC1a ion channels which are coded by Asic1. These ion channels play a vital role in acidosis-sensing mechanisms and mediating amygdala-originated fear in response to CO_2 (Cittaro et al. 2016; Wemmie et al. 2013). Interestingly, the human ortholog of the murine Asic1 gene, ACCN2, is associated with PD, the amygdala's functioning, and CO_2 hypersensitivity (Smoller et al. 2014; Savage et al. 2015).

Overall, this study showed that histone modifications and the correlated consistent ASIC1 expression profiles among RCF mice provide a molecular basis for a gene-environment effect that had been previously identified in humans and animals with CO_2 hypersensitivity (D'Amato et al. 2011a; Spatola et al. 2011). By using a preclinical animal model, this study was able to exclude the gene-environment correlations that often obstruct studies examining human gene-environment interactions (Cittaro et al. 2016). Furthermore, this study demonstrated that intermediate phenotypes of physiological nature, including respiratory responses to CO_2 for PD or SAD, can be used to support parallel investigations in humans and animals. Applying this strategy, specific physiological functions and phenotype characteristic of RCF-exposed animals can be used to identify and understand their implication in human PD. From a developmental approach, the significance of genetic and environmental factors along with their resulting epigenetic modifications can help explain how biological variation and pathophysiology emerge more precisely (Cittaro et al. 2016). Finally, epigenetic marks can constitute a further element of risk prediction for human psychopathology (Davies et al. 2012). Figure 1 highlights the results of the genome-wide altered histone marks study.

When examining the responses to RCF, three notions stand out. Firstly, RCF evokes multiple physiological and behavioural changes. As far as separation anxiety, hyperventilation in response to CO_2, and hypersensitivity to painful stimuli (altered nociception) are concerned, it should be noted that these are three very different phenotypes, so that their copresence in RCF mice cannot be attributed to poor phenomenological discrimination. Secondly, there is longitudinal stability in these changes. Thirdly, the nature of the longitudinal covariation between the different phenotypes that are enhanced by the RCF is a key developmental question, as it may be reconnected to stable changes of expression/regulation of genes that serve multiple systems and disparate phenotypes. Furthermore, can animal data be compared to human data, and is epigenetic transmission of CO_2 hypersensitivity plausible?

To address these questions, we carried out a comparative study of DNA methylation in RCF mice and monozygotic twin pairs discordant (MZD) for CO_2 hypersensitivity (Davies et al. 2012). Together with an RCF-associated intergenerational transmission of CO_2 sensitivity in normally reared mice that have descended from RCF-exposed females (indicating epigenetic inheritance), our study (Davies et al.

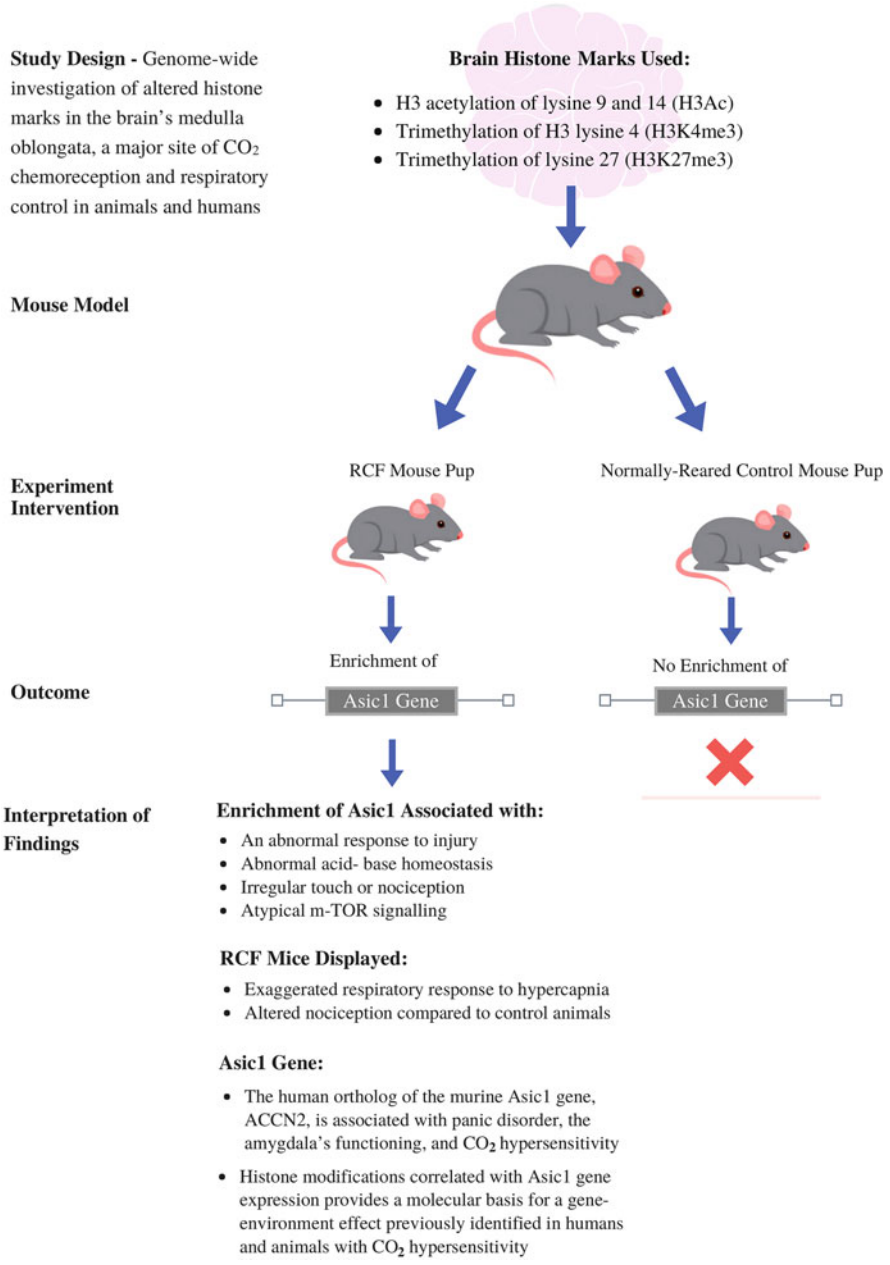

Fig. 1 Protocol and results of the repeated cross-fostering (RCF) genome-wide altered histone marks study

2012) describes multiple accompanying alterations in DNA methylation patterns of the brainstem of RCF vs. control animals. The presence of CO_2 hypersensitivity among normally reared offspring who descended from RCF-exposed females was consistently replicated across three additional independent intergenerational samples and persisted in adulthood (Giannese et al. 2018).

The epigenetic signatures in the medulla oblongata of RCF and control animals were then compared to blood DNA methylation profiles of female MZD discordant for emotional reactivity to a CO_2 challenge. Altered methylation was consistently associated with repeated elements and transcriptional regulatory regions among RCF-exposed animals, their normally reared offspring, and humans with CO_2 hypersensitivity (Giannese et al. 2018). In both mouse and human models, brain regions bearing differential methylation were associated with neurodevelopment, circulation, response to pH acidification processes, the ASIC2 gene, chemoreception, and anxiety. Again, human developmental data reveal some consistency with data from animal models, as children with high separation anxiety are likely to develop a heightened risk for asthma and headaches (Battaglia et al. 2017). Future, multivariate twin studies regarding anxiety, sensory testing, and pain can shed light on the possible parallel nature between animal and human models and the genetic and epigenetic underpinnings of these phenotypes.

2.9 Insights and Developments from Using Preclinical Animal Models of CO_2 Hypersensitivity

One major advantage of using the NMS and RCF protocols, compared to other animal models of human anxiety, is that they elicit respiratory CO_2 hypersensitivity (a key biomarker of human PD and SAD that requires no inference from the researcher). In other words, with these CO_2 sensitivity animal models of PD and SAD, researchers do not need to ask whether the animals are anxious or to what degree is this anxiety relevant to human mental disorders. Another strength of using these protocols is that they are based on evidence that PD, SAD, and CO_2 sensitivity in humans share a large amount of additive genetic variance and that the genes subserving CO_2 sensitivity are largely conserved in mammals including humans (Battaglia et al. 2014).

Both NMS and RCF protocols also confirm the results of human studies that early life adversities are significantly associated with SAD, PD, and their related CO_2 hypersensitivity (Battaglia et al. 2014). Biometric and epigenetic data from RCF studies also establish that there are gene-by-environment interaction mechanisms independent of gene-environment correlations at the basis of CO_2 hypersensitivity. The biometric quantitative evidence not only supports findings from human studies but also highlights the need for further research in genetic and environmental interaction mechanisms, including further investigation of epigenetic mechanisms via molecular tools (Battaglia et al. 2014; Spatola et al. 2011).

Although the NMS and RCF protocols share many similar findings, some differences are evident in adult animal phenotypes. These discrepancies may be due to timing, effects of the protocol used, and other factors that warrant further investigation. Nevertheless, the differences observed between NMS and RCF findings can be applied to solve outstanding questions in human clinical research. For instance, the differences seen in the involvement of the HPA axis in both animal models may help explain why PD patients have a rise in cortisol only after the recurrence of a panic attack (Battaglia et al. 2014). Furthermore, discrepancies between NMS and RCF animal models may explain why cortisol levels in humans with PD appear to be unaffected by 6–7% CO_2 stimulation (Klein 1993).

Having two different protocols with different murine species can also help assess various aspects of SAD and PD that benefit from the species-specific advantages. For example, rat animal models might be more suitable to examine electrophysiological factors, while mice can be used to investigate the molecular genetics of SAD and PD (Battaglia et al. 2014). Non-pharmacological therapeutic interventions can also be explored using these two animal models. For example, it is known that long-term NMS effects such as an increase in HPA activity, anxiety-like behaviour, and susceptibility to ethanol and drug consumption can be reduced by environmental enrichment after weaning (Huot et al. 2001; Meaney et al. 1994, 1996; Meaney 2001; Kosten et al. 2000, 2004; Li et al. 2003; Ploj et al. 2003; Zhang et al. 2005; Francis et al. 2002; Vivinetto et al. 2013). Exposure of NMS animals to environmental enhancement can also have a therapeutic effect on their respiratory response to increased CO_2 concentrations and advance the understanding of the molecular basis of CO_2 hypersensitivity.

One can also develop behavioural strategies that counter CO_2 hypersensitivity in mouse pups exposed to the RCF protocol. For instance, increasing maternal care or maintaining the original biological mother's olfactory cues in the cross-fostered pup's environment during their development may revert the RCF CO_2 phenotype (Battaglia et al. 2014; D'Amato et al. 2011b). Lastly, the respiratory response to CO_2 in NMS and RCF rats can help validate which of these protocols provide a better model of human PD when taking into consideration HPA axis activity and sex-dependent differences (Battaglia et al. 2014).

3 Limitations and Future Research Directions

Given the nature of CO_2 stimulus and its multiple physiological and behavioural associations, it is not surprising to see its response applied to several research fields, including experimental modelling of anxiety in healthy volunteers and its interference of cognitive and executive tasks (Bailey et al. 2011; Diaper et al. 2012). As general predisposition to anxiety may predict part of the individual differences of CO_2 hypersensitivity, some have dismissed responses to CO_2 as unspecific indices of predisposition to anxiety. However, at least two sets of data speak against CO_2 hypersensitivity as a generic index for anxiety. First, Fluharty and colleagues

demonstrated that while proneness to anxiety is associated with greater subjective and physiological responses, general anxiety-prone individuals also have a greater subjective response to placebo conditions (Fluharty et al. 2016). This is not seen in CO_2 responders, who typically show no/minimal placebo reactivity. Secondly, Roberson-Nay and colleagues found a significant degree of independence of genetic factors influencing pre-CO_2 anxiety that become activated by respiratory stimulation (Roberson-Nay et al. 2013). These data show that while anxiety sensitivity may in part explain the response to CO_2 stimulation in humans, CO_2 challenges impinge on systems and genetic backgrounds that cannot simply be reduced to a general predisposition to anxiety. On the other hand, it should be remembered that CO_2 hypersensitivity does not map human SAD or PD with perfect specificity or sensitivity (Schenberg 2016b).

While these aspects should be bore in mind, future research directions regarding SAD and PD are greatly needed, given that anxiety disorders are the most prevalent mental health conditions. According to epidemiological surveys, up to one third of the population is affected by an anxiety disorder during their lifetime, and women are more likely to experience them (Bandelow and Michaelis 2015). Thus, further preclinical studies designed to determine the precise molecular and biological mechanisms, gene-environment interplays, and epigenetic changes related to SAD, PD, and CO_2 sensitivity are reasonable targets for parallel investigations involving human and animal research models.

As children with SAD and adults with PD display elevated emotional and respiratory responses to CO_2, these anxiety disorders may share a distinct sensitivity to low/acidic brain pH levels (Esquivel et al. 2010; Magnotta et al. 2012; Maddock et al. 2013). This creates another research opportunity using preclinical models to examine the role of acid-sensing ion channels (ASICs) and their association with CO_2 hypersensitivity, anxiety disorders, pH detection, and altered nociception in childhood and early adulthood (Wemmie et al. 2013; Ziemann et al. 2009). To explain how early life adversity alters nociception, methylation has been suggested as the primary mechanism (Tran et al. 2013), and our own data support this. This opens another, quite interesting question, on the nature of covariation between altered nociception and anxiety, as chronic pain affects over 20% of adults and mood/anxiety disorders are often comorbid (McWilliams et al. 2004; Battaglia et al. 1995). A preclinical animal model investigating the impact of early life adversities would help understand whether environmentally induced and epigenetically maintained ASICs dysfunction could result in elevated anxiety, CO_2 hypersensitivity, and altered nociception. By the same token, could an ASIC antagonist modulate these responses? If so, could they constitute a viable therapeutic avenue for some human anxiety disorders and pain syndromes?

Evidence that an ASIC antagonist, appropriately administered to reach the brain, could rescue normal CO_2 reactivity and nociception in RCF animals would constitute a stringent validation of these findings. This would provide support to modulatory effects on pH-sensing ASICs and the ability to reduce the excessive respiratory drive induced by hypercapnia and brain extracellular acidification. The ensuing question would then be that of possible therapeutic use of ASIC antagonists in

humans, which should however be envisioned in an appropriate context. For instance, it is known that a variety of agents (most convincingly tricyclic antidepressants and SSRIs) can attenuate the responses to CO_2 stimulation and treat a variety of anxiety disorders. But it has also been shown that a single dose of muscarinic inhibitor biperiden restores normal responses to 35% CO_2 in PD patients (Battaglia et al. 2001). Would this be sufficient to support biperiden as a treatment for panic attacks? Inasmuch as virtually all antidepressants exert at least some anticholinergic effects, adding anti-muscarinic molecules to anxiety treatment would hardly be justifiable. Interestingly, however, some preclinical data show an anxiolytic-like activity of ASIC inhibitors, and hyperventilation induced by hypercapnia is partially under emotional control (Dumont et al. 2011; Dwyer et al. 2009). This indicates that ASIC inhibitors could also exert a beneficial anxiolytic-like action and hence constitute plausible therapeutic tools to add to the current armamentarium.

4 Conclusion

We have suggested using physiological phenotypes of biological and evolutionary significance in both human and preclinical animal research models relating to SAD and PD. Regarding separation anxiety, it applies to multiple forms of distress responses seen in mammals during postnatal development, including separation from a caregiver. Childhood SAD is an important risk factor for developing PD in early adulthood, and both conditions display an increased sensitivity to elevated CO_2 concentrations inhaled from the air. By interfacing epidemiological, genetic, and physiological knowledge with preclinical animal research models, it is possible to map mechanisms that are central to separation anxiety and panic disorders while also suggesting possible therapies. This is a two-way phenomenon from human to animal and back, which we believe is the hallmark of translational research.

Preclinical research models allow for environmentally controlled studies of early parental separation without the interference of gene-environment correlation. Moreover, they have shown that different forms of early maternal separation in mice (RCF) and rats (NMS) induce elevated CO_2 sensitivity, an important biomarker linking SAD and PD. In mice, this is likely due to a gene-environment interaction that affects multiple behavioural and physical phenotypes after exposure to early life adversity (parental separation). Although several questions regarding the causal mechanism of SAD and PD remain unanswered, the identification and improved understanding of biomarkers that link these mental health conditions under the guise of preclinical research models in conjunction with human longitudinal cohort studies can help resolve these issues.

Acknowledgements The authors would like to thank Dr. Richard Kinkead for the discussion and clarifications regarding his findings relating to the NMS paradigm. Moreover, we would like to thank Myeda Kamal for constructing the figure presented in our chapter.

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Imaging and Genetic Approaches to Inform Biomarkers for Anxiety Disorders, Obsessive–Compulsive Disorders, and PSTD

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Contents

The original version of this chapter was revised. The correction to this chapter can be found at DOI 10.1007/7854_2018_64

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Curr Topics Behav Neurosci (2018) 40: 219–292
DOI 10.1007/7854_2018_49
Published Online: 22 May 2018

Abstract Anxiety disorders are the most common mental health problem in the world and also claim the highest health care cost among various neuropsychiatric disorders. Anxiety disorders have a chronic and recurrent course and cause significantly negative impacts on patients' social, personal, and occupational functioning as well as quality of life. Despite their high prevalence rates, anxiety disorders have often been under-diagnosed or misdiagnosed, and consequently under-treated. Even with the correct diagnosis, anxiety disorders are known to be difficult to treat successfully. In order to implement better strategies in diagnosis, prognosis, treatment decision, and early prevention for anxiety disorders, tremendous efforts have been put into studies using genetic and neuroimaging techniques to advance our understandings of the underlying biological mechanisms. In addition to anxiety disorders including panic disorder, generalised anxiety disorder (GAD), specific phobias, social anxiety disorders (SAD), due to overlapping symptom dimensions,

obsessive–compulsive disorder (OCD), and post-traumatic stress disorder (PTSD) (which were removed from the anxiety disorder category in DSM-5 to become separate categories) are also included for review of relevant genetic and neuroimaging findings. Although the number of genetic or neuroimaging studies focusing on anxiety disorders is relatively small compare to other psychiatric disorders such as psychotic disorders or mood disorders, various structural abnormalities in the grey or white matter, functional alterations of activity during resting-state or task conditions, molecular changes of neurotransmitter receptors or transporters, and genetic associations have all been reported. With continuing effort, further genetic and neuroimaging research may potentially lead to clinically useful biomarkers for the prevention, diagnosis, and management of these disorders.

Keywords Anxiety disorders · Biomarkers · Brain imaging · Genetics

Abbreviations

[11C]5-HTP	[11C]5-hydroxytryptophan
[18F]FDG	[18F]fluorodeoxyglucose
5-HT1A	Serotonin type 1A
5-HT1B	Serotonin type 1B
5-HT2A	Serotonin type 2A
5-HTT	Serotonin transporter
5-HTTLPR	Serotonin transporter polymorphism
ACC	Anterior cingulate cortex
BA	Brodmann area
BDNF	Brain-derived neurotrophic factor
BNST	Bed nucleus of stria terminalis
BOLD	Blood-oxygen-level dependent
BZD	Benzodiazepine
CBT	Cognitive behavioural therapy
COMT	Catechol-*O*-methyltransferase
CSTC	Cortico-striato-thalamo-cortical
DAT	Dopamine transporter
DLPFC	Dorsolateral prefrontal cortex
DMPFC	Dorsomedial prefrontal cortex
DRD2	Dopamine D2 receptor
DRD3	Dopamine D3 receptor
DSM-5	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion tensor imaging
EEG	Electroencephalography
FA	Fractional anisotropy
fALFF	Fractional amplitude of low frequency fluctuations
fMRI	Functional magnetic resonance imaging

GABA	γ-Aminobutyric acid
GAD	Generalised anxiety disorder
Glx	Glutamate + glutamine
GM	Grey matter
GWA	Genome-wide association
IFG	Inferior frontal gyrus
IPL	Inferior parietal lobule
ITG	Inferior temporal gyrus
MAOA	Monoamine oxidase A
MCC	Mid cingulate cortex
MEG	Magnetoencephalography
MeTL	Medial temporal lobe
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MTF	Middle temporal gyrus
NAA	*N*-acetylaspartate
NK1	Neurokinin-1
NPY	Neuropeptide Y
OCD	Obsessive–compulsive disorder
OFC	Orbitofrontal cortex
PBMCs	Peripheral blood mononuclear cells
PCC	Posterior cingulate cortex
PD	Panic disorder
PDA	Panic disorder and agoraphobia
PET	Positron emission tomography
PFC	Prefrontal cortex
PSMD9	Proteasome modulator 9
PTSD	Post-traumatic stress disorder
rCBF	Regional cerebral blood flow
rCMRGlc	Regional cerebral metabolic rate of glucose uptake
RGS2	Regulator of G-protein signaling 2
RSFC	Resting-state functional connectivity
SAD	Social anxiety disorder
SLF	Superior longitudinal fasciculus
SMA	Supplementary motor area
SNPs	Single nucleotide polymorphisms
SNRIs	Serotonin and norepinephrine reuptake inhibitors
SP	Simple phobias
SPECT	Single photon emission computed tomography
SPL	Superior parietal lobe
SSRIs	Selective serotonin reuptake inhibitors
STG	Superior temporal gyrus
TMEM132D	Transmembrane protein 132D
TPH2	Tryptophan hydroxylase 2

VBM	Voxel-based morphometry
VLPFC	Ventrolateral prefrontal cortex
VMPFC	Ventromedial prefrontal cortex
WM	White matter

1 Introduction

In this chapter, we review the large amount of available data and have focused in particular on clinical evidence from neuroimaging and genetic measurements as biomarkers in anxiety disorders. We cover generalised anxiety disorder (GAD), panic disorder (PD), social anxiety disorder (SAD), and simple phobias (SP), in order to better understand potential biomarkers involved in their aetiology and treatment. Although both obsessive–compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) have been removed from the anxiety category in the most recent (5th) edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM-5), they are still considered by many to be anxiety disorders due to overlapping phenomenology and therefore they both are also included in the current chapter. More details on the symptomatology and diagnosis of each are given in the respective sections below.

Anxiety disorders are the most common psychiatric illnesses experienced, affecting an estimated 18% of people in the USA, according to epidemiological studies, with the age of onset usually in the mid-twenties and the prevalence twice as high in females as compared to males (Kessler et al. 2005). In the European Union, anxiety disorders are also the most common of all mental disorders with a prevalence rate around 14% (Wittchen et al. 2011). In addition, anxiety disorders are characterised by a chronic and fluctuating clinical course and can be seriously disabling diseases causing impairment in social, personal, and occupational functioning as well as leading to significant loss in quality of life and to an enormous social cost (Mendlowicz and Stein 2000). As demonstrated in recent studies, anxiety disorders are the most costly in the USA, amounting to 46.6 billion dollars, or 31.5% of the total economic costs of mental disorders (Rice and Miller 1998). In the European Union, anxiety disorders had a direct health care cost of 46.3 billion Euros in 2010, which was the highest among various neuropsychiatric disorders (Olesen et al. 2012). Furthermore, in community studies, patients with anxiety disorders were found to use more medical and psychiatric services than control populations (Markowitz et al. 1989). Therefore, the early diagnosing and the availability of more effective, relatively low-cost outpatient treatment could substantially reduce the economic and social burden of these common and often crippling disorders. Despite the high prevalence and dramatic outcomes, anxiety disorders remain underdiagnosed or misdiagnosed, and consequently under-treated. For example, data obtained from a 1-year national telephone survey in the USA revealed that only 23.2% of people with anxiety disorders had used appropriate treatment, including

counselling (Kasper 2006). The same phenomenon was also demonstrated in a multinational study conducted in European countries. Only 32.4% of patients with any anxiety disorders received any psychopharmacologic treatment in a 12-month period (Alonso et al. 2004).

There are several reasons, which may explain why anxiety disorders are often neglected by mental health and primary services. Due to the nature of anxiety disorders, some patients may avoid seeking help, or being misinterpreted because of mimicking symptoms. However, the main problem lies within the biological backgrounds of anxiety disorders. Similar to other mental illnesses, we are still lacking comprehensive understanding about specific biological markers, which would underlie the diagnosing and treatment of anxiety disorders. The identification of such biomarkers has been recently prioritised by psychiatric research as it may significantly improve earlier diagnosing and prevention strategies of mental disorders. It seems especially important in light of treatment efficacy in anxiety disorders, which are notoriously difficult to successfully treat, and where a variety of genetic and environmental factors contribute to their development and severity (Scott et al. 2015).

Although selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) have been shown to be beneficial for the treatment of certain anxiety disorders, not all patients achieve an adequate clinical therapeutic response. For example, only 10% of patients with PD were symptom-free in a 3-year follow-up study (Noyes et al. 1986) and only 12% of PD patients were in full remission after 5 years (Faravelli et al. 1995). The existence of "non-responders" indicates that SSRIs and SNRIs are not the failsafe solution to treating anxiety that clinicians have been looking for. In addition, SSRIs and SNRIs are associated with complications that can limit their use in some patients, including delays in producing the desired clinical reduction in anxiety (often they takes weeks to work) or even potential worsening of the anxiety particularly at the start of treatment leading to dropouts (Ravindran and Stein 2010). Nevertheless, there is strong belief that further search for biological predictors of treatment response, which are also defined as "treatment biomarkers", would contribute to the precision medicine or even the personalised medicine approach, in which biomarkers would guide decision-making and even help to select the most suitable medication for individual patients. Moreover, incorporation of predicting biomarkers into antidepressant treatment algorithms could speed recovery from disease by shortening or eliminating lengthy and ineffective trials (Leuchter et al. 2010). During the last decade, there have been intensive ongoing research efforts applying neuroimaging and genetic approaches for the discovery of pathogenetic biomarkers for anxiety disorders. However, only a few of these studies have specifically addressed treatment predictors.

1.1 Neuroimaging Biomarkers

Many of the major advances in biomarker research have arisen from innovation and development in neuroimaging technology, including structural (magnetic resonance imaging – MRI and diffusion tensor imaging – DTI), functional (functional magnetic resonance imaging – fMRI and positron emission tomography – PET/single photon emission computed tomography – SPECT), biochemical (magnetic resonance spectroscopy – MRS), and neurophysiological (electroencephalography – EEG and magnetoencephalography – MEG) methodologies (http://institute.progress.im/en/content/introduction-brain-imaging). These different neuroimaging techniques have all been used to study whether baseline, pretreatment characteristics or changes in brain functioning and metabolism correlate with symptom improvement following antidepressant treatment. Despite being based on straightforward, quick, and simple methodology, a relatively small number of neuroimaging studies have been conducted in anxiety disorders so far as compared to other mental health problems such as mood or psychotic illnesses. In part, this may reflect the fear that many patients with anxiety disorders, particularly those with PD and phobias, have for the confined space of the neuroimaging scanner.

1.2 Genetic Biomarkers

Increasing efforts are being made to determine genetic factors involved in the onset and development of psychiatric disorders, and also those influencing their response to therapeutic interventions. Given the complex genetic and polygenic nature of anxiety disorders, OCD, and PTSD, a multitude of common genetic variants is expected in this context, with each variant increasing the genetic disease risk by approximately 1–2%. Although the search for genetic biomarkers is facilitated by several approaches including epidemiological (family and twin) studies, molecular (linkage and association) methods, and more recently genome-wide association (GWA) studies, transcriptional and microRNA analyses, gene–environment interaction, and epigenetic approaches, the genetic research of anxiety disorders remains again very modest as compared to other mental health and somatic diseases. It is the same with pharmacogenetic studies, aiming to identify genetic factors underlying prediction of drug response and side effect tolerance. So, in contrast to the wealth of pharmacogenetic studies in depression, the research of genetic markers of treatment response in anxiety disorders is still limited to single, non-replicated candidate-gene trials in relatively small samples.

2 Panic Disorder and Agoraphobia

Briefly, PD is characterised by recurrent panic attacks, which are discrete periods of intense fear or discomfort, accompanied by at least four of 13 somatic and psychic symptoms. About two-thirds of all patients with PD suffer from comorbid agoraphobia, which is defined as fear in places or situations (e.g. crowds and public transport) from which escape might be difficult or in which help may not be available in the event of having unexpected panic attacks.

2.1 Structural Magnetic Resonance Imaging Studies in Panic Disorder and Agoraphobia (See Tables 1, 2, 3, and 4)

Previous and recent MRI studies have revealed both structural and connectivity alterations in multiple brain areas, including the amygdala, the hippocampal and parahippocampal gyri, temporal lobes, and the brainstem nuclei, which are believed to be involved in the pathogenesis of panic disorder and agoraphobia (PDA). In an early study, Fontaine et al. (1990) observed focal abnormalities in the temporal lobes, including areas of abnormal signal activity and asymmetric atrophy. Two independent research groups reported bilateral decrease of amygdala volume in relatively small samples of PD patients in comparison with healthy controls (Massana et al. 2003b; Hayano et al. 2009). Notably, the volume reduction in the right amygdala and the bilateral insular cortex was significantly greater in the males with PD as compared to female patients, while reduction in the right superior temporal gyrus was greater in females (Asami et al. 2009).

Although it is unlikely that the small amygdalar volume can be considered as a specific biological feature of PD, these data are intriguing in light of changes in other often studied areas in PD, namely the orbitofrontal cortex (OFC), due to reciprocal connections between the two structures and evidence that the OFC restrains amygdala activity. Particularly, Atmaca et al. (2012) detected significantly smaller left OFC volumes in 20 PD patients compared with 20 healthy controls. In one study, volume reduction in the right posterior-medial OFC region was only found in PDA patients with an absent or a single posterior orbital sulcus (Roppongi et al. 2010). Lai and Wu (2012a) showed in 30 first-episode, drug-naive, and late-onset PD patients lower grey matter (GM) volumes in left OFC, as compared to 21 healthy controls. Na et al. (2013) showed decreased GM volume in the left medial OFC in 12 patients with both PD and agoraphobia compared to 22 healthy control subjects, but not in 10 patients with PD and without agoraphobia nor in the total sample. As regarding other brain areas, there are reports that PD patients have lower GM volume in several regions, including left parahippocampal gyrus (Massana et al. 2003a), the temporal and frontal lobes (Sobanski et al. 2010), the putamen (Yoo et al. 2005), the right dorsal and the rostral anterior cingulate cortex (ACC) (Asami et al. 2008), and the pituitary gland (Kartalci et al. 2011). However, in the last study the patients with

Table 1 Grey matter alterations associated with anxiety disorders, OCD, and PTSD

Grey matter volume, density, or cortical thickness		PDA		GAD		SP		SAD		OCD		PTSD	
		V	T	V	T	V	T	V	T	V	T	V	T
Frontal	OFC	↓		↓	↓	↑				↑	↑	↓	
	Frontopolar									↓			
	DLPFC									↓			
	DMPFC			↑		↑				↓		↓	
	VLPFC				↑					↓			
	VMPFC				↑							↓	
	Precentral			↑		↑							
Temporal	STG	↓								↓			
	MTG	↓			↑					↓		↓	
	ITG				↑				↑			↓	
	Temporal pole			↑	↓								
	Hippocampus	↓		↓				↓		↓		↓	
	Parahippocampal	↓										↓	
	Fusiform				↓								
	MeTL										↑		
Limbic	Amygdala	↓		↑				↓				↓	
	Insula	↑↓				↑	↑			↓		↓	
	ACC	↓				↑	↑		↓	↓		↓	
	PCC			↓			↑				↑		
Parietal	Precuneus			↑									
	Supramarginal									↓			
	IPL									↓			
Occipital	Right lateral				↑								
	Left lateral				↓								
	Visual						↑						
	Associative									↓			
Subcortical	Putamen	↓		↑						↑			
	Caudate			↑									
	Thalamus									↑			
	Pituitary	↓											
Brainstem	Midbrain	↑											
	Pons	↑											

V volume change, *T* cortical thickness change

agoraphobia had a significantly smaller pituitary volume than those patients without agoraphobia (Kartalci et al. 2011). On the other hand, increased GM volume was demonstrated in the left insula (Uchida et al. 2008) as well as in the midbrain and rostral pons of the brainstem (Protopopescu et al. 2006), perhaps indicating some form of expansion in the presumed panic circuit. Regarding the latter finding, Fujiwara et al. (2011) also showed increased midbrain volume in 38 PD patients compared with the control group of 38 matched healthy subjects. Furthermore, there was a significant positive correlation between relative dorsal midbrain volume and

Table 2 Grey matter areas with alterations associated with treatment effect of pharmacological or psychological interventions in anxiety disorders, OCD, and PTSD

Grey matter volume, density, or cortical thickness		PDA		GAD		SP		SAD		OCD		PTSD	
		V	T	V	T	V	T	V	T	V	T	V	T
Frontal	OFC									•			
	DMPFC	•											
	VLPFC	•											
	VMPFC									•			
	Precentral	•											
Temporal	Hippocampus											•	
	Fusiform	•											
Limbic	Amygdala							•					
	ACC									•		•	•
	PCC									•			
Subcortical	Putamen									•			
	Thalamus									•			
Cerebellum	Cerebellum	•											

V volume change, *T* cortical thickness change

Table 3 White matter alterations associated with anxiety disorders, OCD, and PTSD

White matter volume	PDA	GAD	SP	SAD	OCD	PTSD
Prefrontal	↓	↓			↑	↑↓
Temporal						↑↓
Limbic	↓					↑↓
Parietal					↑	↑↓
Thalamic radiation	↓					
Internal capsule		↓			↑	↓
Cerebellum	↓					
Midbrain		↓				
White matter FA	PDA	GAD	SP	SAD	OCD	PTSD
Frontal-cortical	↓				↓	
Frontal-limbic		↓				
Frontal-occipital fasciculus	↓					
Cingulum bundle	↑					↑↓
Corpus callosum	↑↓				↓	
Thalamic radiation	↑					
Corona radiata	↑					
Internal capsule	↑					
Superior longitudinal fasciculus	↑↓					↑↓
Sagittal stratum	↑					

FA fractional anisotropy

Table 4 White matter areas with alterations associated with treatment effect of pharmacological or psychological interventions in anxiety disorders, OCD, and PTSD

White matter volume	PDA	GAD	SP	SAD	OCD	PTSD
Uncinate fasciculus	•			•		
Fronto-occipital fasciculus	•					
Inferior longitudinal fasciculus				•		

panic severity on the Panic Disorder Severity Scale, what is in good accordance with hypothesis suggesting a key role of midbrain structures in the generation of panic.

Considering possible white matter (WM) abnormalities in PD, the first study to investigate the WM integrity by applying the DTI technique revealed higher structural integrity in terms of greater fractional anisotropy (FA) values in the cingulum bundle (Han et al. 2008). In a recent study with 30 first-episode, medication-naive, and late-onset PD patients, Lai and Wu (2013a) showed reduced integrity in WM tracts of the right inferior fronto-occipital fasciculus, left body of corpus callosum, and left superior longitudinal fasciculus (SLF) when compared with 21 controls. In contrast, Kim et al. (2013) reported no significant difference in WM integrity between 26 PD patients and 26 healthy controls. However, the authors showed increased right-lateralised FA in posterior thalamic radiation, posterior and superior corona radiata, SLF, and sagittal stratum in patients with the catechol-*O*-methyltransferase (COMT) AA/AG genotype group compared to those with the GG genotype. In a subsequent study, they reported decreased FA in frontal WM and the genu of the corpus callosum in 36 short-term medicated patients with PD compared to 27 healthy controls (Kim et al. 2014). Furthermore, increased structural integrity in the internal capsule, corpus callosum, superior and posterior corona radiata, thalamic radiations, sagittal stratum, and SLF were detected in 12 PD patients with a history of suicide attempts compared to 24 PD patients without suicidal attempts (Kim et al. 2015). However, due to the lack of a healthy control group, this result should be interpreted with caution.

Recently, Lai and Wu (2016) compared 53 medication-naive patients with 1st-episode PD, 53 medication-naive patients with 1st-episode MDD, and 54 healthy controls with regard to the WM integrity. The PD group had lower integrity in bilateral SLF and left inferior fronto-occipital fasciculus when compared to controls, whereas MDD patients revealed reductions in the WM integrity when compared with controls in the bilateral SLF, inferior longitudinal fasciculi, inferior fronto-occipital fasciculi, and corpus callosum. The MDD group had lower WM integrity than the PD group in the left anterior thalamic radiation, left uncinate fasciculus, left inferior fronto-occipital fasciculus, and bilateral corpus callosum. Using voxel-based morphometry (VBM), Konishi et al. (2014) demonstrated in 40 PD patients significant volumetric reductions in widespread WM regions including fronto-limbic, thalamo-cortical, and cerebellar pathways compared with 40 healthy controls. One structural imaging study investigated cortical gyrification in PD and detected significant reduction in gyrification in 23 patients with PD in the lateral brain, extending from the fronto-parietal to the temporal areas compared with 33 healthy individuals

(Yoon et al. 2013). Schwartz et al. (2015) demonstrated a significant relationship between behavioural inhibition and hippocampal structure. Behavioural inhibition in childhood predicted reduced hippocampal volumes in adolescents who were offspring of parents with either PD or PD with comorbid major depression, suggesting a role of the hippocampus in anxiety disorder. Finally, Shinoura et al. (2011) reported in a case report study that damages to the dorsal part of the ACC led to repeated panic attacks, indicating that this structure might play an important pathophysiologic role in PD.

There are only a few studies that aim to explore the possible effect of treatment on brain structure changes in PD patients. Recently, these relatively small studies in patients with PD have demonstrated remission stage following 6 weeks of escitalopram treatment that was accompanied by not only a significant increase in the GM volume in the left superior frontal gyrus but also a reduction in the right precentral gyrus. Furthermore, the changes in total GM volume after remission were correlated with changes in clinical scores (Lai and Wu 2013b). Another study using DTI found increased white matter microstructural integrity reflected by fractional anisotropy in some regions of the right uncinate fasciculus and left fronto-occipital fasciculus after escitalopram remission in PD patients (Lai et al. 2013). Earlier, increases of GM volume were showed in left infero-frontal cortex, right fusiform gyrus, and right cerebellum areas in remitted depressive patients with comorbid PD following 6 weeks medication with duloxetine (Lai and Hsu 2011). None of these studies have specifically focused on predictive effect of brain structural measures on treatment response.

2.2 Functional Magnetic Resonance Imaging Studies in Panic Disorder and Agoraphobia (See Tables 5 and 6)

The twin approaches, of both resting-state and emotional reactivity paradigms, have been applied with functional MRI studies to explore neural activation in brain circuits related to PD. One of these studies, examining the differences in the resting-state functional connectivity (RSFC) showed that patients with PD have increased RSFC between the amygdala and the bilateral precuneus as well as altered RSFC between dorsal ACC (dACC) and frontal, parietal, and occipital areas in comparison with healthy volunteers (Pannekoek et al. 2013). In another study with a larger sample of first-episode drug-naive patients with PD, right-lateralised altered local fractional amplitude of low frequency fluctuations (fALFF) signal was observed in the occipital cortex, putamen, and thalamus among patients (Lai and Wu 2012b). It should be noted that ALFF represents the strength or intensity of low frequency oscillations in the BOLD (i.e. blood-oxygen-level dependent) signal, where the fractional ALFF represents the ratio of the amplitude in a low frequency band to the amplitude in the total frequency band. There was also evidence showing abnormal regional homogeneity in the occipital cortex of PD patients compared with

Table 5 Brain activation alterations during fMRI tasks in anxiety disorders, OCS, and PTSD

Activation to emotional or provocation tasks		PDA	GAD	SP	SAD	OCD	PTSD
Frontal	OFC	↑			↑↓	↑	
	Frontopolar		↑		↑		
	DLPFC	↑	↑↓	↑	↑	↑	
	DMPFC	↑	↑↓				
	VLPFC	↑	↑↓				
	VMPFC					↓	↓
	Precentral		↑				
Temporal	Anterior temporal					↑	
	Superior temporal sulcus				↑		
	Hippocampus	↑			↑		
	Parahippocampus			↑			
Limbic	Amygdala	↑↓	↑↓	↑	↑↓	↑	
	Insula	↑	↓		↑	↑	
	ACC	↑	↑↓			↑	↓
	MCC	↑					
	PCC	↑			↓		
Parietal	Precuneus	↑			↓		
Occipital	Visual				↑		
	Associative			↑			
	Lingual	↓			↓		
Subcortical	Putamen					↑	
	Globus pallidus				↑	↑	
	Caudate	↑				↑↓	
	Nucleus accumbens	↑					
	Thalamus	↑			↓		
	Subthalamic nucleus					↑	
	BNST		↑				
Cerebellum	Cerebellum	↓			↓		
Brainstem	VTA		↑				
	Midbrain	↑					
	Brainstem	↑					

controls and decreased inter-hemispheric functional coordination (based on the voxel-mirrored homotopic connectivity) in PD patients in the posterior cingulate cortex (PCC) and precuneus (Lai and Wu 2013c).

Although presentation of words or pictures with threatening contents represents a common paradigm to elicit fear reaction and activation of certain brain regions, it has been noted that PDA patients do not necessarily show an abnormal response to fearful stimuli, unless the stimulus is panic-specific. For example, Engel et al. (2016) found greater activation in PDA patients ($n = 19$) than control subjects in the insular cortices, left inferior frontal gyrus (IFG), dorsomedial prefrontal cortex (DMPFC), the left hippocampal formation, and left caudate, when responses to panic or neutral

Table 6 Brain areas with altered activation during fMRI tasks associated with treatment effect of pharmacological or psychological interventions in anxiety disorders, OCD, and PTSD

Activation to emotional or provocation tasks		PDA	GAD	SP	SAD	OCD	PTSD
Frontal	OFC			•	•	•	
	Frontopolar						•
	DLPFC	•		•	•		•
	DMPFC	•					
	VLPFC	•	•	•			•
	VMPFC						•
	SMA						•
Temporal	STG	•				•	
	Hippocampus	•					
	Parahippocampus			•			
Limbic	Amygdala	•	•	•	•		•
	Insula	•	•	•	•		
	ACC		•	•	•	•	•
	Paralimbic		•				
Parietal	Precuneus				•		
	Postcentral						
	Supramarginal	•					
	SPL			•			
	IPL				•		•
Occipital	Middle occipital				•		
	Associative			•			
Subcortical	Dorsal striatum		•				
	Ventral striatum						•
Cerebellum	Cerebellum				•	•	

pictures were compared. Similarly, higher activation in an extended fear network, involving the brainstem, insula, thalamus, ACC, mid cingulate cortex, and DMPFC, was demonstrated in PD patients ($n = 26$) for panic-related vs neutral scenes (Feldker et al. 2016). Interestingly, the authors did not show any significant differences in amygdala activation between groups; however, subjective levels of anxiety significantly correlated with brainstem activation in PD patients, which is in good accordance with the hypothesis of Gorman et al. (2000). Notably, simple conditioning and safety signal processing results were also related to increased midbrain activation, which was positively associated with anxiety sensitivity, in a large sample of patients with PD and agoraphobia ($n = 60$) (Lueken et al. 2014).

The non-specific role of the amygdala in PD probably explains the inconsistent or rather negative results among the studies examining the neural activation of this brain region in PD patients. In contrast to control subjects, who demonstrated the classic amygdala activation to fearful masked faces, PDA patients ($n = 13$) were characterised by the absence of any significant change in amygdala response, suggesting desensitisation (Ottaviani et al. 2012). By using a face-matching

paradigm in PD patients, Poletti et al. (2015) showed a positive correlation between anxiety sensitivity and neuronal activation during emotional processing in several regions, including DMPFC, ACC, and insula, but not in the amygdala. Nevertheless, activation of the right amygdala and right hippocampus was observed among PDA patients after presentation of words with potentially threatening content (van den Heuvel et al. 2005). On the contrary, during the presentation of anxious and happy faces, PDA patients displayed a lower activation or no responsivity in amygdala, respectively; however, distinguishing changes were found in the ACC (Pillay et al. 2006, 2007). In addition, Demenescu et al. (2013) reported that patients with PD ($n = 14$), but not those with social phobia, showed hypoactivation in the amygdala and lingual gyrus during perception of angry, fearful, happy, and neutral faces, compared to healthy participants. The authors also found a positive correlation between degree of anxiety symptoms and functional connectivity of the amygdala to dACC and to DMPFC during perception of fearful faces.

Regarding other brain regions, increased activation in the left posterior cingulum and the left middle frontal cortex after presentation of words with potentially threatening content was demonstrated in PDA as compared with controls (Maddock et al. 2003). Fear provocation with anxiety-related images was associated with increased activity in the inferior frontal cortex, the hippocampus, the anterior and posterior cingulate, and the OFC (Bystritsky et al. 2001). In another study, PDA patients differed from controls in showing increased activity in the left IFG in response to panic-related relative to neutral words (Dresler et al. 2012). Greater bilateral insula activation to unpredictable aversiveness has distinguished PDA from both healthy control and major depressive disorder, indicating a specific role of the insula in the anxiety pathogenesis (Gorka et al. 2014). Decreased neural activation in the occipital cortex and the cerebellum, and increased activation in the precuneus was observed in PD patients with agoraphobia ($n = 15$) in comparison to healthy volunteers (Petrowski et al. 2014). Finally, Wittmann et al. (2014) investigated the neural correlates of the anticipation of agoraphobic situations in large sample of PD patients with agoraphobia ($n = 72$) using agoraphobia-specific and neutral pictures presented with and without anticipatory stimulus. Higher activations were found in the bilateral ventral striatum and left insula in patients compared to controls during the anticipation of agoraphobia-specific pictures. These enhanced insula responses are consistent with the well-known tendency of PD patients to be supersensitive to changes in bodily symptoms.

A recent fMRI study of patients with PD comorbid with agoraphobia has failed to find any brain regions predictive of cognitive behavioural therapy (CBT) outcome (Hahn et al. 2015). However, an earlier study by Lueken et al. (2013) observed that treatment response to CBT in panic patients was associated with an inhibitory functional coupling between the anterior cingulate cortex and the amygdala, whereas responders and non-responders were characterised by distinct neuronal activation at the baseline. Their later study in the same sample has demonstrated that inhibitory ACC–amygdala coupling during fear conditioning was associated with the long variant of the serotonin transporter polymorphism (5-HTTLPR) in PD responders only. This points toward potential intermediate connectivity phenotype modulating

response to exposure-based CBT (Lueken et al. 2015). A better response to brief CBT was predicted in subjects with an increased pretreatment activation in bilateral insula and left dorsolateral prefrontal cortex (DLPFC) during threat processing (Reinecke et al. 2014). In addition, the greater activation in cortico-limbic circuitry, including superior frontal gyri, anterior insula, superior temporal, supramarginal, and hippocampus, predicted better CBT response in mixed sample of patients with PD and GAD (Ball et al. 2014). Very recently, Liebscher et al. (2016) showed that successful CBT led to a greater decrease in anxiety symptoms and associated reduction in bilateral amygdala activation during processing of agoraphobia-related pictures compared with the patients receiving antidepressants and a waiting list control group. In another randomised, controlled, multicentre clinical trial, Kircher et al. (2013) reported that after CBT, patients with PD and agoraphobia ($n = 42$) revealed reduced activation for the conditioned response in the left IFG compared to control subjects, which was correlated with reduction in agoraphobic symptoms. Patients also demonstrated increased functional connectivity between the IFG and amygdala, insula, as well as ACC after CBT. However, neither of these last two studies explored predictive effect of neural activation on CBT response. Regarding pharmacological intervention in PD, the remission following escitalopram treatment was associated with changes in regional homogeneity of temporo-parietal regions in a recent fMRI study, which, however, again did not specifically explore predictive measures of treatment response (Lai and Wu 2013b).

2.3 Positron Emission Tomography, Single Photon Emission Computed Tomography, and Magnetic Resonance Spectroscopy Studies in Panic Disorder and Agoraphobia (See Tables 7, 8, 9, and 10)

Radiotracer brain-imaging techniques with SPECT and PET, targeting regional cerebral blood flow (rCBF), regional cerebral metabolic rate of glucose (rCMRGlc) uptake or key proteins of neurochemical systems as well as metabolic MRS can contribute to further understanding of the biomarkers underlying pathogenesis of anxiety disorders and efficacy of therapeutic intervention. However again, up to now, a very limited number of these studies have been conducted in anxiety disorders, and particularly in PDA. Neumeister et al. (2004) used PET to study serotonin type 1A (5-HT1A) receptor binding in 16 patients with PD and 15 matched healthy controls. PD patients showed lower values in the anterior and PCC as well as in the raphe nuclei. For the first time, this study provided in vivo evidence for the involvement of serotonin type 1A receptors in the pathophysiology of PD. Nash et al. (2008) performed a 5-HT1A receptor binding study in PD patients at different stages of therapy. Nine drug-naive patients with PD, seven patients who had recovered on SSRI medication, and nineteen healthy volunteers underwent a single PET scan. In untreated patients, both presynaptic and postsynaptic 5-HT1A receptor

Table 7 5-HT system alterations from PET and SPECT studies in anxiety disorders, OCD, and PTSD

Serotonin system radioligand binding			PDA	GAD	SP	SAD	OCD	PTSD
5-HT synthesis	Temporal	Temporal gyrus					↑	
		Hippocampus				↑	↑	
	Limbic	Amygdala				↑		
		ACC				↑		
	Subcortical	Putamen				↑		
		Caudate				↑	↑	
	Brainstem	Raphe nuclei				↑		
5-HTT	Frontal	Precentral						↑
	Temporal	Temporal	↓					
	Limbic	Amygdala						↓
		Insula				↑		
		ACC	↑					
		PCC						↑
	Subcortical	Putamen				↑	↓	
		Caudate				↑		
		Thalamus	↓			↑	↓	
	Brainstem	Midbrain	↑↓				↑	
		Raphe nuclei	↑			↑		
		Pons					↑	
5-HT1A	Frontal	OFC	↓					
		DLPFC						↑
		Ventral prefrontal						↑
		Medial prefrontal						↑
	Temporal	Temporal	↓					↑
		Parahippocampus						↑
	Limbic	Amygdala	↓			↓		↑
		Insula				↓		↑
		ACC	↓			↓		↑
		PCC	↓					↑
	Parietal	Parietal						↑
	Occipital	Occipital						↑
	Brainstem	Raphe nuclei	↓			↓		↑
5-HT1B	Limbic	Amygdala						↓
		ACC						↓
	Subcortical	Caudate						↓
5-HT2A	Frontal	Frontopolar					↓	
		DLPFC					↓	
		Medial prefrontal					↓	
	Temporal	Associative					↓	
	Parietal	Associative					↓	
	Subcortical	Caudate					↑	

Table 8 Dopaminergic system alterations from PET and SPECT studies in anxiety disorders, OCD, and PTSD

Dopamine system radioligand binding			PDA	GAD	SP	SAD	OCD	PTSD
Dopamine synthesis	Frontal	Premotor					↑	
	Temporal	MTG					↑	
	Limbic	PCC					↑	
	Parietal	Precuneus					↑	
	Occipital	Cuneus					↑	
		Lingual					↑	
	Cerebellum	Cerebellum					↑	
Dopamine transporter	Subcortical	Striatum	↑	↓		↑↓		
D1 receptor	Subcortical	Caudate					↓	
D2 receptor	Subcortical	Striatum				↓	↓	

Table 9 GABAergic system alterations from PET and SPECT studies in anxiety disorders, OCD, and PTSD

GABA system radioligand binding			PDA	GAD	SP	SAD	OCD	PTSD
Benzodiazepine–GABAA receptor	Frontal	Prefrontal	↓					↓
	Temporal	Temporal cortex	↓					
		Temporal pole		↓				
		Hippocampus	↑					
		Parahippocampus	↑					
	Limbic	Insula	↓					
		Cingulate cortex	↓					
	Parietal	Parietal	↓					
MR spectroscopy GABA			PDA	GAD	SP	SAD	OCD	PTSD
GABA level	Frontal	OFC					↓	
		Medial prefrontal	↓					
	Limbic	ACC	↓				↓	
	Occipital	Occipital	↓					
	Subcortical	Basal ganglia	↓					

Table 10 Brain areas with glutamatergic/NAA system alterations from PET and SPECT studies in anxiety disorders, OCD, and PTSD

Glutamatergic/NAA MR spectroscopy		PDA	GAD	SP	SAD	OCD	PTSD
Whole brain					•		
Frontal	DLPFC		•				
	Medial prefrontal					•	
Temporal	Hippocampus		•				•
Limbic	Insula				•		
	ACC				•	•	•
Subcortical	Caudate					•	
	Thalamus					•	

binding was reduced predominantly in the raphe nuclei, OFC, temporal cortex, and amygdala. In recovered patients, presynaptic binding (in the raphe) was also reduced, but there was no significant reduction in postsynaptic binding. Maron et al. (2004a) investigated the binding of the serotonin transporter (5-HTT) in patients with PD by SPECT. The authors reported a significant decrease in 5-HTT binding in the midbrain, in the temporal lobes, and in the thalamus in eight acute PD patients, but not in eight remitted patients in comparison to matched controls. Regional 5-HTT binding was negatively correlated with the severity of panic symptoms. Notably, in the same sample of PD patients they also found significantly higher dopamine transporter (DAT) binding in striatum among remitted PD females as compared with both currently ill PD and control females, indicating a possible role of the dopamine system in panicogenesis (Maron et al. 2010b). In a further 5-HTT binding study of PD by PET, Maron et al. (2011) reported increased binding potential of the serotonin transporter in the raphe nuclei and several cortical areas. These findings were observed in male patients, but not in females. The authors concluded that distinctive functioning of the serotonin system in male and female PD patients might underlie the gender-dependent expression of the disease. Importantly, these results were confirmed by another independent PET study of Cannon et al. (2013), which also showed increased regional 5-HTT binding in male PD patients, but not in females.

The benzodiazepine (BZD) receptor system is another main target for neuroimaging studies in PD. Several research groups using PET methods had found decreased BZD receptor binding potential in multiple regions of the frontal, temporal, parietal, cingulate, insular, and limbic areas in subjects with PD as compared with controls (Malizia et al. 1998; Cameron et al. 2007; Hasler et al. 2008). Therefore, abnormal BZD–gamma-aminobutyric acid type A receptor binding in PD may suggest that basal and/or compensatory changes in inhibitory neurotransmission play roles in the pathophysiological mechanism of PD (Hasler et al. 2008). Interestingly, in contrast to other brain areas BZD receptor binding potential was increased in the hippocampus/parahippocampal region of PD patients (Hasler et al. 2008). Considering changes in 5-HTT binding in the same area, these pieces of evidence indicate that the hippocampus may have a different role in panicogenesis than in other parts of the panic neurocircuitry, as was suggested by Deakin and Graeff's hypothesis (1991). It should be noted, however, that opposite changes in the BZD system were found by SPECT studies. For example, increased, but not decreased BZD receptor uptake in the prefrontal cortices was reported (Kuikka et al. 1995), while decreased BZD receptor binding in the hippocampus of PD patients was demonstrated (Bremner et al. 2000b). These inconsistencies between PET and SPECT results raise an important issue about the technical differences between the two methods, probably leading to inconsistent findings, which should be taken into account by researchers. Finally, a single PET study has recently explored the density of neurokinin-1 (NK1) receptors in the brains of panic patients and found a widespread reduction, demonstrating for the first time in living human brain that neurokinin could be involved in the development of PD (Fujimura et al. 2009).

MRS studies of γ-aminobutyric acid (GABA) concentration have also produced conflicting data with early studies showing lower concentration of GABA, particularly in ACC, medial prefrontal cortex, occipital cortex, and basal ganglia of PD patients relative to comparison subjects (see for review Bandelow et al. 2016). However, a recent review and meta-analysis of 1H-MRS studies, involving 81 patients with PD, showed no significant differences in GABA levels (Schür et al. 2016). For a further discussion of the value of GABA MRS in Psychiatric research, see Myers et al. (2014).

A few studies which used the traditional approach of measuring brain baseline glucose metabolism by [(18)F]fluorodeoxyglucose-PET have reported normalisation of decreased metabolic level in global neocortical and limbic areas among PD responders, but not in non-responders after 12 weeks of escitalopram treatment (Kang et al. 2012) as well as adaptive metabolic changes in several cortical areas of PD patients after either successful CBT or antidepressant interventions (Prasko et al. 2004). In addition, no correlation was found between the changes in rCBF and clinical measures in PD patients who completed a CBT course (Seo et al. 2014).

2.4 Genetic Studies in Panic Disorder and Agoraphobia

Although the data from twin and family studies suggest an involvement of genetic factors in the familial transmission of PD with a heritability estimate near 40% (Hettema et al. 2001), the genetic substrate underlying panicogenesis is not yet understood. The linkage studies so far have suggested that chromosomal regions 13q, 14q, 22q, 4q31–q34, and probably 9q31 are associated with the transmission of PD phenotypes; however, only a few genes with a possible role in PD were detected within these loci (see for review Maron et al. 2010a). The molecular genetic research on PD has grown tremendously in the past decade and particularly strengthened by GWA approaches in more recent times. The pioneer association studies have predominantly focused on classic candidate genes, the "usual suspects", or on those putatively relevant to PD pathogenesis, such as genes related to serotonin, cholecystokinin, dopamine, or adenosine systems. Subsequent molecular research has extended the search to candidate genes related to hormonal, opioid, immune, neurotrophic, and other systems. However, most of the results of previous candidate-gene association studies investigating common variants remain inconsistent, negative, or not clearly replicated as the majority of these studies had small sample sizes, different methodological designs and study populations, and/or had limited clinical characterisations of PD. Only the Val158Met polymorphism of the COMT gene has been implicated in susceptibility to PD by several studies in independent samples and confirmed in a recent meta-analysis (Maron et al. 2010a).

Moreover, the available GWA studies of PD have not yet established risk loci, although genetic and functional data have recently implicated a variant in the transmembrane protein 132D (TMEM132D) gene, of which the molecular function is still unclear. Recently, Howe et al. (2016) conducted novel, exploratory meta-

analyses and a robust review of previously published genetic association studies of PD, considering the influences of sex, agoraphobia co-morbidity, and ancestry of origin. Their meta-analyses were performed on 23 variants in 20 genes that have been previously implicated as susceptibility genes in PD pathogenesis. Most of the variants were observed to have null findings with only two genes, COMT and TMEM132D, surviving stringent correction for multiple testing in studies with samples of European ancestry. No significant associations were observed in the secondary analyses considering sex, agoraphobia co-morbidity, and studies with samples of Asian ancestry. Despite not very fruitful results of molecular genetic research in PD, several international research groups are now collaborating to analyse the genetic substrates of panic phenotypes. For example, the recently formed Panic International Consortium (PAN·I·C) is aiming to identify new markers using the largest combined and, therefore, most powered sample of patients to date. The results of this joint effort are likely to shed more light on the genetic underpinnings of PD in the near future.

Among studies exploring genetic markers of therapeutic outcomes in PD, two available reports indicate that better response to SSRI treatment is predicted by the L-form of 5-HTTLPR and the 5-HT1A receptor-1019C/G polymorphism, respectively (Perna et al. 2005; Yevtushenko et al. 2010). In addition, the COMT 158Val allele was associated with greater symptom relief during exposure-based CBT (Lonsdorf et al. 2010), while carriers of the long monoamine oxidase A (MAOA)-uVNTR alleles showed significantly worse outcome after CBT (Reif et al. 2014). Recently, preliminary evidence for poorer CBT treatment outcomes in a subgroup of female traumatised individuals carrying the low-active variant of the MAOA gene was also reported (Trautmann et al. 2017).

3 Generalised Anxiety Disorder

The main feature of this anxiety disorder is excessive and persistent worry, accompanied by somatic anxiety symptoms as well as from restlessness, irritability, difficulty concentrating, muscle tension, sleep disturbances (insomnia), and being easily fatigued.

3.1 Structural Magnetic Resonance Imaging Studies in Generalised Anxiety Disorder (See Tables 1, 2, 3, and 4)

There is good evidence that GAD is characterised by significant anatomical changes in the brain, particularly within regions related to anxiety neurocircuitry. For example, increased GM volume in amygdala has been repeatedly found in GAD patients (Etkin et al. 2009). Notably, increased right amygdala volume in GAD patients,

mostly among females, was associated with prolonged reaction times on a tracking task, indicating attentional impairment. In another study, larger volumes of the amygdala and the DMPFC were observed in GAD females, suggesting that these disturbances in anxiety specific regions may be related to gender predisposition for GAD (Makovac et al. 2016a). In contrast, significantly larger GM volume in the right putamen was found in GAD patients as compared with healthy controls, whereas a significant gender main effect was in the left precuneus/PCC, with larger GM volumes in males compared with females. However, no gender-by-diagnosis interaction effect was found in this study, suggesting that GM volumes in GAD are not influenced by gender (Liao et al. 2014). The same group has also reported that larger GM volumes in the right putamen are positively correlated with childhood maltreatment (Liao et al. 2013). A study in medication-free adolescents suffering from non-comorbid GAD reported increased GM volumes in the right precuneus and right precentral gyrus and decreased grey matter volumes in the left orbital gyrus and posterior cingulate (Strawn et al. 2013). Compared with healthy adolescents, youth with GAD exhibited increased cortical thickness in the right inferolateral and ventromedial prefrontal cortex (VMPFC) (i.e. IFG), the left inferior and middle temporal cortex as well as the right lateral occipital cortex. No relationships were observed between cortical thickness and the severity of anxiety symptoms in the significant regions (Strawn et al. 2014). Additionally, significantly higher GM volumes were found in medication-free GAD subjects mainly in basal ganglia structures and less consistently in the superior temporal pole; however, WM volumes were lower in the DLPFC (Hilbert et al. 2015b). Similarly, significant reduction of the WM volumes in the DLPFC, anterior limb of the internal capsule, and midbrain was observed in GAD patients with working memory dysfunction (Moon and Jeong 2017). Notably, reduced DLPFC volume was negatively correlated with clinical severity and illness duration in GAD, whereas significantly less OFC volume was demonstrated in females compared to male patients (Moon and Jeong 2015). A decrease of hippocampal volumes has also been found in GAD (Abdallah et al. 2013). The distinguishable brain alterations, particularly thinner cortices in the right medial orbitofrontal and fusiform gyri, left temporal pole, and lateral occipital regions, were found in MDD patients with comorbid GAD as compared with those without GAD or controls, supporting the notion that GAD is a distinct clinical entity (Canu et al. 2015). Finally, reduced frontolimbic structural connectivity was demonstrated in patients with GAD in a diffusion tensor imaging study, suggesting a neural basis for emotion regulation deficits in GAD (Tromp et al. 2012).

3.2 Functional Magnetic Resonance Imaging Studies in Generalised Anxiety Disorder (Tables 5 and 6)

The regions traditionally connected to anxiety neurocircuitry and/or emotional regulation, including the amygdala, ACC, medial prefrontal cortex, ventrolateral

prefrontal cortex (VLPFC), DLPFC, and some others, have showed abnormal or changed activities in GAD. In particular, greater amygdala activation was demonstrated in paediatric patients with GAD and positively correlated with anxiety severity (Monk et al. 2008). Earlier, other paediatric GAD studies have shown hyperactivity in the amygdala in response to negative emotional faces (McClure et al. 2007). Also, disruptions in amygdala-based intrinsic functional connectivity networks have been reported to be similar between adult and adolescents with GAD (Roy et al. 2013). Similar findings were also evident in adult GAD patients (Nitschke et al. 2009; Etkin et al. 2010). In another study, GAD patients presented higher amygdala activation than healthy controls in response to neutral, but not angry faces (Hölzel et al. 2013). However, after fear induction in a gambling task, patients with GAD demonstrated decreased activity in the amygdala and increased activity in the bed nucleus of the stria terminalis when compared with controls (Yassa et al. 2012).

Evidence for the involvement of cortical regions was demonstrated in studies showing that in response to angry faces or triggered worry, GAD patients demonstrated increased BOLD responses in the lateral region of the middle frontal gyrus (Blair et al. 2008) and persistent activation in both ACC and prefrontal cortex areas (Paulesu et al. 2010). The exaggerated early neural responses to errors, as reflected by the error-related negativity on EEG, were also linked to ACC abnormalities in GAD (Weinberg et al. 2010). In contrast, hypoactivation of prefrontal cortex (only in female patients) or reduced dACC BOLD activity was observed in response to fearful, sad, angry, and happy facial expressions (Palm et al. 2011; Blair et al. 2012). The BOLD hypoactivation in prefrontal cortex was also demonstrated in both GAD and PD during responding in a reappraisal task, suggesting common neuronal pathways underlying emotion dysregulation in both anxiety disorders (Ball et al. 2013). In contrast, significantly higher neuronal activities were observed in the VLPFC and precentral gyrus BOLD response to anxiety-inducing words (Moon et al. 2015b).

The VMPFC has been shown to have a critical role in threat processing in close association with broader corticolimbic circuit abnormalities, which may synergistically contribute to GAD (Cha et al. 2014b). Moreover, maladaptive threat processing was observed in the ventral tegmental area and the mesocorticolimbic system in female patients with GAD, which may implicate dopaminergic pathways in clinical anxiety (Cha et al. 2014a). Adolescents with GAD showed greater right VLPFC activation to trials containing an angry face as compared to healthy adolescents. This activation was negatively correlated with anxiety severity, suggesting that the BOLD signal increase may serve as a compensatory response (Monk et al. 2006). Functional abnormalities in ventral cingulate and the amygdala, however, seem to be common for both major depression and GAD, perhaps due to shared genetic factors (Etkin and Schatzberg 2011). However, those with comorbid GAD and major depression had modulated hypoactivation in middle frontal regions and the insula to an emotional task which is usually seen in pure depression; this gives additional support to there being different types of emotional information processing in anxiety and depression (Schlund et al. 2012).

RSFC was reported to be lower in prefrontal–limbic and cingulate and higher in prefrontal–hippocampus regions, and both abnormalities were correlated with clinical symptoms severity (Wang et al. 2016). Also recently, the amygdala–prefrontal cortex connectivity underlying worry and rumination in GAD has been linked to autonomic dyscontrol, suggesting overlapping neuronal substrates for cognitive and autonomic dysregulation (Makovac et al. 2016b). Furthermore, amygdala and middle frontal gyrus activation in response to the presentation of emotional faces can distinguish patients with GAD and social phobia, indicating different neural circuitry dysfunctions in these two highly prevalent anxiety disorders (Blair et al. 2008). Previous research also implicates the ACC in emotion regulation through effects on the amygdala and suggests that deficits in ACC–amygdala connectivity may contribute to emotion dysregulation in patients with GAD (Etkin et al. 2010). Different hippocampal connectivity was observed between PTSD and GAD patients, potentially explaining the difference in fear-related memory dysregulation in two anxiety phenotypes. In particular, a robust and selective blunting in both connectivity and task-independent deactivation within the posterior hippocampus/default-mode network (DMN) was found in PTSD patients, which was dissociable from the intact connectivity and deactivation patterns in GAD patients, who were furthermore indistinguishable from healthy cohorts (Chen and Etkin 2013). Increased activation of the medial prefrontal cortex and right VLPFC as well as altered connectivity between the amygdala or VLPFC and regions which subserve mentalisation (e.g. PCC, precuneus, and medial prefrontal cortex) was observed in adolescents with GAD (Strawn et al. 2012). In addition, increased functional connectivity between the hippocampus/parahippocampus and the fusiform gyrus was found in GAD, while a greater functional connectivity between the somatosensory cortex and the thalamus was observed in PD, further suggesting different clinical and psychopathological processes between these two disorders (Cui et al. 2016). Finally, decreased functional connectivity between the left amygdala and left DLPFC and increased right amygdala functional connectivity with insula and superior temporal gyrus were found in adolescents with GAD, confirming that they have abnormalities in brain regions associated with the emotional processing pathways (Liu et al. 2015).

Only a few fMRI studies have measured changes after treatment. The greater pretreatment reactivity to fearful faces in rostral ACC and lesser reactivity in the amygdala predicted better response to medication with venlafaxine for 8 weeks in GAD. However, no differences in neuronal activation within these regions were detected before treatment between patients and controls (Whalen et al. 2008). In addition, higher levels of pretreatment anterior cingulate cortex activity in anticipation of both aversive and neutral pictures were associated with greater reductions in anxiety and worry symptoms following 8 weeks of venlafaxine treatment in GAD (Nitschke et al. 2009). This suggests that ACC–amygdala responsivity could prove useful as a predictor of reuptake blocker treatment response in GAD. A significant increase in right VLPFC activation in response to angry faces following treatment with CBT or fluoxetine was reported in small samples of young patients with GAD (Maslowsky et al. 2010). Greater anticipatory activity in the bilateral dorsal amygdala was shown in GAD and a CBT course led to attenuation of amygdalar and

subgenual anterior cingulate response to fear/angry faces presentation, plus heightened insular activation to happy faces (Fonzo et al. 2014). However, the treatment had no apparent effects on increased amygdalo-insular connectivity and the changes were not associated with symptoms of worry. An interesting study with the BZD alprazolam found that neuronal activation in the amygdala and insula during emotional tests was reduced after acute administration of alprazolam. However, activity returned to baseline levels at week 4 of alprazolam treatment, indicating that the neural mechanisms supporting sustained treatment effects of BZDs in GAD differ from those underlying their acute effects (Brown et al. 2015). Significantly reduced BOLD responses to a pathology-specific worry in prefrontal regions, striatum, insula, and paralimbic regions were reported after 7 weeks of treatment with citalopram in a small sample of patients with GAD (Hoehn-Saric et al. 2004).

3.3 Positron Emission Tomography, Single Photon Emission Computed Tomography, and Magnetic Resonance Spectroscopy Studies in Generalised Anxiety Disorder (Tables 7, 8, 9, and 10)

The first PET report in GAD found lower absolute metabolic rates in the basal ganglia, but increased rates in the left inferior gyrus, Brodmann area (BA) 17, in the occipital lobe, right posterior temporal lobe, and the right precentral frontal gyrus (Wu et al. 1991). Unlike in MDD and other anxiety disorders, there was no change in serotonin reuptake site availability in the brains of GAD patients as measured by tracers such as [123I]nor-beta-CIT and [I]ADAM (Maron et al. 2004b; Lee et al. 2015). In contrast, dopamine reuptake site density measured by [Tc]TRODAT-1 in the striatum was significantly lower in GAD patients than in the healthy controls (Lee et al. 2015). Additionally, significant decreases in GABA-A receptors in the left temporal pole were found in a SPECT study with GAD female patients (Tiihonen et al. 1997b).

The first MRS study demonstrated a higher *N*-acetylaspartate/creatine (NAA/Cr) ratio in the right DLPFC in medication-free patients with GAD as compared to healthy participants (Mathew et al. 2004). A subsequent study reported persistently lower levels of bilateral hippocampal NAA/Cr in GAD after successful treatment with 12 weeks of paroxetine but excluded an association between this hippocampal neuronal marker and anxiolytic response to this medication (Mathew et al. 2010). Interestingly, a significant increase in hippocampal NAA was observed in GAD responders to the glutamate-release inhibitor riluzole, while non-responders had decreases over 8 weeks of treatment (Mathew et al. 2008). A low level of choline/*N*-acetylaspartate in the DLPFC was observed in GAD patients in another recent study and this negatively correlated with anxiety severity (Moon et al. 2015a).

3.4 Genetic Studies in Generalised Anxiety Disorder

So far, only a few association studies have been conducted among patients with GAD phenotype, leaving our knowledge about GAD vulnerability genes without consistent or clear conclusions. Specifically, genetic variants in MAOA and 5-HTTLPR have been demonstrated to be potentially implicated in the pathogenesis of GAD (Tadic et al. 2003; You et al. 2005), while the association of GAD with 5-HTR1A gene variation was partly mediated by comorbidity with major depression (Molina et al. 2011). A recent study showed that the Met allele of the functional brain-derived neurotrophic factor (BDNF) Val66Met polymorphism is associated with GAD risk along with an increase in serum BDNF levels (Moreira et al. 2015). However, Val66Met variation was not associated with GAD nor BDNF plasma levels in a Chinese Han patient population (Wang et al. 2015). In addition, polymorphisms of both regulator of G-protein signaling 2 (RGS2) and neuropeptide Y (NPY) genes have been shown to modify risk of post-disaster GAD under conditions of high stressor exposure among adults living in areas affected by the 2004 Florida Hurricanes (Koenen et al. 2009; Amstadter et al. 2010). In Italian families with type 2 diabetes, a few single nucleotide polymorphisms (SNPs) in a proteasome modulator 9 (PSMD9) gene were associated with GAD (Gragnoli 2014).

Recently, microarray studies of peripheral gene expression signatures have become a powerful and promising approach in the discovery of novel biomarkers via transcriptional and microRNA analyses. For example, a microRNA array study performed in peripheral blood mononuclear cells (PBMCs) has revealed negative correlation between expression levels of miR-4505 and miR-663 and anxiety manifestation in GAD patients. However, the molecular mechanism of this association requires further explanation (Chen et al. 2016). Another genome-wide peripheral gene expression study in quite a large sample of patients with GAD found no significant differential expression in women, but 631 genes, most of which were immune-related, were differentially expressed between anxious and control men (Wingo and Gibson 2015).

Some other promising data have been reported by pharmacogenetic initiatives, where an intensive search for genetic treatment predictors has revealed a few genes, including the pituitary adenylate cyclase-activating peptide, the serotonin transporter, the serotonin 2A receptor gene, corticotropin-releasing hormone receptor 1, dopamine receptor D3 (DRD3), nuclear receptor subfamily group C, member 1, and phosphodiesterase 1A, as potential markers predicting therapeutic response to SSRI medication in patients with GAD. In contrast, none of the investigated polymorphisms within dopamine receptor D2 (DRD2) or DAT 1 genes showed impact on venlafaxine XR treatment response in GAD (see for review Maron and Nutt 2017).

3.5 Specific Phobias (SPs)

SP is characterised by excessive or unreasonable fear of single objects or situations (e.g. flying, heights, animals, seeing blood, etc.).

3.6 Structural Magnetic Resonance Imaging Studies in SPs

Recently, MRI study reported that subjects with an animal type of SP ($n = 10$) exhibited increased cortical thickness in bilateral insular, bilateral pregenual anterior cingulate, and bilateral PCC as well as left visual cortical regions as compared to 20 healthy control subjects (Rauch et al. 2004). The consequent study showed no difference in cortical thickness or insula volume in adults with animal SP ($n = 19$) when compared with healthy controls; however, anxiety severity predicted right anterior insula thickness in patients but not control subjects (Rosso et al. 2010). A recent study showed that patients with dental phobia differed from individuals with snake phobia and healthy controls; they had significantly increased GM volumes in areas including the right subgenual ACC, left insula, left orbitofrontal, and left prefrontal cortices (Hilbert et al. 2015a).

3.7 Functional Magnetic Resonance Imaging Studies in SPs

A systematic review of 24 fMRI studies has revealed a greater activation in the insula, ACC, amygdala, and prefrontal and OFC of patients exposed to phobia-related situations compared to healthy controls, thus supporting the hypotheses of hyperactivation of a neuroanatomic structural network involved in specific phobia (Linares et al. 2012).

Similar to other anxiety disorders, there are a limited number of studies that have investigated the neuronal effect of medication in people with SPs. An fMRI study which examined the effects of acute D-cycloserine during symptom provocation in spider phobia showed increased activation of the PFC, dACC, and insula during exposure to phobia-related images among patients who received medication as compared to those who received placebo. The authors suggested that during the initial phobic symptom inducement, D-cycloserine increases activation in regions involved in cognitive control and interoceptive integration (Aupperle et al. 2009). The other studies demonstrated that upon exposure to phobia-related images prior to CBT sessions, patients with SPs had increased activity in the parahippocampal gyrus, associative visual cortex, right DLPFC, and amygdala (Paquette et al. 2003; Goossens et al. 2007).

After CBT, phobic subjects showed decreased symptoms and increased activity in areas, such as the associative visual cortex, superior parietal lobule, and bilateral

IFG. There was no change in the activation of the parahippocampal gyrus, and right DLPFC activation (Paquette et al. 2003) but a decrease in amygdala, insula, and ACC hyperactivity (Goossens et al. 2007). These findings suggest that the CBT psychotherapeutic treatment has the potential to modify the dysfunctional neural circuitry associated with SP (Linares et al. 2012). In addition, the individuals with spider phobia who underwent CBT psychotherapy demonstrated decreased activity of the insula, amygdala, and ACC, but increased activation in the medial OFC as compared to a waiting list group (Schienle et al. 2007). In an attempt to prove the efficacy of CBT after 6 months of treatment, Schienle et al. (2009) demonstrated that the efficacy of psychotherapy led to increased activity of the medial OFC, and decreased activity in the insula and lateral OFC. The authors highlighted that the results of the medial OFC could be related to the neural basis of the lasting positive outcome of CBT. Finally, the changes of anterior insula activation and fear scores both measured 2 weeks after an exposure therapy compared to 1 week prior to treatment had strong positive correlations with phobia severity measured at the 8-year follow-up (that is, larger reduction in activation or fear score corresponds to lower phobia severity at follow-up) among females with spider phobia (Lange et al. 2016).

3.8 Positron Emission Tomography, Single Photon Emission Computed Tomography, and Magnetic Resonance Spectroscopy Studies in SPs

The first study using PET in SP did not find significant differences in rCBF between patients with animal phobia and healthy volunteers (Mountz et al. 1989). However, subsequent studies have found significant increases in rCBF in several brain regions, including the visual cortex, amygdala, thalamus, and striatum as well as reductions of rCBF in the OFC, temporopolar cortex, PCC, and hippocampus (see for review Linares et al. 2012). Furthermore, in tasks related to imagination and anticipatory anxiety, patients with small animal phobia showed increased rCBF in the anterior cingulate cortex, insular cortex, anterior temporal cortex, and somatosensory cortex and decreased rCBF in the primary visual cortex, indicating the existence of a neurophysiological correlate of avoidant anticipatory coping (Rauch et al. 1995; Wik et al. 1996). The increase in activation of both the ACC and the left hippocampal–amygdaloid area during exposure to phobia-related images associated with aversive noise was also demonstrated in 16 female patients with SPs, suggesting that the amygdaloid area and the ACC form part of a neural system dedicated to attention and orientation to danger (Pissiota et al. 2003). Finally, a study in the same sample observed a significant reduction of the NK1 receptor antagonist tracer binding in the right amygdala during phobic stimulation, with the authors suggesting that a decreased availability of NK1 receptors may reflect the increased release of endogenous substance P (Michelgård et al. 2007).

3.9 Genetic Studies in SPs

The long and more active MAOA uVNTR alleles have been found to be associated with anxiety disorders of the phobic spectrum (Samochowiec et al. 2004). In Han Chinese patients with specific phobias, association with a BDNF gene variation has been reported (Xie et al. 2011).

4 Social Phobia (Social Anxiety Disorder)

SAD is characterised by persistent and unreasonable fear of being observed or evaluated negatively by others in social performance or interaction situations (e.g. speaking in public or being exposed to possible scrutiny by others) and is associated with somatic and cognitive anxiety symptoms.

4.1 Structural Magnetic Resonance Imaging Studies in Social Anxiety Disorder (Tables 1, 2, 3, and 4)

Increased thickness of the left inferior temporal cortex and a negative association between symptom severity and thickness of the right rostral anterior cingulate cortex were found in SAD patients (Frick et al. 2013). Reduced left amygdala volume was noted in SAD patients and was associated with symptom severity (Fisler et al. 2013). Irle et al. (2010) have also found significantly reduced amygdalar and hippocampal volume in comparison to healthy subjects. Further, on the right side, hippocampal volume was significantly related to more severe SAD symptoms. A recent DTI study revealed a significant increase in FA in bilateral uncinate fasciculus and right inferior longitudinal fasciculus in patients with SAD ($n = 33$) following a 10-week cognitive behavioural group therapy (Steiger et al. 2017). Another study found that only reduced amygdala GM volume, and not functional activity, is associated with a clinical response 1 year after CBT. In particular, left amygdala GM volume was more reduced in responders relative to non-responders from pretreatment to 1-year follow-up (Månsson et al. 2017). Earlier, the same research group reported that diminished amygdala GM volume mediated the relationship between decreased neural responsivity and reduced social anxiety after treatment. Thus, these results suggest that improvement-related structural plasticity impacts neural responsiveness within the amygdala, which could be essential for achieving anxiety reduction with CBT (Månsson et al. 2016).

4.2 *Functional Magnetic Resonance Imaging Studies in Social Anxiety Disorder (Tables 5 and 6)*

In a recent and very comprehensive review, Freitas-Ferrari et al. (2010) have summarised the available fMRI studies and found significant and consistent evidence of altered brain functioning in SAD, with a predominance of limbic structures, especially the amygdala, hippocampus, and insula being affected. The studies published after this review demonstrated that patients with SAD have decreased positive connections within the frontal lobe and decreased negative connections between the frontal and occipital lobes when compared with healthy controls (Ding et al. 2011). In a study of the connectivity of the amygdala, a decreased influence from the inferior temporal gyrus to the amygdala was found in SAD, while bidirectional influences between the amygdala and visual cortices were increased compared with healthy controls (Liao et al. 2010). These results suggest that an amygdala dysfunction in SAD is characterised both by a decreased regulatory influence of OFC and increased communication with the visual cortex, according to the authors. A reduction of prefrontal control over amygdalar activation was also found in another study (Sladky et al. 2015). During a social situation task, patients showed a significantly decreased activation in the left cerebellum, left precuneus, and bilateral PCC (Nakao et al. 2011). In an alternation learning task, the highest correlations between the degree of activation and the anxiety scores as assessed by the Liebowitz Social Anxiety Scale were obtained in the left temporal region and in the OFC (Gross-Isseroff et al. 2010). In contrast to healthy controls, habituation to social stimuli was found in the amygdalae, orbital frontal cortex, and pulvinar thalamus (Sladky et al. 2012). According to the authors, this result, which is somehow counterintuitive, is compatible with the assumption that increased effort is needed within modulatory networks in prefrontal brain areas of SAD patients to exert sufficient top-down control over hyperactivation in the amygdala when confronted with unknown and potentially threatening stimuli. To provide more comprehensive understanding of the neural underpinnings of face perception in SAD, Gentili et al. performed a meta-analysis of 23 fMRI studies, including some unpublished data, which totalled 449 patients and 424 healthy control individuals. They identified significant clusters in which faces evoked a higher response in SAD, including the bilateral amygdala, globus pallidus, superior temporal sulcus, visual cortex, and prefrontal cortex; and also demonstrated a higher activity for healthy controls in the lingual gyrus and in the posterior cingulate (Gentili et al. 2016). The authors suggested that the alteration in neural response to face in SAD is not limited to emotional structures but involves a complex network, whereas a dysfunctional face perception process may bias patient person-to-person interactions.

Interestingly, Pantazatos et al. (2014) reported that SAD is characterised by reduced functional connectivity in the left hippocampus–left temporal pole, and that this feature has discriminated SAD from both PD and healthy controls. Moreover, this deficit was rectified following 8 weeks of treatment with paroxetine. These encouraging results provide support for emerging functional connectivity-based

biomarkers for SAD diagnosis and pharmacological treatment effects. In addition, greater right amygdala–pregenual ACC connectivity and greater left amygdala–pgACC coupling encompassing medial prefrontal cortex predicted better symptom improvement after CBT in patients with generalised SAD (Klumpp et al. 2014), which is in good accordance with previous observations that pretreatment cortical hyperactivity to social threat signals can predict CBT success in this disorder (Doehrmann et al. 2013; Klumpp et al. 2013).

Recently, Young et al. (2017) reported that effective CBT enhances impaired amygdala–prefrontal functional connectivity in SAD. Another study exploring the effect of combining escitalopram and internet-delivered CBT on brain reactivity and connectivity in SAD revealed that such a combination, relative to placebo + CBT, resulted in significantly more decreased right amygdala reactivity to emotional faces, and that this effect was more pronounced in treatment responders as compared to non-responders (Gingnell et al. 2016). Furthermore, a meta-analysis of 12 fMRI studies having a total of 295 subjects with SAD before and after treatment showed that effective psychotherapy significantly increased activity in the bilateral precuneus and left inferior parietal gyrus and decreased activity in the left anterior cingulate gyrus, left middle frontal gyrus, and right cerebellum (Li et al. 2016). In addition, successful pharmacotherapy increased activity in the right postcentral gyrus, left middle occipital gyrus, and right medial orbital frontal gyrus and reduced activity in the bilateral insula and left medial cingula. The authors also reported that the improvement in social anxiety symptoms was positively associated with baseline hyperactivity of the bilateral precuneus, left inferior partial gyrus, right medial cingulate, and right postcentral gyrus but negatively associated with baseline hypoactivity of the bilateral insula and right medial cingulate, whereas no difference in amygdala was found (Li et al. 2016). However, the heightened amygdala reactivity to fearful faces in individuals with SAD was attenuated by administration of oxytocin (Labuschagne et al. 2010). Moreover, the oxytocin enhanced functional connectivity of the amygdala with the rostral ACC/medial prefrontal cortex (Dodhia et al. 2014; Gorka et al. 2015).

4.3 Positron Emission Tomography, Single Photon Emission Computed Tomography, and Magnetic Resonance Spectroscopy Studies in SAD (Tables 7, 8, 9, and 10)

In an early SPECT study, rCBF in patients with SAD was not different from healthy controls (Stein and Leslie 1996). There have been a couple of PET studies measuring rCBF with 15O-water in patients with SAD exposed to a tailored phobic situation. The first used personalised scripts for the SAD patients and compared their brain activity with that found in a study of healthy volunteers who had been exposed to anxiety-producing pain stimuli. Both groups showed increased activation in the classic anxiety-related regions of the ACC, and insula/OFC plus the cerebellum.

However, as predicted, the patients also showed unique activation in right anterior prefrontal and left parietal regions, with the former implicated in symptoms of speech block according to the author and the latter perhaps related to mentally scanning the environment in preparation for escape behaviour in SAD patients (Nutt et al. 1998). In a later study, Van Ameringen et al. (2004) required patients to perform in front of a panel of experts. No significant hyperactivations were found during exposure but deactivations were found in the right lingual gyrus and in the right medial frontal gyrus (as Nutt et al. 1998 also found). According to the authors, deactivation of these regions may reflect a strategy of visual avoidance employed by patients to dampen their phobic experience. In another study with 15O-water PET, during public versus private speaking, subjective anxiety increased more in patients with SAD than in a healthy control group. Increased anxiety was accompanied by enhanced regional blood flow in the amygdaloid complex in the social phobics relative to the comparison subjects (Tillfors et al. 2001). The same research group showed that anticipation of a public speaking challenge was accompanied by enhanced cerebral blood flow in the right DLPFC, left inferior temporal cortex, and in the left amygdaloid-hippocampal region, and by reduced cerebral blood flow in the left temporal pole and bilaterally in the cerebellum in the anticipation group (Tillfors et al. 2002).

In another PET study with SAD patients who underwent a public-speaking challenge at baseline and after placebo treatment, salivary cortisol increase was positively associated with hypothalamic rCBF in a midbrain cluster encompassing the hypothalamus with its statistical maximum in the mammillary bodies, while negative covariations were observed in the medial prefrontal cortex as well as in the motor and premotor cortices. These results suggest that stress-induced cortisol secretion in SAD may be inhibited by activity in the medial prefrontal cortex and enhanced by activity in the hypothalamus (Ahs et al. 2006).

In a study with [18F]fluorodeoxyglucose ([18F]FDG) PET, individuals with SAD demonstrated less pretreatment rCMRglu within the ACC and ventral medial PFC at baseline compared with the healthy controls. Different treatment-induced co-activations of rCBF between the left amygdala and the DLPFC as well as the rostral ACC were observed among responders and non-responders to SSRIs and placebo in a large sample of SAD and could be considered as useful neuromarkers, differentiating between successful and unsuccessful anxiolytic treatments (Faria et al. 2014). An earlier study with a small sample of patients with SAD has reported that non-responders to SSRI medication had higher rCBF at baseline in the anterior and lateral part of the left temporal cortex and the lateral part of the left mid frontal regions as compared with responders (Van der Linden et al. 2000). Additionally, the magnitude of treatment response to the GABA reuptake blocker tiagabine was inversely correlated with pretreatment rCMRglu within VMPFC in patients with generalised SAD (Evans et al. 2009). In a PET study, Furmark et al. (2005) demonstrated that the administration of both an NK1 receptor antagonist and citalopram improved SAD and attenuated neural activity in the medial temporal lobe network. Decreased rCBF in the right amygdala and hippocampus and increased blood flow in the left middle occipital, and bilateral lingual gyri and

hippocampi were observed in patients with SAD following treatment with nefazodone (Kilts et al. 2006).

Based on the high rate of social phobia in Parkinson's disease (Riedel et al. 2010), which is characterised by reduced dopaminergic and noradrenergic functioning, abnormal dopamine functioning had been hypothesised in SAD. In a case-control study using SPECT technology, Tiihonen et al. (1997a) reported a lower striatal density of the DAT in SAD patients compared with control subjects. This finding was replicated in another SPECT study of DAT binding (Warwick et al. 2012). Conversely, van der Wee et al. (2008) reported a significantly higher binding potential for the DAT in the striatum of psychotropic medication-naïve patients with SAD compared with healthy controls. Moreover, after escitalopram therapy, DAT binding was increased in patients with SAD, which may be due to an effect of serotoninergic modulation by SSRI on the dopamine system (Warwick et al. 2012). Several studies have investigated dopamine receptor radiotracers. One study using the dopamine D2 and D3 receptor tracer [123I]-iodobenzamide demonstrated lower D2 receptor binding in the striatum of patients with SAD than in healthy controls (Schneier et al. 2000), but in a follow-up study with [11C]-raclopride binding, the authors could not find any significant difference in dopamine D2 receptor availability nor in DAT binding between SAD and controls (Schneier et al. 2009). A more recent study using high-resolution PET and the high-affinity DRD2 antagonist radioligand [11C]FLB 457, before and after CPT, showed a negative correlation between symptom change after CBT and D2 receptor binding, thus indicating a role for the dopamine system in cortical and limbic brain regions in the pathophysiology of SAD (Cervenka et al. 2012).

In a study using [11C]WAY-100635 PET, reduced 5-HT1A binding in the amygdala and mesiofrontal areas was demonstrated in a SAD sample (Lanzenberger et al. 2007). Brain 5-HTT availability was increased in the thalamus, but not in midbrain in patients with SAD in a study using [123I]-beta-phenyl-tropane SPECT (van der Wee et al. 2008). Another PET study showed that dysregulation of cortisol levels might increase the vulnerability for SAD, by altering limbic serotoninergic 5-HT1A receptors. Indeed, SAD patients displayed significantly lower baseline cortisol plasma levels compared with normal controls and strong negative correlations between cortisol levels and 5-HT1A binding in amygdala, hippocampus, and retrosplenial cortex were observed (Lanzenberger et al. 2010). Recently, in a PET study with the [11C]5-hydroxytryptophan ([11C]5-HTP), serotonin synthesis was examined in SAD patients (Frick et al. 2015a). The authors demonstrated increased [11C]5-HTP influx rates in the amygdala, raphe nuclei region, caudate nucleus, putamen, hippocampus, and ACC of patients with SAD compared with healthy controls, supporting an enhanced serotonin synthesis rate. Furthermore, the serotonin synthesis rate in these regions seems to be modulated by tryptophan hydroxylase 2 (TPH2) G-703T polymorphism (Furmark et al. 2016). Notably, increased 5-HTT availability was observed in the patients with SAD in the raphe nuclei region, caudate nucleus, putamen, thalamus, and insula cortex, indicating possible compensatory mechanisms trying to normalise serotonin concentration (Frick et al. 2015a). In related studies from the same team, both citalopram and the NK1 receptor

antagonist attenuated serotonin synthesis rate in the amygdala after treatment, which was associated with reduced amygdala rCBF during public speaking and accompanied by symptom improvement (Frick et al. 2016a). Interestingly, they also demonstrated that NK1 receptor availability is specifically increased in the right amygdala in SAD patients relative to controls, providing additional support for an involvement of NK in SAD (Frick et al. 2015b).

In an MRS study, a method for measuring whole-brain fluoxetine concentrations was tested and showed a higher level of fluoxetine in the brain of SAD patients who responded to treatment as compared to non-responders (Miner et al. 1995). Another study measured choline, creatine, *N*-acetylaspartate, and myo-inositol and found no change of these compounds after clonazepam treatment (Tupler et al. 1997). Phan et al. (2005) found that glutamate (relative to creatine) levels were significantly higher in SAD patients than in healthy controls in the ACC, but not in the occipital cortex. Pollack et al. (2008) found significantly higher whole-brain levels of glutamate and glutamine but no significant differences in GABA concentrations. Recently, Tükel et al. (2016) reported that ratios of NAA/Cr were significantly higher in patients with SAD than in healthy control subjects in the anterior cingulate and insula, with the ratio in the latter region correlating positively with anxiety severity.

4.4 Genetic Studies in Social Anxiety Disorder

Only a few association and pharmacogenetics studies have been conducted in SAD so far. Gelernter et al. (2004) detected significant linkage for SAD on chromosome 16. Since the gene encoding norepinephrine (noradrenaline) transporter, SLC6A2, maps to this broad region, it has been hypothesised that this gene may be a possible candidate for influencing social phobia risk. A significant impact of several polymorphisms in dopamine receptor D2, D3, and D4 in addition to the DAT (SLC6A3) on SAD was excluded in a sample of 17 multiplex social phobia families (Kennedy et al. 2001). Reduction in social anxiety symptoms during SSRI treatment was significantly associated with the 5-HTTLPR genotype in a very small sample (Stein et al. 2006). However, none of the three gene candidates (5-HTT, COMT, and TPH2) predicted response to CBT in another, larger study (Andersson et al. 2013). Recently, the most powerful study (having 346 patients) with SAD has found that two of the four RGS2 SNPs predict remission to sertraline treatment. These data suggest that this genetic variant could be further evaluated as a potential biomarker for the likelihood of benefiting from SSRI medication among patients with social phobia (Stein et al. 2014).

5 Obsessive–Compulsive and Related Disorders

OCD is characterised by recurrent obsessions (concerns involving contamination, harm, or sexual and religious preoccupations) or compulsions (e.g. washing, checking, repeating, ordering, counting, and hoarding), or both, that cause impairment in terms of distress, time, or interference with functioning.

5.1 Structural Magnetic Resonance Imaging Studies in Obsessive–Compulsive Disorder (Tables 1, 2, 3, and 4)

Structural brain abnormalities are frequently reported in OCD, which is characterised by widespread changes throughout the whole brain, but mainly in the fronto-striato-thalamic circuit, especially in the globus pallidus and medial frontal cortex. However, alterations are not limited to fronto-striatal circuits but might extend to temporal and parietal areas (Tan et al. 2013). Thus, it is possible that other regions, e.g. parietal lobe, may also play a role in OCD (Valente et al. 2005), which might contribute to neuropsychological signature and symptomatology of OCD patients. In fact, this was confirmed by a more recent systematic review of VBM studies, where consistent evidence was found for volume reductions of the dorsolateral prefronto-striatal "executive" circuit, which includes the dorsomedial, dorsolateral, ventrolateral, and frontopolar prefrontal cortices, and of reciprocally connected regions, the temporo-parieto-occipital associative areas, as possible signs for altered anatomical connectivity in fronto-subcortical circuitry (Piras et al. 2015). Moreover, increased volumes of the internal capsule and reduced frontal and parietal white matter volumes were reported in this study. Earlier, another meta-analysis of structural studies revealed reduced GM density in parietal and frontal areas (superior frontal gyrus, supramarginal gyrus [BA 40], DLPFC [BA 9], and anterior prefrontal cortex [BA 10]), and increased grey matter in the lateral part of the OFC (BA 47) and in the putamen to be associated with OCD (Rotge et al. 2010). Altered morphology in terms of increased surface area of the tail and head of the caudate and thalamus and increased thickness in the lateral OFC, left medial temporal cortex, and right posterior cingulate were shown not only in patients with OCD but also (to a lesser degree) in their unaffected first-degree relatives (Shaw et al. 2015). The results were compatible with theoretical models of OCD behaviour, involving increased functional activation of subcortical nuclei coupled with reduced cortical inhibition, and suggested that these anatomical changes may represent a useful vulnerability marker for the investigation of genes linked to the disorder.

Interestingly, a direct comparison between OCD and PDA or PTSD showed that decreased bilateral grey matter volumes in the dorsomedial and anterior cingulate gyri were shared across disorders. However, increased GM in the anterior putamen was a unique feature of OCD; in sharp contrast, the same brain region showed abnormally low volumes in PDA and PTSD as compared to healthy volunteers

(Radua et al. 2010). A multicentre study showed smaller medial frontal grey matter volume in OCD patients in BA 6/8/9, extending to the anterior cingulate BA 24/32, and IFG/insula (BA 13/47). OCD patients additionally showed a relative preservation of putamen and OFC with ageing (de Wit et al. 2014). Recently, possible GM abnormalities in the early stage of paediatric OCD in relation to clinical features were explored and the patient group exhibited greater GM volume in the bilateral putamen and left OFC and less GM volume in the left inferior parietal lobule (Cheng et al. 2016). Both alterations were correlated with symptomatic severity. Thus, these findings provide further evidence of brain GM abnormalities in OCD that are not only present in the classical fronto-striatal-thalamic circuit but also in the DMN, which may represent the interaction of abnormal functional organisation of both networks at an early stage of paediatric OCD. Finally, the most powerful meta- and mega-analyses of data from OCD sites worldwide, including 1,830 OCD patients and 1,759 controls, found that adult OCD patients have significantly smaller hippocampal volumes and larger pallidum volumes compared with adult controls (Boedhoe et al. 2017). Both effects were stronger in medicated patients compared with controls. On the other hand, unmedicated paediatric patients had significantly larger thalamic volumes compared with paediatric controls. These findings highlight the potential importance of neurodevelopmental alterations in OCD and indicate different patterns of subcortical abnormalities in paediatric and adult OCD patients, where the pallidum and hippocampus seem to be key structures in adult OCD, but the thalamus in paediatric OCD.

The only study to investigate the predictive effect of brain alterations on treatment response was that conducted by Hoexter et al. (2013). In particular, they investigated structural MRI correlates as potential pretreatment brain markers to predict treatment response in treatment-naïve OCD patients, entering a randomised 12-week clinical trial of either fluoxetine or group-based CBT. This study showed that symptom improvement in the fluoxetine treatment group was significantly correlated with smaller pretreatment GM volume within the right middle lateral OFC, whereas symptom improvement in the CBT treatment group was significantly correlated with a larger pretreatment GM volume within the right medial prefrontal cortex. Although these findings suggest that pretreatment GM volume of distinct brain regions within the lateral OFC and medial prefrontal cortex was differentially correlated to treatment response to fluoxetine versus CBT in OCD patients, the included sample was relatively small and needs replication in a larger set of patients with a prospective design. Recently, Tang et al. (2016) aimed to assess the possible effect of 12 weeks of medication with the SSRI sertraline on brain structure in a relatively small sample of OCD patients. They demonstrated a significant increase in GM volume in several brain structures, including the left anterior and posterior cingulate gyri, thalamus, and putamen, following improvement on medication in their patients, while no significant differences were found on resting fMRI. Additional data is provided by Banks et al. (2015) who revealed that features of ACC structure and connectivity predicted clinical response to dorsal anterior cingulotomy for refractory OCD (Banks et al. 2015). They suggested that the variability seen in individual responses to a highly consistent, stereotyped procedure may be due to neuroanatomical variation in the patients.

5.2 *Functional Magnetic Resonance Imaging Studies in Obsessive–Compulsive Disorder (Tables 5 and 6)*

A common assay to investigate the neural underpinnings of OCD is represented by symptom provocation experimental paradigms in which patients are exposed to individually tailored stimuli designed to induce an anxious or symptomatic state. One of the seminal studies, using PET, found increased glucose consumption in the right caudate, left ACC, and bilateral OFC during symptom provocation (Rauch et al. 1994). The involvement of these and other brain regions, such as lateral frontal, anterior temporal areas, insula, and amygdala, was confirmed by a subsequent study in which symptom provocation was used in conjunction with fMRI (Breiter et al. 1996). More recently, a novel experimental paradigm was designed to achieve live tailored provocation either by placing provoking stimuli (i.e. "contaminated" gloves) in a patient's hands during fMRI scanning or by having real-time video exposure to the experimenter disorganising and littering the patient's home. In this study, effective symptom provocation was associated in OCD patients with deactivation of the caudate and prefrontal circuits and hyperactivation of the subthalamic nucleus and putamen (Banca et al. 2015). Dimensionality of OCD, i.e. the fact that patients have thoughts and compulsions related to different domains (i.e. symmetry, forbidden thoughts, cleaning, and hoarding), has been investigated by Mataix-Cols et al. (2004) using symptom provocation, highlighting that different neural circuits might be implicated in the manifestation of different OCD symptom dimensions.

To explore regional spontaneous activity abnormalities in OCD, an fALFF approach was applied in an fMRI study involving 31 patients with OCD and 32 age- and sex-matched normal controls (Qiu et al. 2017). They found that patients with OCD showed decreased fALFF not only in the cortico-striato-thalamo-cortical (CSTC) circuits like the thalamus, but also in other cerebral systems like the cerebellum, the parietal cortex, and the temporal cortex. Additionally, OCD patients demonstrated significant associations between decreased fALFF and obsessive–compulsive symptom severity in the thalamus, the paracentral lobule, and the cerebellum, suggesting that brain areas outside the CSTC circuits may also play an important role in the pathophysiology of OCD. In addition, resting-state studies demonstrated that by sampling the signal from different areas of the basal ganglia and addressing functional connectivity with the rest of the brain, OCD patients showed an imbalance of connectivity of fronto-striatal loops (Harrison et al. 2009). Namely, reduced functional connectivity was identified along the dorsal axis linking the caudate to the lateral prefrontal cortex and increased connectivity was identified along the ventral axis between the nucleus accumbens and the anterior prefrontal cortex. By adopting a data-driven approach, instead of seed-based analysis, clusters of decreased global connectivity in the left lateral prefrontal cortex were found in OCD patients. Within regions of interest located in subcortical structures, increased global connectivity in the dorsal striatum and anterior thalamus was found, which was reduced in patients on medication (Anticevic et al. 2014). Results have been extended further by showing that pathophysiological changes

within fronto-striatal circuits are common across OCD symptom dimensions. However, it is possible to identify unique functional connectivity "fingerprints" related to symptom dimensions: viz. aggression symptoms were related to altered functional connectivity between the ventral striatum, amygdala, and ventromedial frontal cortex; sexual/religious symptoms involving ventral striatum–insula connectivity; and hoarding modulated the strength of functional connectivity between both ventral and dorsal striatal regions and distributed frontal areas (Harrison et al. 2013). Furthermore, the neuronal deficit of the right caudate body during executive functioning among patients with OCD was confirmed by a recent meta-analysis of 28 peer-reviewed studies using fMRI (Del Casale et al. 2016).

Teasing apart the potential confounding effect of medication, Posner et al. (2014) found that un-medicated OCD patients showed hypo-connectivity along the ventral/limbic circuit possibly pointing to this network as candidate vulnerability marker to test the effect of pharmacological or behavioural treatment. The degree of functional connectivity of relevant brain areas seems to have a clinical relevance for OCD. For example, higher symptom severity was linked to greater hypo-connectivity of the right superior OFC (Meunier et al. 2012). In fact, excessive fronto-striatal connectivity between the nucleus accumbens and the prefrontal cortex was normalised after deep brain stimulation treatment, and the degree of connectivity normalisation significantly correlated with OCD symptoms improvement (Figee et al. 2013). In a longitudinal study, haemodynamic response of the ACC and right OFC to obsession-inducing images was reduced after CBT and associated with reduced symptom severity (Morgiève et al. 2014). In two longitudinal studies, fMRI data of OCD patients were acquired before and after SSRI treatment. Response to SSRIs was correlated with changes in the degree of connectivity of the right ventral frontal cortex after treatment (Shin et al. 2014) as well as pretreatment activation in the right cerebellum and in the left superior temporal gyrus (Sanematsu et al. 2010). In the first study exploring the predictive value of RSFC for the outcome of CBT in OCD, Göttlich et al. (2015) found that degree centrality of the right basolateral nuclei group of the amygdala was positively correlated with the response to subsequent therapy (Göttlich et al. 2015). In particular, their results suggest that the diminished CBT response in patients showing a lower degree centrality of the basolateral amygdala reflects a deficient fear circuit in these patients which may impact fear extinction as a core mechanism of exposure-based CBT.

5.3 Positron Emission Tomography, Single Photon Emission Computed Tomography, and Magnetic Resonance Spectroscopy Studies in Obsessive–Compulsive Disorder (Tables 7, 8, 9, and 10)

Across PET and SPECT studies, the head of the caudate was found to be associated with greater glucose metabolism and reduced cerebral blood flow in OCD patients,

versus controls (Whiteside et al. 2004). A seminal paper hinting at the tight reciprocal influence of cortical and basal ganglia brain areas demonstrated that clomipramine, a tricyclic antidepressant with serotonin reuptake inhibiting properties, led to a decreased metabolic rate in the OFC and caudate nucleus and an increase in the right anterior putamen (Benkelfat et al. 1990). Different studies evaluating the impact of SSRI and using different imaging modalities and methods of analysis have consistently shown that lower pretreatment glucose metabolic rate of the OFC is associated with better response to SSRI in OCD patients (Saxena et al. 1999; Swedo 2002; Rauch et al. 2002). Responders to SSRIs treatment showed greater glucose consumption normalisation in the anterior lateral OFC and caudate nucleus compared with non-responders (Saxena et al. 1999). In addition, activity in the lateral OFC (BA 11 and BA 47) predicted subsequent reduction in OCD symptom severity associated with treatment with SSRIs (Saxena et al. 1999; Rauch et al. 2002). However, this does not directly transfer to behavioural therapy where a better response is associated with higher cerebral metabolic rate in the left OFC (Brody et al. 1998).

A large study in OCD has suggested that baseline increased rCBF in forebrain regions and decreased perfusion in posterior brain regions can potentially predict treatment response to SSRI monotherapy or combined medication with quetiapine (Wen et al. 2013). Furthermore, higher whole-brain perfusion and higher rCBF values in the cerebellum were associated with drug response (Ho Pian et al. 2005). In contrast, no pattern of baseline activation distinguishing responders from non-responders to subsequent SSRI pharmacotherapy was detected in another SPECT perfusion study, which included SAD, OCD, and PTSD samples (Carey et al. 2004). A study comparing response to CBT and fluoxetine had complicated results; correlations between normalised left OFC metabolism and treatment response revealed that higher normalised metabolism in this region was associated with greater improvement in the CBT-treated group, but worse outcome in the SSRI group (Brody et al. 1998). Additionally, no significant differences in pretreatment rCBF were observed in a SPECT study between responders and non-responders to behaviour therapy. However, the post-treatment rCBF values in the left medial prefrontal cortex and bilateral middle frontal gyri were significantly lower in the responders than in the non-responders (Yamanishi et al. 2009).

With respect to the role of the 5-HTT in the neurobiology of OCD, SPECT, and PET, studies have shown inconsistent results, apparently because of differences in tracer properties, but also due to the variability in patient characteristics (gender, 5-HTTLPR genotype, smoking, age at onset, etc.). Hence, no difference in 5-HTT density was observed between unmedicated OCD patients of both genders and healthy controls. However, reduced 5-HTT availability in various brain regions was found in several other studies, and higher midbrain-pons [123I]-beta-CIT binding in one report (for review, see Maron et al. 2012). In a recent study, patients with OCD and healthy controls underwent serial PET scans after administration of escitalopram. The results showed that the patients with OCD exhibited significantly lower 5-HTT binding when drug-free than healthy controls in the putamen and the thalamus, consistent with previous findings in the striatum and thalamus in drug-free

patients (Kim et al. 2016). Thus, this finding could account for the poor response and the high risk of relapse in OCD. Notably, in a study using 5-HTT imaging with [123I]beta-CIT SPECT before and after 1 year of citalopram treatment in OCD, it was shown that higher occupancy of 5-HTT by citalopram was associated with a better clinical response (Stengler-Wenzke et al. 2006).

One PET study using a low selectivity tracer [18F]altanserin showed significantly higher 5-HT2A receptor distribution in the caudate nuclei in untreated OCD patients as compared with age- and gender-matched healthy volunteers. 5-HT2A receptor binding did not correlate significantly with the severity of OCD and appeared not to be different from the findings in healthy controls after treatment with SSRIs (Adams et al. 2005). Other PET studies used a selective radioligand [11C]MDL 100907. One showed a significant reduction of cortical 5-HT2A receptor availability in prefrontal cortices, temporal and parietal associative areas in drug-naïve patients, which correlated negatively with clinical severity (Perani et al. 2008). However, Simpson et al. (2011) failed to detect group differences in [11C]MDL 100907 binding potential in any region of interest and demonstrated no correlation between regional 5-HT2A availability and OCD severity, indicating that OCD is not characterised by major changes in 5-HT2A availability in the cortical or limbic brain regions.

Using a PET method for estimating brain regional 5-HT synthesis, [11C]-AMT K* trapping, Berney et al. (2011) reported that medication-free patients exhibited significantly greater [11C]-AMT K* trapping in the right hippocampus and in the left temporal gyrus relative to age- and sex-matched controls. Furthermore, these differences were more robust in male patients, who also had higher [11C]-AMT K* values in the caudate nucleus. In addition, there were significant and positive correlations between OCD symptom severity and 5-HT synthesis rate in the caudate and the temporal lobe of the patients, suggesting that increased 5-HT neurotransmission could be a contributing factor to the pathophysiology and symptom profile of OCD.

Regarding the dopamine system, Perani et al. (2008) reported reduced D2 receptors binding in the whole striatum, particularly in the ventral portion, possibly reflecting endogenous dopaminergic hyperactivity. In addition, the study of Hsieh et al. (2014) suggests the involvement of increased dopaminergic neuronal function in OCD. They showed that OCD patients had increased dopaminergic metabolism in the left frontal premotor cortex, along with trends toward an increase in the left posterior cingulate gyrus, bilateral cuneus, bilateral lingual gyri, right precuneus, right middle temporal gyrus, and bilateral cerebellum compared to the healthy subjects. The binding of [(11)C]-SCH23390 at D(1) receptors in OCD patients was also significantly reduced in both caudate nucleus and putamen compared with healthy controls; however, no correlations were found between D(1) binding potential and symptom measures (Olver et al. 2009). In a subsequent study, the authors demonstrated significantly reduced D(1) binding in the ACC of a small sample of drug-free OCD patients as compared with matched healthy controls (Olver et al. 2010). Finally, a significant decrease of DAT binding in the right basal ganglia was observed in OCD patients following treatment with SSRI (Kim et al. 2007).

In vivo quantification of specific neurochemicals in various brain regions by means of MRS in OCD has received limited research attention to date, and findings should be regarded as preliminary (Brennan et al. 2013). Several factors might limit the results, for example, small sample sizes, varying severity, illness duration, and treatment as well as suboptimal technical methodologies (i.e. low magnetic field and single voxel assay). Further technological advances and the possibility of coupling several imaging techniques show promise for generating important findings in the future. A Canadian study found that OCD children and adolescents had higher levels of right prefrontal white matter choline and NAA, with levels of NAA, creatine, and myo-inositol being positively and significantly correlated with severity of symptoms (Weber et al. 2014). In the same year, an American study showed compromised white matter integrity and reduced myelination in some brain regions of children with OCD, particularly the corpus callosum and fibre tracts that connect the frontal lobes to widespread cortical and subcortical targets (Rosso et al. 2014).

More recently, a Spanish study did not confirm the hypothesis of differences in glutamate plus glutamine concentrations in the ACC between children and adolescents with OCD and healthy controls; however, the authors found differences in the glutamine concentration in OCD patients depending on the duration of illness (Ortiz et al. 2015). Adult patients with OCD showed significantly decreased glutamate level in medial prefrontal cortex and right thalamus, and significantly increased choline compounds in left thalamus as compared to healthy subjects, indicating glutamatergic signaling dysfunction in OCD (Zhu et al. 2015b). The other large study showed that individuals with OCD had significantly decreased GABA concentration in the OFC area and a trend for a decrease in the ACC (Zhang et al. 2016). Earlier, Tükel et al. (2015) reported that the NAA/Cr levels were significantly lower in patients with OCD than in healthy controls in the anterior cingulate and in the caudate, but this difference disappeared after patients took 12 weeks of sertraline, suggesting that reductions in NAA can be reversed with SSRI treatment, which may indicate an improvement in neuronal integrity.

5.4 Genetic Studies in Obsessive–Compulsive Disorder

Although OCD is familial and heritable, the genetic factors responsible for its pathogenesis remain largely unknown despite the numerous candidate gene studies conducted. Genes related to the serotonergic neurotransmitter system have been examined extensively in the genetic risk of developing OCD given the role of serotonin in the proposed neurocircuitry, and the significance of SSRIs as the first-line pharmacological treatment of OCD. A recent meta-analysis of OCD genetic association studies showed significant results for a 5HTTLPR and the serotonin 2A receptor rs6311 marker (Taylor 2013). Also, in an early onset paediatric study, the 5HTTLPR LA allele conferred risk (Walitza et al. 2014). However, two recent GWASs of OCD did not detect any significant markers related to this system (Stewart et al. 2013b; Mattheisen et al. 2015).

Other evidence has implicated abnormalities in the glutamatergic system as part of the aetiology of OCD, with the most robust genetic results being from genes that are involved in this system. A meta-analysis by Taylor (2013) reported a non-significant trend in a glutamatergic system gene, the neuronal glutamate transporter gene (SLC1A1). Furthermore, although the first published GWAS of OCD by the International OCD Foundation Genetics Collaborative group did not detect genome-wide significant association between any tested markers and OCD diagnosis (Stewart et al. 2013b), interesting trends were observed in several glutamatergic system genes including the discs large (drosophila) homologue-associated protein 1 and glutamate receptor, ionotropic, and kainate 2 genes. Another recent but more in-depth meta-analysis by Stewart et al. (2013a) examining nine SNPs across the 3′ region of the SLC1A1 gene and OCD illness revealed a consistent nominally significant finding in one of the SNPs, rs301443, with another SNP showing modest association when controlled for gender (rs12682897). The second GWAS conducted by the OCD Collaborative Genetics Association also did not report any genome-wide significant results (Mattheisen et al. 2015) but detected a trend in the top-hit marker on chromosome 9 near the protein-tyrosine phosphatase, receptor-type, delta gene, which promotes glutamatergic synaptic differentiation.

Interest in the dopaminergic system stems from the treatment of OCD using dopamine receptor blocking agents as augmenting treatments alongside SSRIs (Fineberg et al. 2013). Although Taylor (2013) in his recent meta-analysis of OCD genetic association studies showed a nominally significant finding for the COMT rs4680 marker and non-significant trends for two additional dopaminergic system genes (DAT1 and DRD3), no other genetic studies support a dopamine genetic link to OCD. In addition, genes involved in the GABAergic system have been investigated with inconsistent and non-replicated results (Taylor 2013). Similarly, a lack of support was reported for the BDNF gene including the most studied Val66Met (rs6265) variant (Zai et al. 2015) and genes related to neuroplasticity (Taylor 2013). The recently formed International Obsessive Compulsive Disorder Foundation Genetics Collaborative (IOCDF-GC) has conducted a meta-analysis from the two previous consortia, investigating a total of 2,688 individuals of European ancestry with OCD and 7,037 genomically matched controls (International Obsessive Compulsive Disorder Foundation Genetics Collaborative (IOCDF-GC) and OCD Collaborative Genetics Association Studies (OCGAS) 2017). The several variants located in or near the genes ASB13, RSPO4, DLGAP1, PTPRD, GRIK2, FAIM2, and CDH20, identified in linkage peaks and the original GWASs, were among the top signals. Notably, this joint analysis constituting the largest single OCD genome-wide study to date represents a major integrative step in elucidating the genetic causes of OCD.

Although several pharmacogenetic studies have been conducted in OCD, no definitive results support a single genetic variation or gene that determines the response to an SSRI. However, the most intriguing pharmacogenetic findings involving both pharmacokinetic and pharmacodynamic lines of evidence have emerged from studies in OCD and have been reviewed in detail (Zai et al. 2014). As the authors summarised, only two cytochrome P450 liver enzyme genes,

CYP2D6 and CYP2C19, have been studied in relation to the SSRI response in OCD. This showed that non-responders appear to be more common among non-extensive metabolisers according to genetic status of CYP2D6, suggesting that genes regulating metabolism of drugs may play an important role in treatment response (Brandl et al. 2014). Regarding the pharmacodynamic studies in OCD, available data are still inconsistent, preliminary, or not yet replicated in independent and well-powered samples. Among various candidates, a number of genes related to the serotonin, glutamate, and dopamine systems, in addition to neurotrophic factors, have been identified as promising genetic predictors of treatment response to antidepressants in OCD (Zai et al. 2014). Furthermore, OCD remains the only anxiety disorder, where the GWAS approach was applied to detect novel biomarkers of treatment response. Many new loci were identified as top hits in the recent GWAS of SSRI response in OCD patients, including GRIN2B, glypican 6, dispatched homologue 1 (Drosophila), ankyrin repeat and fibronectin type III domain containing 1, and arrestin domain containing 4 (T-cell lymphoma invasion and metastasis 1, protocadherin 10) (Qin et al. 2016). However, a great deal of further research is required to clarify their functional status and their potential role in the treatment response.

6 Post-traumatic Stress Disorder

PTSD develops after a terrifying ordeal including that which involves exposure to actual or threatened death, serious injury, or sexual violence via experiencing by oneself, witnessing in person, or learning close family members or friends are exposed to such events. It is characterised by recurrent and intrusive distressing recollections of the event(s), nightmares, dissociative flashback episodes, distress at exposure to cues that resemble the traumatic event, avoidance of stimuli associated with the trauma, estrangement from others, sleep disturbances, irritability, difficulty concentrating, hypervigilance, and exaggerated startle response.

6.1 Structural Magnetic Resonance Imaging Studies (Tables 1, 2, 3, and 4)

According to a recent review of studies investigating white matter volume in PTSD, volume reductions were reported more often than increases in these populations. However, these findings require further replication as the heterogeneity of the exact locations indicated only a weak overlap across published studies (Daniels et al. 2013). In the same review, when addressing DTI studies, differences were found consistently in the cingulum and the SLF. However, the directions of the difference (i.e. increases in or decreases in fractional anisotropy) were found equally.

Several MRI studies and a meta-analysis (Karl et al. 2006) consistently showed that PTSD is associated with reduced hippocampal volumes, which is in good line with the hypothesis suggesting that lower volume in hippocampus is the result of exposure to trauma (Bremner 2001). PTSD patients also showed broad reduction of GM volume in different brain regions, including VMPFC, dACC, left ACC, left insula and right parahippocampal gyrus, medial prefrontal cortex, left middle temporal gyrus, and right superior frontal gyrus (see for review Lebois et al. 2016). The decreased volume of the inferior temporal cortex was found to be inversely related to self-reported anxiety level in PTSD patients (Kroes et al. 2011). Findings of reduced hippocampal and medial OFC volumes were also independently shown by another study but no difference was found for amygdala volumes (Levy-Gigi et al. 2013). However, an investigation of a larger sample of nearly a hundred PTSD patients was characterised by decreased amygdala volumes (Morey et al. 2012). Volume reduction was not associated with the PTSD chronicity, trauma load, and severity of depressive symptoms.

In relation to treatment effect, the structural MRI studies in PTSD have reported that a better response to CBT is predicted by greater right hippocampal GM volume (Levy-Gigi et al. 2013) and larger rostral ACC volume (Bryant et al. 2008). Both regions are linked to threat processing and fear memory activation and seem to be involved in mediation of CBT effectiveness. War veterans who did not respond to trauma-focused CBT were reported to have smaller left hippocampi prior to treatment than those who were PTSD remitters (van Rooij et al. 2015b). However, correlation analyses indicated no significant relationship between hippocampal volume and PTSD severity either pre- or post-treatment. Another study in PTSD showed that symptom improvement was correlated with and predicted by cortical thickness in the right subgenual ACC (Dickie et al. 2013).

6.2 *Functional Magnetic Resonance Imaging Studies in Post-traumatic Stress Disorder (Tables 5 and 6)*

Several brain regions, including the amygdala, VMPFC, ACC, insula, and hippocampus, have been identified by fMRI studies as key neuronal structures involved in PTSD. Increased activation has been consistently observed in amygdala, dACC, and insula in response to different emotional tasks and stimuli, and some studies also reported a positive correlation between amygdala activity and PTSD symptom severity (see for review Lebois et al. 2016). Furthermore, in an earlier meta-analysis of fMRI studies in PTSD, SAD, and specific phobia, greater activity was found in the amygdala and insula in all three disorders but to a lesser degree in PTSD than in the other two disorders when compared to matched comparison subjects. In contrast, hypoactivation in the VMPFC and the ventral ACC have often been reported in PTSD and suggested to be main factors contributing to the maintenance of traumatic memories and impaired emotion regulation (Etkin and Wager 2007).

One resting-state fMRI study in PTSD patients has revealed that regional spontaneous activity of precuneus and OFC could be a potential prognostic indicator for chronic treatment with antidepressant drugs (Zhu et al. 2015a). In another study, PTSD responders to psychotherapy showed increased pretreatment activation of the left inferior parietal lobe during contextual cue processing compared with non-responders. This activation predicted percentage symptom improvement and therefore could potentially serve as a valuable predictive biomarker for PTSD treatment response (van Rooij et al. 2015a). An earlier fMRI study had demonstrated that poor improvement after CBT course was associated with greater bilateral amygdala and ventral anterior cingulate activation in response to masked fearful faces, suggesting that excessive fear processing of emotional stimuli may be a key factor in limiting responses to psychotherapy of PTSD (Bryant et al. 2008).

Recently, MacNamara et al. (2016) demonstrated that SSRI treatment increased activation in both the left DLPFC and supplementary motor area (SMA) during emotion regulation, although only changes in the SMA occurred over time in veterans with PTSD and not those without PTSD. Furthermore, less activation of the right VLPFC/IFG during pretreatment emotion regulation was associated with a greater reduction in PTSD symptoms with SSRI treatment, irrespective of pretreatment severity. Two further studies were conducted in the same sample of PTSD patients. One reported that psychotherapy increased lateral frontopolar cortex activity and connectivity with the VMPFC/ventral striatum and that greater increases in frontopolar activation were associated with improvement in hyperarousal symptoms and psychological well-being (Fonzo et al. 2017b). The other showed that at baseline, individuals with larger treatment-related symptom reductions demonstrated greater dorsal prefrontal activation and less left amygdala activation during emotion reactivity. In addition, greater VMPFC/ventral striatal activation was noted during emotional conflict regulation in comparison with the waiting list condition (Fonzo et al. 2017a). These studies conclude that changes in frontopolar function during deliberate regulation of negative affect is one key mechanism of adaptive psychotherapeutic change in PTSD, whereas capacity to benefit from prolonged exposure in PTSD is gated by the degree to which prefrontal resources are spontaneously engaged when superficially processing threat and adaptively mitigating emotional interference, but not when deliberately reducing negative emotionality.

6.3 Positron Emission Tomography, Single Photon Emission Computed Tomography, and Magnetic Resonance Spectroscopy Studies in Post-traumatic Stress Disorder (Tables 7, 8, 9, and 10)

Radioisotope studies of the 5-HT system showed reduced 5-HTT availability in the amygdala (Murrough et al. 2011b) but up-regulation of brainstem and forebrain 5-HT1A receptors (Sullivan et al. 2013) in PTSD patients. Additionally, reduced

functioning of the 5-HT1B receptor in the caudate, amygdala, and anterior cingulate cortex following early trauma exposure was reported (Murrough et al. 2011a). However, no difference in regional 5-HT1A receptor binding was reported in another PET study in PTSD (Bonne et al. 2005). A recent interesting PET study, exploring the serotonin and substance P/neurokinin-1 (SP/NK1) systems individually as well as their overlapping expression in PTSD, revealed an increased number of NK1 receptors, but unchanged availability of 5-HTT in amygdalae (Frick et al. 2016b). However, increased 5-HTT availability was detected in the precentral gyrus and PCC of PTSD patients. Moreover, patients, relative to controls, displayed a lower degree of overlapping expression between 5-HTT and NK1 receptors in the putamen, thalamus, insula, and lateral orbitofrontal gyrus, where lower overlap was associated with higher PTSD symptom severity. Thus, these results suggest that aberrant serotonergic-SP/NK1 couplings contribute to the pathophysiology of PTSD and, consequently, that normalisation of these couplings may be therapeutically important (Frick et al. 2016b).

A reduction in BZD binding in the brain, localised to the medial prefrontal cortex, was found in a PET study (Bremner et al. 2000a). Recently, Holmes et al. (2017) demonstrated significantly higher cortical mGluR5 availability in PTSD in a PET study and positive correlations between mGluR5 availability and avoidance symptoms. In a postmortem sample, they also observed up-regulation of SHANK1, a protein that anchors mGluR5 to the cell surface, as well as decreased expression of FKBP5, implicating aberrant glucocorticoid functioning in PTSD (Holmes et al. 2017). The results of this study provide insight into the molecular mechanisms underlying PTSD and suggest that mGluR5 may be a promising target for a mechanism-based treatment aimed at mitigating this disorder.

The available MRS studies reported so far find significantly lower glutamate + glutamine/creatine (Glx/Cr) levels in the ACC in paediatric PTSD patients relative to remitted patients as well as significantly lower Glx/Cr levels in the ACC in remitted patients relative to healthy controls (Yang et al. 2015). PTSD patients also exhibited significantly lower NAA in bilateral hippocampi, and significantly higher Glu and Glu/NAA in the right hippocampus (Rosso et al. 2017). Importantly, the metabolic changes were strongly correlated with re-experiencing symptoms, indicating that hippocampal neuronal integrity and glutamate metabolism may reflect biomarkers of clinically significant disease variation in PTSD. Another MRS study showed that although Glx concentrations within the dACC did not differ between recently traumatised and non-traumatised control groups, a positive linear relationship was observed between Glx concentrations and current stress disorder symptoms in traumatised individuals (Harnett et al. 2017). Moreover, Glx concentrations showed a positive linear relationship with future stress disorder symptoms (i.e. assessed 3 months post-trauma), suggesting that glutamate concentrations, particularly in the dACC, may play a role in both acute and future PTSD symptoms following a traumatic experience.

No pattern of baseline activation distinguishing responders from non-responders to subsequent SSRI pharmacotherapy was detected in a SPECT perfusion study, which included SAD, OCD, and PTSD samples (Carey et al. 2004). This study is in

good accord with the results of Seedat et al. (2004) who also observed no significant pretreatment differences between PTSD responders and non-responders in anterior cingulate perfusion and even deactivation in the left medial temporal cortex following medication with citalopram was irrespective of clinical response. Interestingly, these conflicting pieces of evidence drawn from imaging studies with pharmacological treatment are in line with results from trials that have applied psychological interventions. However, treatment effects of brief eclectic psychotherapy on PTSD symptoms did correlate positively with activation in the left superior temporal gyrus, and superior/middle frontal gyrus (Lindauer et al. 2008).

6.4 *Genetic Studies in Post-traumatic Stress Disorder*

Candidate-gene association studies for PTSD have been recently summarised by Koenen et al. (2013). Available studies showed that various neurotransmitter systems may confer susceptibility to PTSD, including the serotonin system (SLC6A4 promoter region polymorphism, 5-HTTLPR, and 5-HTR2A), the HPA axis (the FK506 binding protein 5 gene, which is coding for a protein influencing glucocorticoid receptor sensitivity, CRHR1), the dopaminergic system (COMT, DRD2, and DAT1), the endocannabinoid system (the cannabinoid receptor 1 gene), and the GABA system (GABA receptor subunit α2 gene). In addition, an involvement of the adrenergic system, in particular the norepinephrine transporter, in the pathogenesis of PTSD was also reported (Pietrzak et al. 2015). A study by Smith et al. (2011) addressed DNA methylation as a possible mediator of persistent changes in gene function following chronic stress in PTSD. The author demonstrated that global methylation was increased in subjects with PTSD and that several genes which were differentially methylated in PTSD patients were inflammation-related. Together, these results suggest that psychosocial stress may alter global and gene-specific DNA methylation patterns potentially associated with peripheral immune dysregulation.

The first GWAS of PTSD identified a genome-wide significant association between PTSD and an SNP (rs8042149) in the retinoid-related orphan receptor gene (Logue et al. 2013), which was later found to be predisposing in individuals with a history of child abuse to PTSD (Lowe et al. 2015). Importantly, the association between this gene SNP rs8042129 and PTSD was replicated in a cohort of Florida hurricane survivors (White et al. 2013). Another GWAS conducted in a sample of European Americans and African Americans has revealed that a gene for Tolloid-Like 1 or TLL-1, a zinc dependent metalloprotease (which plays an important role in remodeling of the extracellular matrix), may be a determinate risk of PTSD development (Xie et al. 2013). The involvement of UNC13C and DSCAM genes within the non-Hispanic black group as well as TBC1D2, SDC2, and PCDH7 genes within the non-Hispanic white group was reported by another GWAS with a multiracial sample largely composed of the US veterans (Ashley-Koch et al. 2015). The largest GWA study of PTSD to date, carried out in a US military sample, found a

significant association with ANKRD55, a gene involved in autoimmune and inflammatory disorders (Stein et al. 2016). Furthermore, a multi-ethnic/racial GWAS of PTSD provided evidence for the phosphoribosyl transferase domain containing 1 gene or PRTFDC1, encoding an enzyme in the purine metabolic enzyme family, to be involved in PTSD pathology (Nievergelt et al. 2015).

There have been very few studies looking at the genetics of treatment response in PTSD. One in particular found that the LL genotype of 5-HTTLPR was associated with greater responsiveness to sertraline treatment and with lower drop outs due to adverse events among PTSD patients (Mushtaq et al. 2012). Additionally, Bryant et al. (2010) reported that the shorter allele of 5-HTTLPR may be associated with poorer long-term treatment response to imaginal exposure-based CBT in individuals with PTSD. Two small studies indicate a possible predictive role of BDNF and glucocorticoid receptor gene BCLI polymorphisms on CBT treatment outcome in PTSD. However, due to sample limitations these data need further validation (Felmingham et al. 2013; Yehuda et al. 2014).

7 Summary

In terms of grey matter volume, density, or cortical thickness (Table 1), all four anxiety disorders plus OCD and PTSD have alterations in limbic structures, such as the amygdala, insula, ACC, and PCC. Changes over the frontal regions are also common in these disorders except SAD, and temporal lobe grey matter changes are also frequently reported except in SP. However, grey matter changes over other cortical brain regions, such as the parietal and occipital areas, and over the subcortical structures, such as the basal ganglia and thalamus, were also noted. Regarding grey matter changes associated with response to pharmacological or psychological interventions, the findings associated with PDA are focused on prefrontal cortices, and those associated with OCD treatment response are mostly concentrated on the cingulate cortices, putamen, thalamus, and frontal regions (Table 2). White matter abnormalities including volume or fractional anisotropy changes as compared with healthy subjects are most widely spread across various neural tracts in patients with PDA. GAD and OCD are the two other disorders that also demonstrated considerable white matter changes (Table 3). Interestingly, in terms of white matter volume and changes associated with treatment effect, SAD showed findings implicating the uncinate fasciculus and inferior longitudinal fasciculus, while PD showed findings implicating the uncinate fasciculus and fronto-occipital fasciculus (Table 4). Brain activations during emotional or symptom provocation fMRI tasks are also noted to be different from healthy controls mostly in PDA, GAD, SAD, and OCD patients. The changes in brain reactivity are centered in prefrontal cortices, limbic structures, and the striatum (Table 5). In addition, brain activations during emotional or symptom provocation tasks, which are associated with treatment response, are also mainly found in the prefrontal cortex and limbic areas (Table 6).

The serotonin system is one of the most studied neurotransmitter systems in anxiety disorders, OCD, and PTSD (Table 7). In terms of 5-HT synthesis alterations, SAD and OCD exhibited increased synthesis in the hippocampus, caudate, limbic structures, and raphe nuclei in the brainstem. Serotonin transporter levels are also altered in patients with PDA, SAD, OCD, and PTSD and are mostly found in the basal ganglia, thalamus, limbic structures, and midbrain. Regarding 5-HT1A receptor changes, PTSD patients have widespread increases in receptor availability in various frontal, temporal, parietal, occipital, and limbic areas as well as the raphe nuclei in the brain stem. On the contrary, PDA and SAD patients showed a decreased number of 5-HT1A receptors mainly in the limbic structures and the raphe nuclei. Reduced 5-HT1B receptor availability is noted in PTSD patients in the amygdala, ACC, and caudate nucleus. OCD patients demonstrated a reduced number of 5-HT2A receptors in prefrontal areas, temporal and parietal associative areas, and caudate nucleus. Dopaminergic system changes are summarised in Table 8. OCD patients showed increased dopamine synthesis in frontal, temporal, parietal, occipital, limbic cortices, and the cerebellum. Dopamine transporter availability changes are noted in PDA, GAD, and SAD in the striatum with either increased or decreased levels. OCD patients also showed a decreased number of D1 receptors in the caudate nucleus while both SAD and OCD patients demonstrated reduced D2 receptor availability in the striatum. Changes in the GABAergic system are summarised in Table 9. Changes of either the BZD–GABAA receptor availability or GABA concentration are mostly observed in PDA patients in frontal, temporal, and limbic regions. Finally, Table 10 summarised the changes associated with the glutamatergic/NAA system which are mainly found in frontal, temporal, and limbic regions in GAD, SAD, OCD, and PTSD patients.

From the current review, it is evident that genetic and neuroimaging studies have revealed new information about the underlying biological alterations associated with anxiety disorders, OCD, and PTSD. However, these findings are currently still inadequate to fulfil the role of valid and reliable clinical biomarkers to guide the diagnosis, prognosis, treatment, and prevention for anxiety disorder, OCD, and PTSD. Anxiety disorders are known to have a very high rate of comorbidity with other mental disorders such as major depression (Hirschfeld 2001), but most independent studies tend to recruit a group of patients as homogeneous as possible. This approach can strengthen the statistical power to detect differences compared to healthy controls but at the same time increase the difficulty for its application in real-world clinical practice where, more often than not, the patient presented may have other psychiatric comorbidities. In addition, minor differences in subject characteristics, experimental design in fMRI tasks or imaging parameters, or the nomenclature or reference atlas selected to report anatomic locations all further complicate the comparison or combination of valuable existing findings. One possible solution to this is to use standardised and widely accepted experimental design, imaging parameters, data structures, analysis pipelines, and report format to collect genetic, biochemical, imaging, and behavioural data from every possible subject, whether with comorbidity or not, and to share the data with the whole research community. With this "big data" approach, a large amount of compatible data which

is impossible to collect independently, can be fed into innovative machine learning or deep learning algorithms, and may potentially help to unearth multidimensional clinical biomarkers useful in clinical practice. At the same time, biomarkers discovered by this "data-driven" approach may then provide interesting targets for further study. For example, in more traditional approaches which focus on a more homogenous group of study samples, to test relevant hypothesis to understand the underlying pathogenetic and pathophysiological mechanisms. In turn, this new understanding of pathological mechanisms can again inform a more refined big data algorithm. These two approaches for biomarker studies may then form a virtuous cycle to generate findings that may fuel the realisation of precision or personalised medicine. For further discussion of the potential approaches to realise clinical useful biomarkers, see Milham et al. (2017).

8 Conclusions

Over the past 30 years, brain-imaging methods and genetic analyses have increasingly been applied in anxiety research. The widespread use of neuroimaging techniques has produced an increasingly diverse picture of the involvement of different brain structures and neurotransmitter systems in the processing of fear. However, despite a plethora of high-quality publications in the field, imaging research has not yet succeeded in reliably identifying neuroanatomical, functional, or metabolic alterations, which can be unequivocally associated with discrete anxiety disorders. Several inconsistencies in the reported findings may be due to heterogeneity in diagnoses, paradigms, study designs, image acquisition methods, or analysis, in addition to many various confounding factors. Nevertheless, imaging suggests specific circuits particularly the amygdala and related structures that may underpin the expression of anxiety and others such as the medial prefrontal cortex that may help restrain it. There is also PET and SPECT data supporting an involvement of GABA-A – 5-HT and NK1 receptors in some anxiety disorders, which does accord with our understanding of current treatment mechanisms.

Similarly, although genetic factors are known to play a major role in anxiety disorders, OCD, and PTSD, genetic research has not unequivocally succeeded in identifying genes reliably associated with any one of these disorders. In linkage studies, LOD scores of over three, which mean that a linkage is being considered as significant, have not yet been found for any chromosomal regions with candidate genes in anxiety disorders, OCD, or PTSD, underlining the complex genetic background of these disorders entailing multiple gene polymorphisms and emphasising the likely role of environmental and epigenetic factors. We predict in future gene-×-environmental factors coming to prominence, perhaps with other biomarkers such as hormones and imaging.

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Deconstructing Schizophrenia: Advances in Preclinical Models for Biomarker Identification

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Curr Topics Behav Neurosci (2018) 40: 295–324
DOI 10.1007/7854_2018_48
Published Online: 2 May 2018

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 behaviour can help in identifying biologically relevant pathways that are attractive for informing clinical studies and biomarker development. In this chapter, we emphasize 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 characterization 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).

Keywords Behavioural biomarkers · Biochemical biomarkers · Cognition · Forward translation · Genetic mouse models · Glutamate · Imaging biomarkers · NMDA receptor · Oscillations · Reverse translation · Risk factors · Thalamic reticular nucleus

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 personalized 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. 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 et al. 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 Lydon-Staley and Bassett 2018; Hunter and Lawrie 2018; Herron et al. 2018; and Notter, 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 et al. 2018, 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 et al. 2011; Ayhan et al. 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 et al. 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 and quality of life (Green et al. 2000, 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) which is a 'new way of classifying mental disorders based on dimensions of observable behaviour 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. 2013; 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 (PFC) 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 (Fig. 1).

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

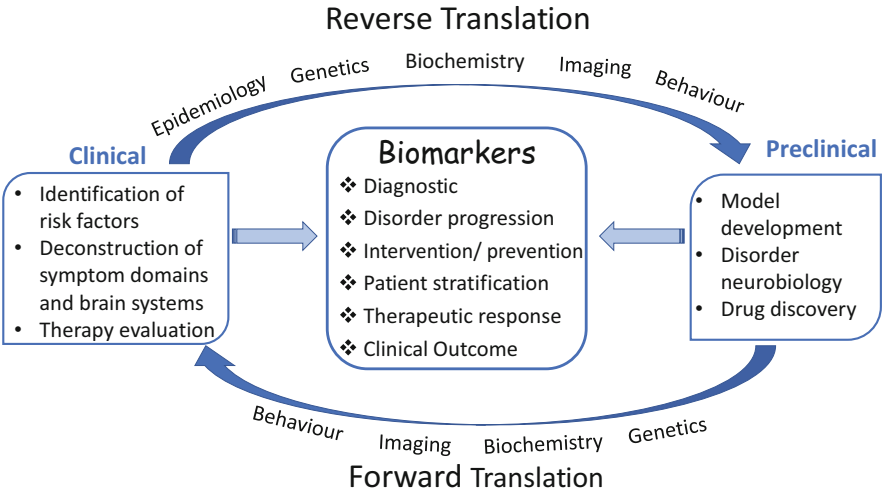

Fig. 1 Framework for biomarker development through the adoption of forward and reverse translation approaches. The importance of adopting a reciprocal forward and reverse translation approach that is iteratively updated when additional new information is gained, either preclinically or clinically, offers the 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: (1) diagnostic – where they can aid in predicting risk and diagnosis, (2) prognostic – where they can provide a signpost for clinical course and (3) predictive of drug response/therapeutic intervention – whether beneficial or adverse, and thereby provide a potential means of patient stratification

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.

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 and 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 et al. 2014; Hall et al. 2015; Harrison 2015; Reddaway et al. 2018, this volume).

Although, there is accumulating evidence for an impact of schizophrenia genetic risk upon the glutamatergic synapse, the huge investment in NMDA receptor antagonist models, particularly for drug discovery, began ~30 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. 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 (Jentsch and Roth 1999; Morris et al. 2005; Large 2007; Jones et al. 2011; Pratt et al. 2012). 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 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 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 and 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 PFC 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, 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 subchronic 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 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 2018, 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 et al. 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; 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 et al. 2010; Tomasi and Volkow 2014). Many of these alterations appear to be conserved in 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 (see Dawson et al. 2015a 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 Copy Number Variants (CNVs) 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 (i.e. between humans and rodents) and across imaging modalities (i.e. 2-DG, fMRI, EEG, electrophysiology and MEG) (Dauvermann et al. 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 (Dawson et al. 2012, 2014b). These results were gained by analysing ^{14}C-2-deoxyglucose (2-DG) 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 et al. 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 (hypofrontality) 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 et al. 2015b). Whilst 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. 2015b). 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) (Dickerson et al. 2010) and a model of impaired neurodevelopment (using the mitotic toxin methylazoxymethanol acetate; MAM) (Phillips et al. 2012a, b; Belujon et al. 2013). To date, the analysis of connectivity in these animals has been limited to electrophysiological characterization 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 coordination 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 (0–4 Hz)

through to fast gamma (30–80 Hz). 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 et al. 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 psychosis, 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 represents 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 coordinating 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 et al. 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. (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 and/or (2) excitatory drive to pyramidal cells is low because of a reduced number of dendritic spines. This in turn leads to a compensatory reduction in strength of feedback inhibition from PV basket cells (Gonzalez-Burgos et al. 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. For example, acute NMDA receptor blockade (PCP and ketamine) leads to increased gamma power (Phillips et al. 2012a, b) 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 knockdown mice, which express less than 10% of the obligatory NMDAR1 subunit. Repeated NMDA receptor blockade with PCP altered theta power but not gamma power whereas NR1 knockdown mice showed an increase in baseline gamma power (similar to acute PCP) and disrupted gamma–theta band cross-frequency phase-coupling between hippocampus and PFC (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 PFC 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, 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 typically 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 et al. 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 subdomains: affective flattening (reduced intensity and range of emotional expression), poverty of speech (alogia), anhedonia and motivational deficits. Subdomains such as anhedonia and motivational deficits are readily accessible in rodent models and have been widely reviewed elsewhere (Millan et al. 2014; Reddy et al. 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 Slaney et al. 2018, 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; MacQueen et al. 2018, this volume). Table 1 summarizes 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, organization, 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).

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 an 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 et al. 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.

Table 1 Tasks proposed by CNTRICS as possessing high construct validity and potential for further development

Cognitive domain	Construct selected of relevance to schizophrenia	Human	Rodent tasks	References
Attention	Control of attention (esp. input selection) Ability to guide and refocus attention in accordance with internal goals and representations	Continuous performance tasks	5-Choice serial reaction time task (5-CSRTT) 5-Choice continuous performance test (5-C-CPT) Distractor condition sustained attention task (dSAT)	Luck and Gold (2008) and Lustig et al. (2013b)
Executive function	Rule generation and control	ID/ED task	Reversal learning and attentional set-shifting task	Gilmour et al. (2013) and Young and Geyer (2015)
Working memory	Goal maintenance and interference control	Goal maintenance Interference control	Operant delayed non-match to position task N-back task	Barch and Smith (2008), Barch et al. (2009)
Long-term memory	Reinforcement learning Declarative memory (episodic memory)	Probabilistic learning tasks Paired associate learning (in CANTAB battery)	Probabilistic learning No clear consensus Novel/Spontaneous object recognition – not recommended Touchscreen task: object–location paired-associated learning Further development and validation recommended	Ragland et al. (2009) and Bussey et al. (2013)
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 Recognition of multidimensional nature of social cognition and challenges of developing animal model Further development and improvements recommended	Carter et al. (2009) and Millan and Bales (2013)

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

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 PPI and mismatch negativity (MMN) have been proposed to align with the human construct (Siegel et al. 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 et al. 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 emphasize 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). Whilst 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). Whilst 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 PFC, 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 et al. 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 et al. 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 et al. 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 1β, soluble interleukin 2 receptor, interleukin 6 and TNFα in patients (Potvin et al. 2008; Miller et al. 2011; Upthegrove et al. 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 et al. 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 PFC 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 et al. (2018) (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 behavioural 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 PCP, to produce symptoms of schizophrenia on acute administration in humans has led to many acute administration studies in rodents (Morris et al. 2005). Once it became clear that these drugs cause a profound increase in glutamate release and metabolic activity in the rodent PFC (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 PCP (Cochran et al. 2003) develop metabolic hypofrontality and PFC 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 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 et al. 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. 2014a, b) and as a result of mutation in the Disc1 gene (Dawson et al. 2015b) 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 2017). The fact that the rodent model was able to predict this pathological feature of the human condition 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).

10 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.

Acknowledgements JAP, BJM and ND jointly hold an MRC research grant (MR/N012704/1). JAP and BJM currently hold MRC grants MR/N012704/1 and IMPC-74593/1 and an academic research grant from Servier. Previous support which has contributed to the development of this work includes funding from Mitsubishi Pharma and a Pfizer Translational Medicine research programme.

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Genomic and Imaging Biomarkers in Schizophrenia

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Curr Topics Behav Neurosci (2018) 40: 325–352
DOI 10.1007/7854_2018_52
Published Online: 24 April 2018

Abstract Recent large-scale genomic studies have confirmed that schizophrenia is a polygenic syndrome and have implicated a number of biological pathways in its aetiology. Both common variants individually of small effect and rarer but more penetrant genetic variants have been shown to play a role in the pathogenesis of the disorder. No simple Mendelian forms of the condition have been identified, but progress has been made in stratifying risk on the basis of the polygenic burden of common variants individually of small effect, and the contribution of rarer variants of larger effect such as Copy Number Variants (CNVs). Pathway analysis of risk-associated variants has begun to identify specific biological processes implicated in risk for the disorder, including elements of the glutamatergic NMDA receptor complex and post synaptic density, voltage-gated calcium channels, targets of the Fragile X Mental Retardation Protein (FMRP targets) and immune pathways. Genetic studies have also been used to drive genomic imaging approaches to the investigation of brain markers associated with risk for the disorder. Genomic imaging approaches have been applied both to investigate the effect of polygenic risk and to study the impact of individual higher-penetrance variants such as CNVs. Both genomic and genomic imaging approaches offer potential for the stratification of patients and at-risk groups and the development of better biomarkers of risk and treatment response; however, further research is needed to integrate this work and realise the full potential of these approaches.

Keywords Copy number variant · Genomic · Imaging · Polygenic · Schizophrenia

1 Introduction

Schizophrenia (SZ) is a complex neuropsychiatric condition currently defined syndromically in the major international classification systems (Cheniaux et al. 2009). A lack of understanding of the fundamental biological causes of these symptoms has hindered the development of biomarkers of illness and new treatments for this disabling syndrome. There is substantive evidence for a significant genetic component to risk for SZ as assessed by family, twin and adoption studies, with

heritability for the disorder estimated at 70–80% (Sullivan et al. 2003; Owen et al. 2016). However, SZ is not a genetically deterministic condition and there is substantive evidence for a significant environmental component of risk for the syndrome (van Os et al. 2010; Owen et al. 2016). Nevertheless, genetic approaches potentially offer an unbiased route to understanding risk for SZ and to developing biomarkers and improving the stratification of patient populations.

Genomic information may itself represent a biomarker for prediction and the stratification of SZ patients according to shared biological risk. In this chapter, we therefore first review progress in genomic understanding of SZ and discuss the potential for genomically based prediction, stratification and treatment. Genomic information may also be of value in guiding the development of biomarkers in other modalities including blood-based biomarkers and imaging markers. In the latter part of the chapter, we focus on results from genomic imaging studies looking at the collective polygenic effect of common risk variants for SZ and the impact of rarer but more penetrant variants on brain structure and function. These studies give an indication of which elements of the brain structure and function changes seen in SZ may relate to primary genetic risk for the disorder, informing the search for reliable biomarkers of risk. Given the major challenges faced in the development of novel therapeutic approaches in SZ, and the key role that reliable biomarkers will play in this process, integrating genomic and phenotypic information in this way to refine the search for valid biomarkers remains a critical endeavour in SZ research.

2 Genomic Findings in Schizophrenia

2.1 Introduction

It has for some time been appreciated that genetic risk for SZ does not conform to simple Mendelian inheritance, but instead follows the pattern associated with complex polygenic inheritance (Gottesman and Shields 1967, 1972). This complexity meant that the genetic architecture of SZ long remained elusive. However, there has been major progress in understanding genomic risk for SZ over the last decade thanks both to advances in technology and collaboration to generate large sample sizes such as those assembled by the Psychiatric Genomic Consortium (PGC) (Schizophrenia Working Group of the Psychiatric Genomics 2014). These studies have identified three main types of genetic variation which contribute to the development of the disorder: (1) Common variants; (2) Rare copy number variants (CNVs) and Chromosomal rearrangements; (3) Rare coding variants (Rees et al. 2015; Singh et al. 2017). Identification and analysis of genetic variations within SZ provides insight into the basic biology underlying the disorder, indicates potential drug targets and aids the development of biomarkers (Javitt et al. 2008; Pickard 2015). In addition, genomic progress has allowed the development of polygenic risk scores which aggregate risk and identify persons with a higher probability of developing SZ (Poletti et al. 2017). Below we review these different classes of

genetic risk before considering potential uses of genetic approaches in the biological stratification of SZ.

2.2 Common Variants

Genetic variants present in >1% of the population are classified as common variants, typically represented by single nucleotide polymorphisms (SNPs). SNPs have been estimated to represent a large portion of the total genetic variation seen in SZ (Pardiñas et al. 2018). Genome-wide association studies (GWASes) compare the frequency of SNPs in large patient and control populations using microarray methods to genotype SNPs at hundreds of thousands of loci across the genome. Early GWASes of SZ were underpowered for the full exploration of polygenic risk for schizophrenia, although initial successes encouraged the continued application of this approach (O'Donovan et al. 2008). However, the establishment of the PGC has allowed much larger sample sizes to be examined involving tens of thousands of patients and controls.

In a seminal GWAS, the PGC identified 108 distinct loci with genome-wide significance associated with SZ (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). The loci identified do not necessarily correspond to one specific gene but instead comprise associated chromosomal regions which may or may not be protein coding. 83% of the loci found to be associated with SZ by the PGC contained protein-coding genes or were within 20 kb of one. These genes implicate several pathways in pathophysiology of SZ notably: (1) Glutamatergic neurotransmission (e.g. GRM3, GRIN2A, SRR and GRIA1); (2) Voltage-gated calcium channels (e.g. CACNA1C, CACNB2 and CACNA1I); (3) Dopamine pathway members (e.g. DRD2); (4) Targets of the Fragile X Mental Retardation Protein (FMRP targets); (5) Immune pathways. The finding of genetic association with genes involved in glutamatergic and dopaminergic neurotransmission is in line with prior theories of the pathological basis of SZ and reflects known treatment targets (Kapur and Mamo 2003; Moghaddam and Javitt 2012), although the dopaminergic signal is weaker than that seen for glutametergic synapses, and is primarily represented by association in DRD2 (which encodes the target of antispychotic medication). However much of the genetic signal identifies new pathways which have not been previously associated with the disorder and are likely to both illuminate pathology and indicate novel routes to therapy.

Since the 2014 PGC paper, further studies have identified additional common variants associated with SZ and have attempted to further characterise the loci found by the PGC (e.g. Sekar et al. 2016; Pardiñas et al. 2018). The most significant single locus in the 2014 paper lies within the major histocompatibility complex region on chromosome 6 (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014; Mokhtari and Lachman 2016). This region is genetically highly complex, however in a study designed to tease apart the multiple risk loci within the

MHC region and identify specific immune pathways implicated in SZ, Sekar et al. showed that genetic variation in the multi-allelic member of the complement system, C4, is associated with SZ and specifically that the C4A allele was enriched in those with the disorder (Sekar et al. 2016).

More recently, Pardinas and colleagues have conducted a further meta-analysis of GWAS studies including the addition of >11,000 new cases of SZ, identifying more than 50 additional genome-wide significant loci (Pardiñas et al. 2018). In addition, they also demonstrate that SNPs associated with SZ are also enriched in loss of function (LOF) intolerant genes, accounting for 30% of all SNP-based SZ heritability. Genes with a high probability of being loss of function intolerant have high expression in the brain, high levels of protein-protein-protein interaction and appear to be essential for core biological functions (Pardiñas et al. 2018). Pardiñas and colleagues also show how variation in these genes can nevertheless be maintained in the population through background selection.

2.3 Chromosomal Rearrangements and Copy Number Variants (CNVs)

There has been evidence for some time that cytogenetically detectable chromosomal rearrangements can be associated with psychiatric disorders including SZ (Pickard et al. 2005). Perhaps the best characterised example is a large Scottish family with a high incidence of psychiatric conditions across four generations. Within this family approximately half the family members carry a balanced translocation between chromosomes 1 and 11 (t[1:11]). Twenty of these individuals with the translocation have a major psychiatric disorder (including seven with SZ), representing a highly significant increase in risk compared to those without the t[1:11] karyotype (Chubb et al. 2008). Following the identification of the association of t[1:11] and psychiatric disturbances, work began to identify the gene/s being altered at the junction of the translocation and led to the discovery of disrupted in schizophrenia 1 (DISC1) (Blackwood et al. 2001; Klar 2002). Characterisation of DISC1 showed its role as a molecular scaffolding and ability to regulate neuronal intracellular trafficking of mRNA, mitochondria, receptors, and neurotransmitter-containing vesicles (Bradshaw and Porteous 2012; Devine et al. 2016). However, this translocation appears to be unique to this family and generally large cytogenetically detectable chromosomal rearrangements are considered a rare cause of SZ.

An additional finding of micro-array-based GWAS studies of SZ was the demonstration that smaller, sub-microscopic chromosomal rearrangements known as CNVs are enriched in SZ cases (International Schizophrenia Consortium 2008; Bergen et al. 2012; Szatkiewicz et al. 2014). This extended the earlier demonstration that genetic variation at the 22q11.2 locus, associated with velo-cardio-facial syndrome, is also associated with very high rates of SZ (Murphy et al. 1999; Henry et al. 2002). CNVs are structural variations of genome within a population arising from

duplication and deletion events. CNVs are sub-microscopic, but the variations often span multiple genes and can be larger than 1,000 kb. As an example, the 16p11.2 SZ associated CNV is over 600 kb long and spans 17 genes expressed in the CNS. CNVs therefore can significantly impact a wide range of developmental and regulatory processes, especially those sensitive to dosage effects (Chang et al. 2017; Sullivan 2017). CNVs typically confer odds ratios for SZ that are considerably greater than those seen for common variants (ranging from 2 to 30+) (Rees et al. 2014b). A number of recurrent CNV loci have been identified which show statistical association with SZ and are also often associated with other neurodevelopmental disorders including autism, ADHD and intellectual disability (Kirov et al. 2014; Rees et al. 2014a, 2016; Marshall et al. 2017). These include CNVs at chromosomes 1q21.1, 2p16.3, 3q29, 15q11.2, 15q13.3, 16p11.2 and 22q11.2. Most of these CNVs affect multiple genes although the 2p16.3 CNV specifically impacts the NRXN1 gene, which is also strongly associated with autism. Collectively these recurrent CNVs are identified in approximately 2–3% of SZ cases (Rees et al. 2015).

Large case–control studies have confirmed that in addition to these recurrent CNVs associated with SZ there is a broader burden of individually rare CNVs in the disorder. Whilst these are too rare to achieve individual significance, pathway analysis has revealed an enrichment of genes in these CNVs affecting in particular synaptic genes at both excitatory synapses (including genes encoding proteins in the glutamatergic NMDA receptor complex, ARC complex and post-synaptic density) and inhibitory synapses (including genes encoding GABA receptor subunits), as well as genes previously implicated in behaviour and conditioning (Pocklington et al. 2015; Marshall et al. 2017). These findings support earlier studies of de novo (presumed pathogenic) CNVs in trios and together strongly implicate synaptic processes in the pathogenesis of SZ (Kirov et al. 2012; Pocklington et al. 2015; Marshall et al. 2017).

2.4 Rare Coding Mutations

Single nucleotide variants (SNVs) and small nucleotide insertions or deletions (indels) can potentially generate damaging missense and loss of function (LOF) mutations. Exome sequencing of SZ patients and healthy controls has identified a polygenic burden of rare disruptive mutations in the disorder present in $<0.1\%$ of the population (Fromer et al. 2014; Purcell et al. 2014). Whilst larger sequencing studies including not just exomes but whole genomes are required to fully appreciate the impact of rare mutations in the development of SZ, several studies focussing on rare variants have been conducted. These investigations have identified an enrichment of mutations in specific pathways, showing evidence of convergence with those implicated by studies of common variants and CNVs. In particular, there is evidence for an increased burden of rare variants impacting synaptic genes (NMDA and ARC complexes and post-synaptic density), FMRP targets, voltage-gated calcium channels and genes involved in chromatin remodelling (Fromer et al. 2014;

Purcell et al. 2014; Singh et al. 2017). Whilst most of the individual genes do not yet-reach single-gene exome-wide significance, there is significant evidence for the genes SETD1A and TAF13 at exome-wide thresholds (Singh et al. 2016; Nguyen et al. 2017). Notably mutations in SETD1A, which are also associated with other neurodevelopmental disorders, implicate epigenetic processes in the pathogenesis of SZ (Singh et al. 2016). More recent work has shown that the burden of rare damaging mutations is greater in those with schizophrenia who have marked intellectual disability although those who have schizophrenia without such cognitive impairment also show an excess of damaging mutations compared to controls (Singh et al. 2017).

2.5 Genetics of Schizophrenia, Patient Stratification and Biomarker Development

In addition to establishing the genes involved in risk of developing schizophrenia (gene discovery), a major aim of both common and rare variant genetic studies is to identify groups of patients with similar genetic risk characteristics. In doing so, such studies can begin to dissect the complexity of neuropsychiatric conditions by distinguishing more homogeneous sub-groups based on genetic variables. This approach may then offer insights into re-classification based on shared aetiological mechanisms, as well as informing tailored treatments, the ultimate aims of stratified medicine strategies.

Approaches based on common genetic variation have shown some promise as a basis for stratification. Polygenic scoring methods, which sum the number of risk alleles an individual has and weight each allele by its effect on risk for SZ, allow investigators to create a risk profile score for each individual which represents their cumulative common genetic risk for SZ. These methods have enabled researchers to address important research questions related to stratification since for the first time they offer a way of quantifying individuals' cumulative common variant genetic risk. These and related methods have demonstrated shared genetic risk across psychiatric disorders which presents challenges to current diagnostic categorization but also raises the possibility of stratification across disorders, indeed such approaches are beginning to be informative (Lee et al. 2013). In a recent study, Allardyce and colleagues demonstrated that schizophrenia polygenic risk was able to distinguish between those with sub-types of bipolar disorder (Allardyce et al. 2018). There was a gradient of polygenic risk ranging from the lowest association for those with bipolar II disorder, to those with bipolar I disorder through to those with schizoaffective affective disorder and then schizophrenia following the predicted diagnostic continuum (Owen et al. 2016). Within bipolar disorder, those with psychosis had elevated polygenic scores and scores were higher still in those with mood-incongruent psychotic symptoms (more schizophrenia-like psychosis). This demonstrates the potential of polygenic risk scores for informing classification of conditions.

In non-psychiatric disorders, greater polygenic loading has been shown to be a predictor of response to treatment, specifically in cardiovascular disease predicting stain therapy response (Natarajan et al. 2017). It does not seem that schizophrenia polygenic risk predicts lack of response to antipsychotic therapy (Wimberley et al. 2017), although this field is in its early stages and larger studies are required to establish the utility of polygenic scores in the context of treatment response prediction. There is also the potential for cumulative polygenic risk to be informative about underlying biology by quantifying biological risk within particular sets or pathways of genes. Such approaches are in a theoretical stage of development though they offer promise for genetically informed stratification of schizophrenia and its targeted treatment.

Stratification on the basis of genetic rare variants is challenging at the present time given available sample sizes for discovery and we do not yet know how productive diagnostic genetic testing may prove to be in this context. The low frequency of such variants in schizophrenia may argue for more targeted strategies to support implementation of testing and stratification in clinical contexts. Cognitive impairment is perhaps the most promising clinical marker that may serve as a means of targeting those at increased risk of carrying relevant rare variants since when grouping together carriers of rare variant classes, both CNV and rare single nucleotide variant carriers show compromised neurocognitive functioning (Rees et al. 2016; Singh et al. 2017). This is perhaps to be expected since CNVs associated with schizophrenia also increase risk of developing other neurodevelopmental disorders all of which are associated with impaired cognition (Kirov et al. 2015).

Information about both common and rare variants has also been used to inform studies of other risk-associated biological markers. In the next sections, we review the use of polygenic risk scores and rare variant approaches to guide the identification of imaging biomarkers associated with risk for schizophrenia and related disorders through genomic imaging approaches.

3 Polygenic Risk Imaging Studies and Biomarker Identification in Schizophrenia

3.1 Introduction

As discussed above, GWASes show that a significant proportion of SZ genetic liability is conferred by common variants that span across the genome (Ripke et al. 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). While work into establishing biological pathways enriched for common SZ risk variants continues (Hall et al. 2015), progress has been made in establishing relationships between SZ-related traits and the cumulative effect of risk GWAS SNPs for SZ (Mistry et al. 2017). Individual GWAS-identified SNPs associated with SZ only confer a small effect on SZ risk, posing a challenge to the design of

well-powered studies to explore relationships between single loci and SZ-related imaging traits (Carter et al. 2017). By summing the number of risk alleles an individual has (and by weighting each allele by its effect on risk for SZ), investigators can create a risk profile score (RPS) for each individual which represents their cumulative common genetic risk for SZ. RPSs were first employed to show that the genetic architecture of SZ was polygenic and predicted more variance in the diagnosis than any individual common loci (International Schizophrenia Consortium 2009). As SZ GWAS sample sizes increase, so does the variance in diagnosis explained by the SZ-RPS that an investigator creates using the summary data (Dudbridge 2013). Recent estimates suggest that SZ-RPS explained ~7% of all SZ risk (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014), and it has been associated with several SZ-linked traits such as education, personality and specific symptom dimensions (McIntosh et al. 2013; Roussos et al. 2015; Hagenaars et al. 2016; Jones et al. 2016). These observations suggest that SZ risk alleles may confer risk via brain circuits that support these processes. In order to establish biological mechanisms by which the cumulative effects of common risk alleles for SZ confer risk, investigators have explored the relationship between SZ-RPS and neuroimaging phenotypes also associated with SZ. These studies may help to establish which neuroimaging measures are sensitive to genetic risk for SZ and which imaging measures are genetically independent (suggesting the imaging trait is a consequence of the disease). Measures that are directly related to genetic risk for the disorder may have particular value as biomarker of risk for the disorder.

3.2 Common Risk Alleles for Schizophrenia and Brain Structure

There are reliable associations between SZ and brain morphometry (van Erp et al. 2015), which have motivated investigators to explore if SZ-RPS explains variance associated with this observation. There is now a growing consensus that SZ-RPS explains limited variance in subcortical volumes (see Table 1). However, emerging evidence suggest that SZ-RPS may be negatively associated with cortical thickness.

3.3 Common Risk Alleles for Schizophrenia and Brain Function

Neuroimaging studies have also used SZ-RPS to probe brain function by measuring the blood-oxygen-level dependent (BOLD) signal in the brain either (a) in relation to a specific psychological event (task-based fMRI: contrast/parameter estimate) or (b) as a network of temporal coherence, explored at rest (functional connectivity; rs-fMRI). These studies explore the combined impact of SZ risk alleles on BOLD

Table 1 Association of SZ-RPS with brain volume and cortical thickness

Phenotype	Region(s)	RPS-P_T	Finding	Sample size(s)	Study
Volume	GMV; WMV; ICV	0.002–0.4	–	$N = 142$	van Scheltinga et al. (2013)
	GMV; WMV; ICV	5×10^{-8}–0.4	n/s	$N = 122$	Papiol et al. (2014)
	GMV; WMV; ICV	5×10^{-8}–0.05	n/s	$N = 763$; $N_R = 705$	Van der Auwera et al. (2015)
	WMV	5×10^{-8}	–	$N_D = 94$; $N_R = 89$	Oertel-Knöchel et al. (2015)
	Subcortical; ICV	5×10^{-8}–0.05	n/s	$N = 11{,}840$	Franke et al. (2016)
	Hippocampus	0.01–1	–	$N = 65$	Harrisberger et al. (2016)
Cortical thickness	Total cortical thickness	0.01–1	–	$N = 34$	Neilson et al. (2017)
	STS	0.1–0.5	–	$N = 560$	Ohi et al. (2014)

– negative association, *n/s* not significant, *RPS-PT* P-threshold(s) of schizophrenia GWAS data, N_D discovery sample size, *GMV* grey matter volume, *WMV* white matter volume, *ICV* intracranial volume, *STS* superior temporal sulcus

across a series of paradigms that measure brain activation across key neurocognitive domains of the Research Domain Criteria (memory (working / episodic), reward processing, social cognition and emotion processing). Initial reports suggest that SZ-RPS is associated with altered BOLD signal in a range of brain regions including prefrontal cortex, hippocampus and subcortical regions, depending on the exact task utilised (Table 2). However, as fMRI studies are usually smaller than structural imaging studies, it is currently not known whether these associations are influenced by chance or bias or represent true associations.

There have been a limited number of studies of the effect of SZ-RPS on other imaging biomarkers of brain function including electroencephalogram (EEG) measures (Liu et al. 2017; Ranlund et al. 2018). These have however only so far been conducted in relatively small sample sizes and have failed to show significant effects. Notably, however, there have been no studies that the authors are aware of investigating the relationship between SZ-RPS and brain activity as assessed by magnetoencephalography (MEG) measures, or of the impact of SZ-RPS on brain neurochemistry as assessed by magnetic resonance spectroscopy (MRS).

3.4 RPS Limitations and Future Directions

While genome-wide SZ-RPS generated with a liberal P-threshold (e.g. $P_T < 0.05$) explains the most variance in the clinical diagnosis (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014), it is not known whether this P-threshold performs optimally for imaging traits related to SZ. Furthermore, as

Table 2 Association of SZ-RPS with BOLD signal during psychological events in a range of cognitive domains

Phenotype	Region(s)	RPS-PT	Finding	Sample size(s)	Study
Memory (tb-fMRI)					
SIRP (WM)	DLPFC	$N_{SNPS} = 41$	+	$N = 178$	Walton et al. (2012)
SIRP (WM)	DLPFC; ACC	0.01	+	$N = 206$	Walton et al. (2013)
n-back (WM)	IFG; MPFC	0.05		$N = 181$	Kauppi et al. (2014)
n-back (WM)	–	5×10^{-8}	n/s	$N = 578$	Erk et al. (2017)
Numerical (WM)	DLPFC	5×10^{-8}	–	$N = 99$	Miller et al. (2017)
Mnemonic memory	Hippocampus	0.05	+	$N_D = 191$; $N_{R1} = 76$; $N_{R2} = 137$	Chen et al. (2018b)
Episodic (tb-fMRI)					
Encoding	–	5×10^{-8}	n/s	$N = 578$	Erk et al. (2017)
Recall	–	5×10^{-8}	n/s	$N = 578$	
Recognition	ACC	5×10^{-8}	–	$N = 578$	
Social cognition (tb-fMRI)					
Theory of mind	PCC; PCU	5×10^{-8}	+	$N = 578$	Erk et al. (2017)
Emotion processing (tb-fMRI)					
Face-matching task	–	5×10^{-8}	–	$N = 578$	Erk et al. (2017)
Verbal fluency (tb-fMRI)					
Hayling sentence completion	LPFC	0.5	+	$N = 57$	Whalley et al. (2015)
Reward processing (tb-fMRI)					
Reversal learning: decision phase	FP; VS	0.5	–	$N = 83$	Lancaster et al. (2016a)
Anticipation/receipt	VS	0.01–0.5	+	$N = 1{,}525$	Lancaster et al. (2016b)
Anticipation	VS	5×10^{-8}	n/s	$N = 578$	Erk et al. (2017)
Reversal learning: reward receipt	ACC; VS; Hippocampus	0.05	+	$N = 183$ (R_bG)	Lancaster et al. (2018)
Temporal coherence (rsfMRI)					
	Insula-AG Insula-DLPFC	0.01–0.5	+/–	$N_D = 360$; $N_R = 323$	Wang et al. (2017)

+ positive association, – negative association, *n/s* not significant, *RPS-PT* g = P-threshold(s) of schizophrenia GWAS data, N_D discovery sample size, N_R replication sample size, *SIRP* Sternberg Item Recognition Paradigm, *RbG* recall-by-genotype, *DLPFC* dorsolateral prefrontal cortex, *IFG* inferior frontal gyrus, *MPFC* medial prefrontal cortex, *ACC* anterior cingulate cortex, *PCC* posterior cingulate cortex, *PCU* precuneus, *FP* frontal pole, VS ventral striatum, *AG* angular gyrus, R_bG recall-by-genotype

the SNPs/alleles used to calculate the SZ-RPS are located across the entire genome, it is difficult to establish imaging–genetic relationships that implicate targetable biological pathways. Pathway-based RPS approaches may help to address these challenges. Emerging genetic–imaging studies are now exploring how individual variation in brain structure and function relate to alleles i) within a SZ risk gene (e.g. within *CACNA1C* (Erk et al. 2014)), within a broader SZ locus (such as 6p22.1 (Chen et al. 2018a)), across a co-expression network (e.g. *DRD2* co-expression (Pergola et al. 2017)) or variants in genes targeted by a SZ locus (such as mir137 (Cosgrove et al. 2018)). However, these studies may be challenged by similar power issues as the single variant imaging studies. Linkage disequilibrium (LD) score regression is a promising novel approach that allows researchers to explore genetic correlations between heritable traits, provided the phenotypes have been explored via a well-powered GWAS (Bulik-Sullivan et al. 2015). As meta-analytic imaging–genetic consortia such as ENIGMA Network are beginning to uncover the genetic architecture of neuroimaging traits, we can quantify the degree of overlap between common SZ risk alleles and genetic variation that influences individual variation in brain structure and function (Thompson et al. 2017). While SZ alleles appear to have little association with subcortical volume (Franke et al. 2016), preliminary evidence suggests they may be linked with the alleles that influence individual variation in cortical thickness.

Although variance explained by GWAS data is increasing, sample size is still a challenge, especially for investigators using novel or experimental fMRI paradigms. One way to overcome this challenge is the recall-by-genotype (RbG) approach where a SZ-RPS is generated for a large population sample. Individuals located in the extreme percentiles of the SZ-RPS distribution (very lowest / highest SZ-RPS) can be recalled for specific phenotyping. This approach helps to overcome power issues and also helps to reduce confounding (Corbin et al. 2018). The first RbG for a SZ-RPS study largely conforms to the hypothesis that SZ risk alleles may influence brain function, rather than brain structure (Lancaster et al. 2018). Other genomic techniques such as Mendelian randomisation and conditional false discovery rate (cFDR) may also provide insight into putative shared genetic aetiology between brain imaging measures and SZ risk, informing the development of imaging biomarkers of risk for SZ (Gage et al. 2017; Smeland et al. 2017).

4 Rare Variant Imaging and Biomarker Identification in Schizophrenia

4.1 Introduction

Over the past two decades, evidence has accumulated that in addition to common variants of small effect, rare but highly penetrant genetic variants contribute to the aetiology of SZ. The best-defined rare variants are currently the so-called copy

number variants (CNVs). Studying patients with CNVs offers the opportunity to investigate the effects of genetic variation on brain structure and function, and to relate this to psychopathology, in a relatively homogenous at-risk group. Longitudinal designs comparing imaging metrics between high-risk individuals who later develop SZ and those who do not may help to identify neuroimaging biomarkers for SZ that not only inform further research into disease mechanisms but also improve clinical diagnosis and provide important prognostic information to patients and their families.

22q11.2 Deletion Syndrome (22q11.2DS), also known as velo-cardio facial syndrome (VCFS), is one of the most common and penetrant CNVs associated with SZ. It is present in at least 1 in 4000 live births (Oskarsdottir et al. 2004) and confers an approximately 25% lifetime risk of SZ (Murphy et al. 1999). The 22q11.2 deletion is also associated with other neurodevelopmental manifestations including cognitive impairment, autism spectrum disorders and attention deficit hyperactivity disorder (Niarchou et al. 2014; Schneider et al. 2014), a degree of phenotypic heterogeneity seen in many of the SZ-associated CNVs. Interestingly, the reciprocal 22q11.2 duplication is less common in SZ cases than controls (Rees et al. 2014a) suggesting a possible protective effect, although this duplication is associated with other adverse neurodevelopmental outcomes (Wenger et al. 2016). The 22q11.2 region contains over 40 genes, including *COMT*, *PRODH*, *PIK4CA*, *RTRN4* and *DGCR8,* which have putative roles in brain development, neurotransmitter metabolism and micro-RNA processing. Since the association between 22q11.2 deletions and SZ was discovered, structural and functional neuroimaging methods have been employed to investigate the possible mechanisms by which the CNV confers risk of psychosis and to identify neuroimaging biomarkers for psychiatric outcomes including SZ. As 22q11.2DS has so far received much greater imaging investigation that the other SZ risk-associated CNVs, we focus the following discussion on this variant.

4.2 Qualitative Neuroimaging Studies

Early MRI studies identified qualitative differences between patients with 22q11.2DS and typically developing controls. These include an increased prevalence of midline anomalies such as the cavum septi pellucidi and cavum vergae (Chow et al. 1999; Van Amelsvoort et al. 2001; Shashi et al. 2004); polymicrogyria (Ghariani et al. 2002; Sztriha et al. 2004; Ehara et al. 2005) and pachygyria (Koolen et al. 2004; Ehara et al. 2005); ventricular enlargement (Schreiner et al. 2013) and white matter hyperintensities (Chow et al. 1999; Van Amelsvoort et al. 2001). Taken together, these studies demonstrate widespread structural abnormalities in 22q11.2DS, affecting both grey and white matter, and suggest that early cortical development and neuronal migration may be disrupted in 22q11.2DS. However, these findings are not specific to 22q11.2DS and there is no evidence that they are predictive of future psychotic disorder. Qualitative structural abnormalities have also

been reported in reciprocal rearrangements of 16p11.2 (Blackmon et al. 2017) and 1q21.1 (Bernier et al. 2016) but these preliminary findings require replication in larger samples.

4.3 Cross-Sectional Structural MRI Studies

Quantitative neuroimaging studies of specific CNVs have employed both region-of-interest and whole-brain approaches to better characterize grey and white matter structure in CNV carriers. In 22q11.2DS, cross-sectional studies report overall reductions in total grey and white matter volume in 22q11.2DS, with differences between patients and controls being most marked in the white matter (Eliez et al. 2000; Kates et al. 2001). A number of studies report regional volume differences between patients with 22q11.2DS and typically developing controls. A meta-analysis of these studies found significant volume reductions of the hippocampus and cerebellum in patients with the 22q11.2 deletion. Conversely, the volume of the corpus callosum was increased in 22q11.2DS (Tan et al. 2009). This contrasts with findings in idiopathic SZ, where the corpus callosum is relatively reduced in size compared to healthy controls (Arnone et al. 2008). In children with 22q11.2DS, enlargement of the corpus callosum is associated with better neurocognitive functioning, suggesting that the enlargement may be due, at least in part, to compensatory mechanisms (Shashi et al. 2012a).

The effects of 22q11.2 rearrangements on brain volume seem to be gene dosage dependent. A recent study comparing people with 22q11.2 deletions, those with 22q11.2 duplications and healthy controls found that 22q11.2 gene dosage varied positively with intracranial, grey and white matter volumes. Subcortical differences were also seen: 22q11.2 duplication carriers had a significantly larger right hippocampus but reduced right caudate and corpus callosum volume than 22q deletion carriers (Lin et al. 2017). Specific genotypes have also been shown to affect regional brain volume in 22q11.2DS. Individuals who are hemizygous for the Val functional polymorphism of *COMT* at locus rs4680 have larger frontal lobes and increased grey matter density in the cerebellum, brainstem and parahippocampal gyrus; decreased white matter density in the cerebellum (Van Amelsvoort et al. 2008); and smaller volumes of the corpus callosum (Shashi et al. 2012a) than those with the Met polymorphism. Variants in *ZDHHC8* (rs175174*)* and *UFD1L* (rs5992403) are also associated with corpus callosum volume (Shashi et al. 2012a).

Cortical thickness and surface area have been measured in several studies of 22q11.2DS (Bearden et al. 2006, 2008; Schaer et al. 2009). These studies have found cortical thinning in the parietal, occipital and anterior cingulate cortices. There have been discrepant findings in the frontal regions with one study reporting cortical thinning in the inferior frontal gyrus (Bearden et al. 2006) and another study reporting increased prefrontal thickness in preadolescents with 22q11.2DS, which normalizes by the end of adolescence. In this study, differences in cortical thickness were related to cognitive performance in children and adolescents, and to SZ in

adults (Schaer et al. 2009). Regional differences have also been reported for cortical thickness and surface area in 22q11.2DS (Jalbrzikowski et al. 2013) with thickness of the right orbitofrontal cortex being correlated with positive symptom severity. Gene dosage has been found to affect both cortical thickness and surface area; Lin et al. (2017) found a negative relationship between gene dosage and cortical thickness but a positive relationship with cortical surface area.

Abnormal patterns of gyrification have been reported in patients with SZ (Sallet et al. 2003) and those at high risk of SZ (Harris et al. 2004). Similar patterns have also been found in 22q11.2DS. Reductions in cortical complexity, measured using the gyrification index (GI), have been reported in the frontal and parietal lobes (Schaer et al. 2006; Srivastava et al. 2012) with increased complexity being found in the occipital lobes (Bearden et al. 2008). Better understanding of cortical morphology in 22q11.2DS and its association with psychopathology will aid in the understanding of how the 22q11.2 deletion affects neuronal migration and cortical maturation.

In order to identify risk markers common to those with genetically and clinically defined ultrahigh-risk states, Bakker et al. (2016) compared structural brain imaging metrics between people with 22q11.2DS (a genetic ultrahigh-risk group) and those at clinically defined ultrahigh-risk for psychosis and controls. The authors found that 22q11.2DS was associated with lower total grey matter volume and surface area than the clinical UHR group and controls (Bakker et al. 2016). However, cortical thickness in the rostral middle frontal gyrus was negatively associated with symptom severity in both high-risk groups.

These cross-sectional structural findings suggest a deviant developmental trajectory in 22q11.2DS affecting many different brain regions with the magnitude of deviance being associated with psychopathology and genotype. However, because of the relative rarity of 22q11.2DS and the frequency of MRI contraindications in this population (in particular, a history of cardiac surgery to repair congenital malformations which often involves metallic implants), sample sizes to date have so far been modest and there have been some discrepant findings between research groups. However, efforts are currently underway to combine data between multiple sites internationally through the ENIGMA Network (http://enigma.ini.usc.edu/ongoing/enigma-22q-working-group/). It is hoped that by improving the power of neuroimaging studies to detect differences between people with 22q11.2DS and controls as well as between deletion carriers with and without psychopathology, reliable MRI biomarkers of psychosis risk may be identified.

Other SZ-associated CNVs have been much less extensively studied using brain imaging with only a handful of studies being published to date. Like 22q11.2, the effects of reciprocal rearrangements of 16p11.2 on the brain also appear to be gene dosage dependent with the number of genomic copies being negatively correlated with brain volume (Maillard et al. 2015), surface area (Qureshi et al. 2014) and focal cortical thickness (Blackmon et al. 2017). The 15q11.2 deletion is a relatively common CNV but has a much lower penetrance for SZ than either the 22q11.2 deletion or the 16p11.2 duplication. Despite this, a study of population controls carrying this CNV showed impairments in cognitive function and a pattern of brain

structural abnormalities similar in some respects to that seen in first-episode psychosis (Stefansson et al. 2014). Further studies will be required to characterise the MRI signatures of these and other high-risk CNVs.

4.4 White Matter Microstructure

Studies of white matter microstructure using diffusion tensor imaging techniques have found widespread differences between patients with 22q11.2DS and typically developing controls (Barnea-Goraly et al. 2003; Simon et al. 2005, 2008; Sundram et al. 2010; da Silva Alves et al. 2011b; Kikinis et al. 2012, 2017; Radoeva et al. 2012; Ottet et al. 2013; Villalon-Reina et al. 2013; Jalbrzikowski et al. 2014; Olszewski et al. 2017; Roalf et al. 2017; Tylee et al. 2017). These include the white matter pathways connecting the frontal and temporal lobes and limbic structures as well as fronto-occipital connections, echoing findings in the SZ literature. Furthermore, graph theory has been employed, using data from structural and diffusion MRI, providing preliminary evidence for alterations in the structural organization of brain networks in 22q11.2DS (Ottet et al. 2013).

Several groups have related DTI findings to the presence of psychotic symptoms in 22q11.2DS (da Silva Alves et al. 2011b; Jalbrzikowski et al. 2014; Kates et al. 2015; Kikinis et al. 2017; Olszewski et al. 2017; Roalf et al. 2017; Tylee et al. 2017). These have found associations between the severity of psychotic symptoms and altered diffusion tensor imaging (DTI) metrics in a number of regions including the inferior longitudinal fasciculus (da Silva Alves et al. 2011b; Jalbrzikowski et al. 2014; Olszewski et al. 2017; Tylee et al. 2017), inferior fronto-occipital fasciculus (Olszewski et al. 2017), internal capsule (Perlstein et al. 2014; Kikinis et al. 2017), cingulum bundle (Kates et al. 2015; Roalf et al. 2017) and uncinate fasciculus (Perlstein et al. 2014; Roalf et al. 2017). Abnormal white matter microstructure has also been associated with cognitive decline in 22q11.2DS (Nuninga et al. 2017) and *Nogo-66* receptor genotype (Perlstein et al. 2014).

4.5 Functional Neuroimaging Studies

Patients with 22q11.2DS have impairments over a range of cognitive domains including working memory, executive function, social cognition and emotion regulation. Functional MRI studies have sought to explore the neural correlates of these deficits. These studies have shown abnormal activation patterns during spatial (Azuma et al. 2009) and non-spatial (Kates et al. 2007; Harrell et al. 2017) working memory, response inhibition (Gothelf et al. 2007a), facial processing (Andersson et al. 2008), reward processing (Van Duin et al. 2016) and emotion regulation tasks (Coman et al. 2010).

Self-referential processing has also been investigated in 22q11.2DS using fMRI (Schneider et al. 2012). Compared to typically developing adolescents, adolescents with 22q11.2DS had reduced activation of cortical midline structures during the processing of self-related information. The severity of medial frontal hypoactivation was significantly associated with positive psychotic symptoms. Behavioural studies have previously shown that individuals with 22q11.2DS tend to attribute internally generated information to external sources (Debbané et al. 2008, 2010), and this has also been observed in SZ (Larøi et al. 2004). The authors hypothesize that hypoactivation of medial frontal areas during self-referential processing may contribute to source-monitoring errors which may predispose to the development of positive psychotic symptoms.

Studies of resting-state functional connectivity in 22q11.2DS have shown differences between patients with 22q11.2DS and controls across a number of networks including the default-mode, sensorimotor, visuospatial, self-referential and visual networks (Debbané et al. 2012; Schreiner et al. 2013; Scariati et al. 2014; Padula et al. 2015; Mattiaccio et al. 2016). Atypical connectivity in the default-mode network has been correlated with thought disturbance (Mattiaccio et al. 2016), neuropsychological performance (Debbané et al. 2012) and social competence (Schreiner et al. 2013). The relationship with prodromal psychotic symptoms is less clear with one study reporting an association (Debbané et al. 2012) and another by the same group failing to replicate this (Padula et al. 2015).

4.6 Neurochemical Imaging

To date, there have been relatively few neurochemical imaging studies of high-risk CNVs making it difficult to determine whether there are any neurochemical biomarkers for psychosis risk in CNV carriers. Da Silva Alves et al. (2011a) used MRS to measure cortical neuro-metabolite concentrations in adults with 22q11.2DS and healthy controls. While there were no whole-group differences in metabolite concentrations between those with 22q11.2DS and controls, 22q11.2DS patients with a SZ diagnosis had significantly increased concentrations of glutamate/Glx in the hippocampal region compared to participants without a SZ diagnosis (both 22q11.2DS and controls). This is an intriguing finding as dysregulation of the glutamatergic system has been implicated in SZ. Furthermore, the gene *PRODH* is located in the 22q11.2 region and encodes proline oxidase, which is involved in converting proline to glutamate. However, the sample sizes in this study were small and those with a SZ diagnosis were all taking antipsychotic medication, which is an important confound. In order to determine whether hippocampal glutamate/Glx concentration is a potential biomarker for SZ, replication is required in a larger, unmedicated sample. In another MRS study, Shashi et al. (2012b) studied a sample of non-psychotic children with 22q11.2DS and found that compared to controls, absolute levels of N-acetylaspartate (a marker of cortical maturation) from the dorsolateral prefrontal cortex were significantly elevated in children with 22q11DS

compared with control participants. The elevations were associated with poor global functioning and higher rates of comorbid attention-deficit/hyperactivity disorder. Longitudinal follow-up studies will be required to examine an association with later development of SZ.

In addition to the glutamatergic system, the dopaminergic system has been implicated in SZ. People with 22q11.2DS have reduced dosage of the *COMT* gene, which encodes an enzyme responsible for the degradation of dopamine. It has therefore been hypothesised that increased psychosis risk in 22q11.2DS is due to dopamine dysregulation. Vingerhoets et al. (2018) used single-photon emission computed tomography (SPECT) with ^{123}I-labelled iodobenzamide ([^{123}I]IBZM) to measure striatal dopamine $D_{2/3}$ receptor binding potential between patients at clinical ultrahigh-risk for psychosis and patients with 22q11.2DS. All patients were non-psychotic and were antipsychotic and psychostimulant naïve. There were no significant between-group differences in dopamine $D_{2/3}$ receptor binding potential suggesting that if dopamine dysregulation plays a role in mediating psychosis risk in 22q11.2DS, the pathology may be presynaptic.

Due to the paucity of studies, no neurochemical biomarkers for SZ risk in CNV carriers have yet convincingly been identified. Future studies in larger pre-symptomatic patient samples, followed up through the period of risk, may prove more fruitful. In particular, studies investigating concentrations of excitatory and inhibitory neurotransmitters and the relative balance between these would provide useful insights.

4.7 EEG Studies

EEG has been used to investigate potential biomarkers for SZ in 22q11.2DS. Comparison between resting-state networks in patients with SZ, patients with 22q11.2DS and healthy controls found abnormalities of salience and resting-state networks in both patient groups suggesting that EEG microstates might constitute a marker for SZ (Tomescu et al. 2014).

Abnormal auditory processing has also been reported in 22q11.2DS in both change detection (Baker et al. 2005; Zarchi et al. 2013; Larsen et al. 2018) and steady-state paradigms (Larsen et al. 2017). Mismatch negativity (MMN) is a response to change detection and abnormalities in this response have been reported in chronic SZ (Catts et al. 1995; Michie 2001; Umbricht and Krljes 2005; Näätänen and Kähkönen 2009), first-episode psychosis (Atkinson et al. 2012), as well as first-degree relatives (Jessen et al. 2001; Michie et al. 2002). Baker et al. (2005) found a reduction in the MMN response in patients with 22q11.2DS. Two other groups have failed to replicate this finding (Zarchi et al. 2013; Larsen et al. 2018); however, Zarchi et al. (2013) found associations between smaller MMN amplitudes and scores on the positive and negative symptom scale (PANSS). Larsen and colleagues failed to find any group differences but found that people with 22q11.2DS had greater sensitivity to auditory tones.

The 40 Hz auditory steady-state response (ASSR) has been robustly associated with SZ (Thuné et al. 2016), making it an attractive potential biomarker. Larsen et al. (2017) investigated the ASSR in young people with 22q11.2DS and found a reduction in gamma oscillations in response to 40 Hz auditory stimuli. Gamma band activity is thought to arise from synchronised activity between local networks of neurons and to be driven by the activity of parvalbumin-containing interneurons. Disrupted interneuron migration and placement have been reported in murine models of 22q11.2DS (Meechan et al. 2012). Impaired cortical network activity as a result of disruption to the balance of excitatory and inhibitory activity in the brain has been proposed a potential mechanism for psychosis risk in 22q11.2DS and indeed for other neurodevelopmental disorders including ADHD and ASD. This is, therefore, an exciting finding that merits replication and longitudinal investigation.

4.8 Longitudinal Studies

Identifying reliable and reproducible imaging biomarkers for SZ requires longitudinal studies in large samples scanned before the onset of symptoms and followed up for a sufficient period of time in order to differentiate between those who go on to develop SZ and those who do not. Such designs also have the advantage of controlling for many confounding variables that affect cross-sectional designs such as the effects of psychotropic medication on the brain. Longitudinal studies of 22q11.2DS are currently underway and several reports have already been published, albeit in modestly sized samples with relatively short follow-up periods.

The first longitudinal study of 22q11.2DS used structural neuroimaging data from a small cohort of children with 22q11.2DS who were followed up 5 years later (Gothelf et al. 2007b). Reduction in grey matter volume in the left dorsal prefrontal cortex predicted the severity of psychotic symptoms at follow-up. The magnitude of this change was related to COMT genotype and to change in verbal IQ. Using multivariate pattern analysis, the authors were able to predict risk for psychotic symptoms with >94% accuracy (Gothelf et al. 2011).

A larger study of young people with 22q11.2DS, siblings and community controls, followed up over 3 years, found that reduction in temporal lobe grey matter volume and verbal IQ predicted the presence of positive psychotic symptoms at follow-up (Kates et al. 2011). In the third published longitudinal study in 22q11.2DS, Flahault et al. (2012) compared hippocampal development between patients with 22q11.2DS and control participants over a 3-year period. They did not find any significant differences between the groups in hippocampal development; however, the size of the hippocampal head at baseline was associated with the presence of hallucinations at follow-up. A longitudinal study of cortical complexity in 22q11.2DS found that longitudinal change in gyrification index (GI) scores in the left occipital region was negatively correlated with positive prodromal symptoms (Kunwar et al. 2012).

Although these studies suggest associations between regional brain volumes and psychotic symptoms, replication in larger samples and over longer time periods will be necessary in order to determine whether the same regional variations in childhood/adolescence can reliably predict the development of psychotic disorders in adulthood. If this can be demonstrated, in addition to informing our understanding of the pathophysiology of SZ, serial structural scanning has the potential to provide valuable prognostic information for clinicians and families.

4.9 Future Neuroimaging Studies in Rare Variants

Neuroimaging studies of 22q11.2DS have revealed structural and functional abnormalities in this high-risk group and have provided preliminary evidence for associations between these abnormalities and psychopathology. To further investigate this relationship, multimodal longitudinal neuroimaging studies in large cohorts of children, followed up through the risk period for psychosis, will be required. In addition, further work building on the small literature on neurochemical imaging in 22q11.2DS will help advance mechanistic understanding of the disorder. There has thus far been very little neuroimaging research in other high-risk CNVs, but extending work to these variants may help to identify mechanisms that are common across genetic risk variants.

Studying individuals at high-genetic risk of SZ offers significant advantages over neuroimaging studies of patients with idiopathic SZ as a relatively homogenous, high-risk group can be identified prior to the development of symptoms and the initiation of antipsychotic medication, which confound neuroimaging studies in the non-deleted population. Such an approach offers the potential to identify biomarkers of psychosis risk as well as novel therapeutic targets. Furthermore, as CNVs like the 22q11.2 deletion confer risk across neurodevelopmental disorders, the neural mechanisms underlying this shared risk can be studied.

5 Discussion and Conclusions

The last decade has seen rapid progress in understanding the genomic architecture of SZ. This exciting progress has opened up a range of opportunities for understanding the biological basis of this syndrome. It is already clear that SZ is highly polygenic, and that genetic risk overlaps with other psychiatric syndromes. In addition, it is apparent that the specific genetic risk factors in operation may vary considerably between affected individuals. This may explain some of the variability in the presentation and course of the condition across patients, and suggests that it may be possible to stratify individuals into more homogeneous biological categories of risk. Genomic measures in themselves hold up considerable potential to act as such biological markers for both prediction and stratification. However genomic

information can also be used to inform the development of biomarkers in other modalities, including brain imaging, as illustrated in this chapter, and such derived markers may eventually be of greater utility in clinical practise. Overall it is likely that biomarker development in SZ will be an iterative process, with genomic information informing the identification of potential biomarkers, which are then further refined through additional genetic and biological studies. Progress along this pathway of geomically informed biomarker development is an imperative for the field given the urgent need for better biological understanding of SZ and the development of reliable biomarkers to guide the development of novel therapies.

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Imaging and Genetic Biomarkers Predicting Transition to Psychosis

Stuart A. Hunter and Stephen M. Lawrie

Contents

Abstract The search for diagnostic and prognostic biomarkers in schizophrenia care and treatment is the focus of many within the research community. Longitudinal cohorts of patients presenting at elevated genetic and clinical risk have provided a wealth of data that has informed our understanding of the development of schizophrenia and related psychotic disorders.

Imaging follow-up of high-risk cohorts has demonstrated changes in cerebral grey matter of those that eventually transition to schizophrenia that predate the onset of symptoms and evolve over the course of illness. Longitudinal follow-up studies demonstrate that observed grey matter changes can be employed to differentiate

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Curr Topics Behav Neurosci (2018) 40: 353–388
DOI 10.1007/7854_2018_46
Published Online: 6 April 2018

those who will transition to schizophrenia from those who will not prior to the onset of the disorder.

In recent years our understanding of the genetic makeup of schizophrenia has advanced significantly. The development of modern analysis techniques offers researchers the ability to objectively quantify genetic risk; these have been successfully applied within a high-risk paradigm to assist in differentiating between high-risk individuals who will subsequently become unwell and those who will not.

This chapter will discuss the application of imaging and genetic biomarkers within high-risk groups to predict future transition to schizophrenia and related psychotic disorders. We aim to provide an overview of current approaches focussing on grey matter changes that are predictive of future transition to illness, the developing field of genetic risk scores and other methods being developed to aid clinicians in diagnosis and prognosis.

Keywords Biomarkers · Genetics · High risk · Imaging · Psychosis · Schizophrenia · Transition

1 Introduction

Predicting the transition of high-risk individuals from a state of wellness or subthreshold psychotic symptoms to state of illness has been the pursuit of many within psychosis research for over half a century (Yung and Nelson 2013). The development of "high-risk" research paradigms has facilitated the study of cohorts at significantly elevated risk of making the transition to illness when compared to matched controls. Risk of transition to psychotic disorder varies but can rise as high as 45% depending on cohort and inclusion criteria (Mechelli et al. 2011). It however remains difficult to determine who will transition to illness based on symptom criteria alone (Mechelli et al. 2011).

Targeted early detection and intervention within high-risk groups has been proposed as the most effective way of improving outcomes in patients who eventually transition to psychotic disorder (Fusar-Poli et al. 2017a). Engagement with early intervention services during first-episode psychosis (FEP) has been demonstrated to reduce duration of untreated psychosis (11 days compared to 1 year), reduce hospital admissions (46% vs 68%) and reduce compulsory admission to psychiatric care (30% vs 62%) (Valmaggia et al. 2015). In addition, patients engaged with early intervention services experience shorter time to diagnosis and shorter duration of hospital admissions (Fusar-Poli et al. 2016). A currently proposed model of clinical staging allows for targeted and specific intervention delivery within a high-risk early intervention service (Fusar-Poli et al. 2017a), but even in those patients at highest clinical risk, transition rates of 39% at 2 years (Fusar-Poli et al. 2016a) and 51% at 3 years (Fusar-Poli et al. 2016b) remain too low to be of clinical utility (Fusar-Poli et al. 2017b).

Low rates of transition and difficulties reliably ascertaining high-risk groups pose difficult questions when configuring and delivering early intervention services, such

as who will benefit most from active treatment and what is the opportunity cost? Evidently, with more than 60% of high-risk individuals not transitioning at 2 years, a blanket approach of prophylactic treatment would be undesirable and potentially unsafe and is therefore currently not recommended (McGorry et al. 2002; McGlashan et al. 2006). The inclusion of imaging and genetic biomarkers within a high-risk paradigm is a potentially powerful method of decreasing the uncertainty associated with identifying those individuals who will transition to illness (Smieskova et al. 2010; Fusar-Poli et al. 2011).

2 Cohorts

The second half of the twentieth century witnessed the emergence of a familial high-risk paradigm based on a growing awareness that schizophrenia, in particular, and psychotic disorders generally are highly heritable conditions (Shah et al. 2013; Gottesman 1991). This gave rise to a number of longitudinal familial high-risk cohorts of first- and, less frequently, second-degree relatives of patients being followed up over a number of years, often undergoing regular neuropsychological testing and clinical assessment (Erlenmeyer-Kimling et al. 1997; Jørgensen et al. 1987; Johnstone et al. 2000; Kendler et al. 1993; Gur et al. 2007).

In parallel the concept of a clinical high-risk state gathered increasing purchase during the early 1990s (Yung and Nelson 2013; Yung et al. 2004a, b). The development of the at-risk mental state (ARMS) criteria (at the PACE Clinic in Melbourne; Yung et al. 2004b) for identifying those at highest risk of transitioning to psychosis (see Thompson et al. (2016) for an overview of criteria and Fusar-Poli et al. (2013a) for a comprehensive review) was adopted and modified by several international research teams, giving rise to comparable cohorts of patients presenting to services at clinical high risk (Mechelli et al. 2011; Koutsouleris et al. 2014; Riecher-Rössler et al. 2007; Thompson et al. 2011; O'Donoghue et al. 2015; Fusar-Poli et al. 2013b).

Established on the basis of likely prodromal illness (Yung and McGorry 1996) that does not meet diagnostic criteria for an established psychotic disorder, the selection criteria identify patients at high risk of transitioning to diagnosable psychotic disorder: a recent meta-analysis identified transition rates of 18% at 6 months increasing to 36% at 3 years (Fusar-Poli et al. 2012). Patients present with an array of subthreshold psychotic symptoms that are in the main either too mild or too brief to meet diagnostic criteria (Riecher-Rössler et al. 2009). Follow-up in these cohorts has further contributed to our understanding of the neurobiological changes that take place during the period of transition to illness (Pantelis et al. 2003).

Despite notably different recruitment strategies, there are notable similarities between patients in these clinical high-risk (CHR) and familial high-risk (FHR) cohorts. Most strikingly, about 10–15% of most CHR samples have a family history of psychotic disorder and approximately 50% of FHR samples display subthreshold psychotic symptoms at some stage (Yung et al. 2004a; Johnstone et al. 2002). Despite FHR studies reporting lower transition rates than CHR (Johnstone et al. 2005), there

is substantial overlap in findings of structural abnormality on repeated neuroimaging and the application of these results to predictive diagnostic models (see Table 1) (Smieskova et al. 2010, 2013).

3 Imaging Background

Early structural neuroimaging studies identified reductions in cerebral substance in groups of patients with established schizophrenia in the late 1970s using computed tomography scanning techniques (Johnstone et al. 1976). Observed ventricular enlargement (Johnstone et al. 1976; Van Horn and McManus 1992) and reduced grey matter (Lawrie and Abukmeil 1998) in patients with schizophrenia spurred a wave of research using a new imaging technique, magnetic resonance imaging (MRI). Throughout the 1990s it became apparent that those experiencing a first episode of schizophrenia also demonstrated similar structural brain changes (Suddath et al. 1990; Lawrie and Abukmeil 1998). It was unclear however if these alterations in grey matter were dynamic and altered with illness progression or static and present from an early age. The Edinburgh High-Risk Study (EHRS) was designed to address this question and if any such changes could be used to predict the onset of schizophrenia. Although it was not the first study to introduce imaging in FHR designs (Jørgensen et al. 1987), it was the first high-risk cohort study to include routine repeated imaging during a prolonged follow-up period (Johnstone et al. 2000). The EHRS, amongst others, has provided data demonstrating that changes in grey matter volume evolve during the period of transition to a diagnosis of schizophrenia (Johnstone et al. 2005; Lawrie et al. 2008; Job et al. 2005; Bois et al. 2015). Many of the elements of the EHRS relating to study design, namely, the inclusion of repeated imaging through follow-up, are similar to several more recent international clinical high-risk cohorts (Riecher-Rössler et al. 2007; Fusar-Poli et al. 2013b; Pantelis et al. 2003; Cannon et al. 2015; Velakoulis et al. 1999).

4 Image Analysis

Transition to psychotic disorders is widely believed to involve dynamic changes in brain structure and function (Smieskova et al. 2010). Although commonly held this view is not universal. Weinberger and Radulescu (2016), call for caution when attributing significance to structural changes observed under research conditions. They suggest that some of the observed differences between patients and controls may be more reasonably attributed to potential confounders (lifestyle, medication use and habitus) than the underlying pathophysiology of psychotic disorders. Noting that MRI in itself lacks the fidelity to directly measure brain structure on a cellular level, they suggest that greater care is taken in the reporting future findings and the conclusions that are drawn from the existing body of evidence. This said the authors go to lengths to stress that the primary aim of the commentary is to highlight the danger of using jargonised and unclear language and to underestimate the potential

Table 1 Overview of studies utilising neuroanatomical biomarkers as illness predictors

Author/Year	Imaging processing and statistical analysis	Cohort (city)	High-risk criteria	Population size	Diagnostic breakdown (transition definition)	Transition rates	Additional variables	Accuracy	Grey matter abnormalities discriminating between groups
Pantelis et al. (2003)	VBM Univariate statistical analysis	PACE (Melbourne)	PACE-ARMS (Yung et al. 1998)	75	Schizophrenia – 8 Schizoaffective disorder – 2 Brief psychotic episode – 1 Psychosis NOS – 1 Bipolar affective disorder with psychotic symptoms – 6 Major depression with mood incongruent psychotic disorder – 4 Psychosis with affective features – 1 (transition defined as DSM-IV psychotic disorder)	23 of 75 (31%)	N/A	N/A	*Baseline GMV reductions in HR individuals who transitioned* A right medial temporal region including the hippocampus and parahippocampal cortex 1. Right lateral temporal region encompassing superior temporal gyrus 2. A right inferior frontal region including the orbital portion of the inferior frontal gyrus, the adjacent parts of the insula and basal ganglia 3. A cingulate region which included the anterior and posterior cingulate gyrus bilaterally *GMV reductions at follow-up* 1. Bilateral reduction in GM volume in cingulate gyri 2. Left parahippocampal gyrus 3. Left fusiform gyrus 4. Left orbitofrontal cortex 5. Left cerebellar cortex *Increased volume* 1. Right cuneus

(continued)

Table 1 (continued)

Author/Year	Imaging processing and statistical analysis	Cohort (city)	High-risk criteria	Population size	Diagnostic breakdown (transition definition)	Transition rates	Additional variables	Accuracy	Grey matter abnormalities discriminating between groups
Job et al. (2006)	VBM Univariate statistical analysis	EHRS (Edinburgh)	EHRS (Johnstone et al. 2000)	65	Schizophrenia – 8 (transition defined as ICD-10 schizophrenia)	8 of 65 (12%)	N/A	PPV 60% NPV 92% LR+ 10.69 LR− 0.65	1. Left inferior temporal gyrus 2. Right cerebellum 3. Left uncus
Koutsouleris et al. (2009)	VBM SVM	FETZ (Munich)	BSABS (Klosterkötter et al. 2001) PACE–ARMS (Yung et al. 1998)	45 HR 25 HC 45 HC for validation of model	ICD 10 Transition defined as emergence of diagnosable schizophrenia spectrum disorder	17/45 (38%)	N/A	Sensitivity 83% Specificity 80% BAC 82% PPV 83% NPV 80%	1. Cerebellum 2. Thalamus 3. Lateral prefrontal cortex 4. Lateral temporal cortex bilaterally 5. Medial temporal cortex bilaterally 6. Inferior temporal cortex bilaterally
Mechelli et al. (2011)	VBM and ROI Univariate statistical analysis	OASIS (London) FePsy (Basel) FETZ (Munich) PACE (Melbourne)	PACE–ARMS (Yung et al. 1998) BSABS (Klosterkötter et al. 2001)	182 HR 167 HC	Not specified	48/182 (26%)	N/A	Sensitivity 68% Specificity 66% BAC 67%	1. Anterior left hippocampal gyrus (−21, 6, −27) 2. Prefrontal cortex 3. Anterior cingulated gyrus
Koutsouleris et al. (2012b)	VBM SVM	FePsy (Basel) Validated on FETZ (Munich)	BSIP–ARMS (Riecher-Rössler et al. 2008)	37 HR 22 HC MUNICH validation cohort 17T and 17NT	Melbourne ARMS criteria for transition to psychotic disorder	16/37 (43%)	N/A	Sensitivity 81% Specificity 87.5% BAC 84.2% LR+ 6.5 Sensitivity 82.4% Specificity 94.1% BAC 88.2%	1. Dorsomedial, rostromedial and cingulate cortex, bilaterally 2. Medial orbitofrontal, precuneal and premotor 3. The dorsolateral prefrontal GM and WM; the right 4. Parahippocampal and inferior temporal cortex 5. The thalamus, bilaterally

Borgwardt et al. (2013)	VBM SVM	FePSY (Basel)	BSIP–ARMS (Riecher-Rössler et al. 2008)	23 FEP 16 ARMS-T 22 HC	Melbourne ARMS criteria for transition to psychotic disorder	N/A	N/A	*HC vs ARMS-T* Sensitivity 75% Specificity 86% BAC 80.7%	*HC vs ARMS-T* 1. Prefrontal cortex 2. Limbic lobe (amygdala and olfactory regions) 3. Cerebellum
								ARMS-T vs FEP Sensitivity 91.3% Specificity 68.8% BAC 80%	*ARMS-T vs FEP* 1. Thalamus, putamen, pallidum 2. Cerebellum
Koutsouleris et al. (2014)	VBM SVM	FETZ (Munich) FePsy (Basel)	BSABS (Klosterkötter et al. 2001) BSIP–ARMS (Riecher-Rössler et al. 2008)	73 ARMS	DSM-IV (Munich) OPCRIT (Basel)	45.2%	N/A	Sensitivity 75.8% Specificity 85% BAC 80.4% LR+ 5.1 LR− 0.29 OR 17.7	1. Bilateral reduced GM volume in prefrontal cortices (−10%) covering dorsomedial, ventromedial and orbitofrontal areas and extending to the cingulate and right intra- and perisylvian structures (inferior frontal gyrus, rolandic operculum, insula temporal pole) 2. GM reduction right BG (11.3%), vermal lobule 10 and the cerebellar lobules 7b, 8, crus 1 and 2 3. Increased GM volume in right perisylvian and temporal structures (supramarginal, inferior parietal, angular cortex) and subcortical structures right pallidum, vermal lobules 6–9 and cerebellar lobules 4,5,6,9 and 10
Zarogianni et al. (2017a)	VBM SVM	EHRS (Edinburgh)	EHRS (Johnstone et al. 2000)	16 HR-symp 16 HR-ill	ICD-10	N/A	RISC RAVLT	*Neuroimaging* Sensitivity 76%	1. Cerebellum 2. Lateral and medial temporal lobe

(continued)

Table 1 (continued)

Author/Year	Imaging processing and statistical analysis	Cohort (city)	High-risk criteria	Population size	Diagnostic breakdown (transition definition)	Transition rates	Additional variables	Accuracy	Grey matter abnormalities discriminating between groups
								Specificity 100% BAC 88% *Inclusion of RISC and RAVLT* Sensitivity 100% Specificity 88% BAC 94%	3. Amygdala 4. Medial frontal lobe 5. Putamen 6. Superior parietal lobe covering the supramarginal gyrus bilaterally and extending to the fusiform gyrus
Zarogianni et al. (2017b)	VBM SVM	FePSY (Basel)	BSIP–ARMS (Riecher-Rössler et al. 2008)	35 ARMS	Melbourne ARMS criteria for transition to psychotic disorder	16/35 (45.7%)	N/A	Sensitivity 62.5% Specificity 84.2% BAC 74.2%	1. Cerebellum 2. Superior temporal pole bilaterally 3. Right anterior cingulated cortex 4. Right superior medial frontal cortex 5. Left orbitofrontal cortex 6. Insula bilaterally

Voxel-based morphometry (VBM), Personal Assessment and Crisis Evaluation defined at-risk mental state (PACE-ARMS) (Yung et al. 1998), grey matter volume (GMV), high-risk transition/non-transition (HR-T/NT), Edinburgh High-Risk Study (EHRS) (Johnstone et al. 2000), positive/negative predictive value (PPV/NPV), likelihood ratio positive (LR+) (sensitivity/1 − specificity), likelihood ratio negative (LR−) (1 − sensitivity/specificity), support vector machine (SVM), Früherkennungs- und Therapiezentrum (FETZ, Early Detection and Intervention Centre, Ludwig Maximillian University, Munich), Bonn Scale for Assessment of Basic Symptoms (BSABS) (Klosterkötter et al. 2001), balanced accuracy (BAC), region of interest (ROI), Outreach and Support in South London (OASIS), Fruherkennung von Psychosen (FePsy), Basel Screening Instrument for Psychosis defined at-risk mental state (BSIP-ARMS) (Riecher-Rössler et al. 2008), first-episode psychosis (FE), at-risk mental state-transition (ARMS-T), high-risk patients with subthreshold symptoms (HR-symp), high-risk patients transitioned to schizophrenia (HR-ill)

impact of confounders on results rather than to challenge the credibility of the findings themselves.

The concerns raised are partially addressed in a 2017 paper by Brugger et al., in a meta-analysis of 108 papers (including 3,800 patients and 3,609 controls) using structural MRI measurements in patients experiencing a first episode of psychosis (Brugger and Howes 2017). They conclude that greater heterogeneity is observed between subjects experiencing a first-episode psychosis than between healthy controls in a number of cortical regions (putamen, temporal lobe, thalamus and third ventricle) and a greater degree of homogeneity observed when analysing anterior cingulate cortex volumes. They suggest these findings, greater heterogeneity and homogeneity, may indeed be features of the disorder itself with the anterior cingulate being a key area in the development of illness and heterogeneity being one aspect of the illness from its onset. The breadth and scope of the analysis allows the authors to address some of the common concerns regarding confounders (effectively excluding a linear relationship with duration of illness and treatment status) and provides greater evidence of the biological heterogeneity of schizophrenia as a disorder. Outwith the scope of this chapter but also worth commenting on are known risk factors for schizophrenia that may indeed influence cortical structure to a greater degree than variables previously mentioned such as obstetric complications, childhood adversity and cannabis misuse, factors that require further examination through the use of large data sets (Lawrie 2017).

Grey matter abnormalities localised to frontal, cingulate and temporal cortices, hippocampal structures and the cerebellum have been identified as being potentially predictive in patients who will develop a full-blown diagnosable psychotic disorder (Mechelli et al. 2011; Smieskova et al. 2010; Koutsouleris et al. 2009, 2012a, 2014; Job et al. 2005; Zarogianni et al. 2013, 2017a). Two main techniques have been used – region of interest (ROI) approaches and voxel-based morphometry (VBM). Notably both techniques differ in measurement parameters and analysis process; VBM measures within voxel concentrations of grey matter across subjects, while ROI measures absolute grey matter volumes (Giuliani et al. 2005). Region of interest (ROI) analysis (Phillips et al. 2002; Velakoulis et al. 2006) is labour intensive as it requires hand-tracing and/or semiautomated volume extraction in prespecified cortical and/or subcortical areas for investigation (Giuliani et al. 2005). During the early 2000s, with advances in image analysis routines, voxel-based morphometry (VBM), an automated whole-brain MRI measurement technique, greatly increased in popularity, as it facilitated relatively quick investigation of the whole brain utilising parametric and non-parametric statistical analysis. VBM is therefore more suitable for identifying between group differences in large cohorts (Fusar-Poli et al. 2011; Giuliani et al. 2005; Ashburner and Friston 2000; Borgwardt et al. 2011). It is worth noting that the vast majority of papers discussed below in terms of their predictive value have employed a VBM approach – although at least one has used FreeSurfer (Bois et al. 2015) which is largely automated but benefits from user checking and interaction. The following discussion focuses specifically on volumetric abnormalities that are statistically significant in the context of differentiating between high-risk individuals who transition to psychotic disorder and those who do not. As such these findings contribute to our

understanding of biomarkers that are predictive of emerging schizophrenia and related psychotic disorders.

5 Prefrontal Lobes

Both historical and recent meta-analysis have identified the prefrontal lobes bilaterally as regions of volumetric grey and white matter reduction in patients with established schizophrenia (Wright et al. 2000; Haijma et al. 2013). This finding has been reinforced in a recent meta-analysis using data from patients at risk of developing the disorder who have not transitioned to full-blown illness suggesting that such reductions are present prior to illness onset (Fusar-Poli et al. 2011). Similar findings have been demonstrated in longitudinal studies specifically designed to track prefrontal grey matter changes in high-risk individuals, which may indicate the presence of a progressive pathological process underlying illness development (Sun et al. 2009). These findings are further supported in a recent meta-analysis of longitudinal ROI studies which identified progressive grey and white matter loss after onset in the prefrontal cortex (Olabi et al. 2011). This is in line with an established body of research indicating that dynamic changes within the prefrontal lobes are present in the high-risk state, developed during FEP and on to full-blown schizophrenia (Smieskova et al. 2010, 2013; Fusar-Poli et al. 2011, 2012; Bois et al. 2015; Borgwardt et al. 2008; Farrow et al. 2005).

Although there is no clear consensus on any specific regional grey matter reduction that is predictive of future transition to psychotic disorder, several regions have repeatedly demonstrated statistical significance within high-risk cohorts. Reductions in the volume of the orbitofrontal cortex (OFC) generally and the medial portion specifically have been demonstrated as predictive of onward transition to psychotic disorder. Pantelis et al. (2003) identified statistically significant reductions in GMV within the right inferior frontal region including the orbital portion of the inferior frontal gyrus in patients who transitioned to psychosis. This was partially supported by Mechelli et al. (2011) who demonstrated grey matter loss within the medial orbital gyrus when comparing CHR patients with controls, although this finding was not replicated when comparing CHR patients who transitioned to psychotic disorder to those who did not. This pattern of change was subsequently endorsed by Koutsouleris et al. (2012b) who identified medial orbitofrontal GMV loss and orbitofrontal volume loss in 2014 as statistically significant when differentiating between those who did or did not make the transition (Koutsouleris et al. 2014).

Although less frequently replicated, other regions have demonstrated predictive power when included in multivariate models, suggesting that GMV abnormalities are not confined to the OFC in high-risk groups. In a series of papers (2009, 2012, 2014), Koutsouleris et al. identified a bilateral overall reduction in GMV within the PFC of around 10%, as well as reductions in dorsolateral prefrontal cortex (DLPFC) volume and dorsomedial volume reductions as biomarkers potentially capable of differentiating between CHR who transition to schizophrenia and those who do not

when included in a wider model of volumetric change (Koutsouleris et al. 2009, 2012b, 2014). These findings are again supported by a 2013 study by Borgwardt et al. which demonstrated decreased prefrontal cortex grey matter as being statistically significant when it came to constructing final the neuroanatomical decision function delineating between healthy controls and CHR patients who transition to illness (Borgwardt et al. 2013).

Of the eight papers that have utilised structural MRI in model development to aid prediction to psychotic disorder (Table 2), seven demonstrate statistically significant reduction in frontal lobe grey matter volume (Mechelli et al. 2011; Koutsouleris et al. 2009, 2012b, 2014; Zarogianni et al. 2017a). As such, there are indications that reductions in prefrontal grey matter volume, perhaps particularly the OFC, may contribute to the development of clinically useful biomarkers given the dynamic pattern of change within most high-risk cohorts examined thus far. It is however evident given some variation in results that further research is required to clearly elucidate the pattern of change that occurs during the transition period.

6 Temporal Lobes

The temporal lobes have repeatedly been identified as regions of volume reduction across various stages of developing schizophrenia. Early structural imaging studies in established illness and first-episode psychosis identified overall grey matter volume reduction within the temporal lobes in schizophrenia (Lawrie and Abukmeil 1998; Suddath et al. 1990; Turetsky et al. 1995). Subsequent research identified temporal lobe volume reductions in patients at genetic high risk experiencing psychotic symptoms (Lawrie et al. 2002), a finding in keeping with a large body of research indicating a correlation between psychotic symptom severity and volume reduction in the temporal lobes, most specifically the superior temporal gyrus (STG) (Shenton et al. 1992; Sumich et al. 2005; Sumner et al. 2017). These findings are in line with the established body of evidence in schizophrenia and supported by several large meta-analytical studies (Smieskova et al. 2010; Fusar-Poli et al. 2011, 2012; Bois et al. 2015; Takahashi et al. 2009). Indeed, meta-analysis of longitudinal cohorts has identified the temporal lobes as one of the most frequently isolated areas of abnormality during the transition from health to psychotic disorder (Smieskova et al. 2010).

Six papers have identified the temporal cortices as foci of dynamic change occurring throughout an individual's transition from familial/clinical high-risk status to psychotic disorder (Table 2) (Koutsouleris et al. 2009, 2012b, 2014; Zarogianni et al. 2017a; Job et al. 2006). However there is marked heterogeneity across studies; one paper found increases in grey matter predictive of onward transition to psychotic disorder (Koutsouleris et al. 2014) and several identified multiple foci of reduction within the temporal lobes (Pantelis et al. 2003; Koutsouleris et al. 2009, 2012b; Zarogianni et al. 2017a, b; Job et al. 2006).

Of the papers illustrated in Table 2, the medial and lateral temporal lobes have been identified most frequently as areas of volumetric reduction that differentiates between

Table 2 Table of statistically significant grey matter changes differentiating high-risk groups (Talairach coordinates of voxels of maximum significance (x, y, z))

Paper	Cohort	Imaging modality	Imaging analysis	Frontal cortex	Temporal cortex	Cingulate cortex	Hippocampal structures	Cerebellum	Additional findings
Pantelis et al. (2003)	PACE	sMRI	VBM	*GMV abnormalities at baseline*					
				↓ GMV Right inferior frontal lobe including the orbital portion of the inferior frontal gyrus and the adjacent areas of the insula and basal ganglia	↓ GMV Right medial temporal region Right lateral temporal region encompassing superior temporal gyrus	↓ GMV A cingulate region including the anterior and posterior cingulate gyrus bilaterally	↓ GMV Hippocampus and parahippocampal cortex	–	–
				GM reductions at follow-up					
				↓ GMV Left orbitofrontal gyrus (−17,60, −23)	–	↓ GMV Cingulate gyrus (1, −14, 28)	↓ GMV Left parahippocampal gyrus and fusiform gyrus (−31, −42, −25)	↓ GMV Left cerebellar cortex (−41, −65, −25)	↓ GMV Increased volume in right cuneus (−74, 19, 127)
Job et al. (2005, 2006)	EHRS	sMRI	VBM	–	↓ GMV Left inferior temporal gyrus (−51.1, −16.1, −31.2)	–	↓ GMV Left uncus (−24.6, −10.2, −28.9)	↓ GMV Right cerebellar region (40.6, −44.6, −28.9)	–
Koutsouleris et al. (2009)	FETZ	sMRI	VBM	↓ GMV Lateral prefrontal cortex	↓ GMV Medial, lateral and inferior temporal bilaterally	–	–	↓ GMV	↓ GMV Thalamus

Mechelli et al. (2011)	FETZ, OASIS, PACE, FePSY	sMRI	VBM and ROI	↓ GMV Prefrontal cortex	–	↓ GMV Anterior cingulate cortex	↓ GMV Left anterior parahippocampal gyrus (−21, 6, −27)	–	–
Koutsouleris et al. (2012a, b)	FePSY	sMRI	VBM	↓ GMV Dorsomedial Rostromedial Medial orbitofrontal and dorsolateral prefrontal cortices	↓ GMV Inferior temporal cortex	↓ GMV Cingulate cortex bilaterally	↓ GMV Right parahippocampal cortex	–	↓ GMV Precuneal and premotor areas
Borgwardt et al. (2013)	FePSY	sMRI	VBM	*HC vs ARMS-T*					
				↓ GMV Prefrontal cortex	–	–	–	↓ GMV	↓ GMV Limbic lobe
				ARMS-T vs FE					
				–	–	–	–	↓ GMV	↓ GMV Thalamus Pallidum Putamen
Koutsouleris et al. (2014)	FePSY + FETZ	sMRI	VBM	↓ GMV Bilateral PFC (−10%) including dorsomedial, ventromedial, orbitofrontal	–	↓ GMV Cingulate	–	↓ GMV Vermal lobule 10 and the cerebellar lobules 7b, 8 crus 1 and 2	↓ GMV Perisylvian structures Inferior frontal gyrus Rolandic operculum Insula temporal pole ↓ GMV Right BG (11.3%)
					↑GMV Right perisylvian and temporal				↑GMV And subcortical structures including right

(continued)

Table 2 (continued)

Paper	Cohort	Imaging modality	Imaging analysis	Frontal cortex	Temporal cortex	Cingulate cortex	Hippocampal structures	Cerebellum	Additional findings
					structures (supramarginal, inferior parietal, angular cortex)				pallidum, vermal lobules 6–9 and cerebellar lobules 4, 5, 6, 9 and 10
Zarogianni et al. (2017a)	EHRS	sMRI	VBM	↓ GMV Medial frontal lobe	↓ GMV Lateral and medial temporal lobe	–	–	↓ GMV Cerebellum	↓ GMV Putamen Superior parietal lobe covering the supramarginal gyrus bilaterally and extending to the fusiform gyrus
Zarogianni et al. (2017b)	FePSY	sMRI	VBM	↓ GMV Right superior medial frontal cortex Left orbitofrontal cortex	↓ GMV Superior temporal pole bilaterally	↓ GMV Right anterior cingulate cortex	–	↓ GMV Cerebellum	↓ GMV Insula bilaterally

Structural MRI (sMRI), voxel-based morphometry (VBM), region of interest (ROI), decrease/increase grey matter volume (↓/↑GMV), Personal Assessment and Crisis Evaluation Clinic (PACE), Edinburgh High-Risk Study (EHRS), Früherkennungs- und Therapiezentrum (FETZ, Early Detection and Intervention Centre, Ludwig Maximillian University, Munich), Outreach and Support in South London (OASIS), Fruherkennung von Psychosen (FePsy, Basel early detection of psychosis study)

those who do versus do not make a transition. Pantelis et al. (2003), Koutsouleris et al. (2009) and Zarogianni et al. (2017a) identified statistically significant grey matter volume reductions within the medial temporal lobes as predictive of future group classification, while Koutsouleris and Zarogianni also isolated reductions in lateral temporal lobe volume.

Other findings of statistical significance include reductions in the inferior temporal gyrus (ITG) (Job et al. 2006), inferior temporal cortex (Koutsouleris et al. 2012b) and the superior temporal gyrus (STG) (Pantelis et al. 2003). Interestingly, and in contrast to the established body of evidence, Koutsouleris et al. (2014) identified increases in grey matter volume within right perisylvian and temporal lobe structures, including the supramarginal, inferior parietal and angular cortices, in clinical high-risk patients that eventually transitioned to psychotic disorder.

Although there is a degree of heterogeneity associated with temporal lobe findings, there is a growing body of evidence that suggests grey matter changes in the temporal lobes play a significant role in the pathogenesis of schizophrenia and evolve over time. Despite the dynamic nature of volume reduction, it is not clear that it correlates with clinical presentation on an individual level or with level of functioning in established illness (Cobia et al. 2012). In this regard further evidence generated from longitudinal cohorts focussing on outcomes will serve to inform both diagnostic models and models focussing on prognostication.

7 Cerebellum

There is a body of evidence that implicates the cerebellum in the pathogenesis of schizophrenia. Abnormalities in cerebellar structure and volume were initially identified in patients with chronic schizophrenia on post-mortem and CT in the late 1970s, and this was subsequently confirmed in later studies utilising MRI (Heath et al. 1979; Sandyk et al. 2009; Martin and Albers 1995; Okugawa et al. 2002). Functional and gross volume abnormalities are also recognised as playing a role in the development of cognitive symptoms and neurological soft signs often seen in chronic schizophrenia (Lungu et al. 2013). In the context of the recognised body of evidence in established illness, a pattern of cerebellar volume reduction and structural abnormalities in FEP has emerged (Farrow et al. 2005; Bottmer et al. 2005; Thomann et al. 2009).

Findings of cerebellar volume reduction in FEP have been replicated in multiple high-risk cohort studies and observed more frequently in participants who go on to develop psychotic disorder (Pantelis et al. 2003; Job et al. 2005; Borgwardt et al. 2008, 2011). Volume reductions are significantly different between patients presenting with a clinical high-risk state versus FEP and in turn versus established psychotic disorder (Smieskova et al. 2010; Pantelis et al. 2003; Job et al. 2005; Borgwardt et al. 2008). As such the evolving nature of cerebellar volume reduction theoretically lends itself to predictive modelling of psychosis onset.

Amongst the models currently published, and discussed in Table 1, four papers found that cerebellar volume reduction was a statistically significant predictor of onward transition to psychosis (Koutsouleris et al. 2009, 2014; Zarogianni et al. 2017a; Borgwardt et al. 2013). Koutsouleris et al. (2009) identified a pattern of change including cerebellar abnormalities as potentially discriminative when differentiating between controls and CHR participants (Koutsouleris et al. 2014). Borgwardt et al. (2013) found that alterations in grey matter structure within the cerebellum added to the overall decision function when discriminating between those at risk who became ill versus controls. A 2014 paper by Koutsouleris et al. further built on this work and identified more specific volume reductions within the cerebellum (including cerebellar lobules 7b, 8, Crus 1 and 2) that when incorporated into an overall pattern of change were able to discriminate between a CHR population that transitioned to psychotic disorder and those who did not (Koutsouleris et al. 2014). These findings have been further supported recently by Zarogianni et al. (2017a) who identified a network of grey matter reduction including cerebellar changes capable of discriminating between a patient presenting with symptoms and without in a genetic high-risk paradigm. Thus, although less frequently observed than frontal and temporal abnormalities, cerebellar volume reduction has been demonstrated as potentially predictive of future transition to psychotic disorder in multiple high-risk cohorts. It is therefore possible that cerebellar abnormalities when incorporated into an overall pattern of change will serve to add model accuracy when differentiating between patients who will transition to psychotic disorder and those who will not.

8 Hippocampal Complex

Structural abnormalities within the hippocampal complex have repeatedly been demonstrated in patients with established schizophrenia (Velakoulis et al. 1999, 2006; Nelson et al. 1998) as well as family members (Seidman et al. 1999). These findings have been replicated during first-episode psychosis (Lawrie et al. 1999) although they have been observed less frequently within a population during the transition period (Smieskova et al. 2010). With the addition of data from longitudinal cohort studies, a picture of dynamic change has emerged (Pantelis et al. 2003), although only one model identified alterations in hippocampal volume as predictive of transition to psychotic disorder (Koutsouleris et al. 2012b). This being said there remains substantial evidence that hippocampal abnormality plays a role in the pathogenesis of schizophrenia and the lack of findings in current studies may simply reflect an earlier stage of disease.

9 Cingulate Cortex

Abnormalities in the cingulate cortex, although less frequently observed, have also been repeatedly demonstrated as predictive of onward transition to psychosis (Mechelli et al. 2011; Koutsouleris et al. 2012b, 2014). Although the evidence base is less robust during the transition period, there is a body of data that indicates abnormalities in the cingulate cortex are common in individuals with established psychotic disorder (Pantelis et al. 2003; Baiano et al. 2007; Glahn et al. 2008). Alterations in cingulate cortex volume have been implicated in the development of impairment in emotional processing and higher cognitive performance in established schizophrenia (Fusar-Poli et al. 2011; Baiano et al. 2007).

Overall, the evidence for structural brain abnormalities in overlapping regions and their progressive nature is substantial. However, this is not to say that these changes are necessarily clinically significant. An emerging pattern of multiple affected areas that evolves over the course of an individual's illness and allows for the discrimination between those who are actively ill, those who will transition and those who are well is of value in terms of diagnosis, prognosis and onward care is highly desirable (Zarogianni et al. 2013).

10 Functional MRI

There is, as discussed above, a well-established body of evidence that indicates grey matter reduction is common in patients with schizophrenia and evolves over the course of illness. Although functional abnormalities have been less frequently investigated, there is a growing body of evidence indicating that functional change is common and contributes to overall illness burden and an individuals' social and occupational functioning (Minzenberg et al. 2009; Kronbichler et al. 2017; Fusar-Poli et al. 2009). Indeed functional abnormalities reported in patients with established schizophrenia often correlate with cognitive deficits (Alústiza et al. 2017), impaired emotional processing (Gur et al. 2002) and memory impairment (Kraguljac et al. 2013). Recent work in longitudinal cohorts indicates that functional change is present prior to illness onset in patients presenting at elevated genetic (Baig et al. 2010; Whitfield-Gabrieli et al. 2009; Whyte et al. 2006; Whalley et al. 2004, 2006) and clinical risk (Marjoram et al. 2006; Smieskova et al. 2012; Fusar-Poli et al. 2010).

To date a single paper has employed functional MRI scanning in a predictive group classification capacity. Whalley et al. (2006) demonstrated multiple functional deficits in a familial high-risk population. Using the Hayling Sentence Completion Test during fMRI, the team were able to discriminate between those patients at high risk that stayed well and those who subsequently developed schizophrenia. Notably the team recorded increased activity in the parietal lobe as well as decreased activity in the lingual gyrus and bilateral temporal lobes with increasing test complexity in subjects who subsequently transitioned to ICD-10 schizophrenia.

The overall model achieved a ROC area under the curve of 0.99 with positive and negative predictive values of 0.80 and 1.00 through the combination of parietal lobe ROI data and lingual gyrus ROI. The findings were in line with previous studies from the same study (the EHRS) showing parietal overactivation in those at familial high risk experiencing isolated psychotic symptoms (Whalley et al. 2004). Despite the low rates of transition observed after an fMRI scan (6%), the discriminative power of the final within-study model is excellent and serves to highlight the potential value of additional measures over sMRI in isolation; however replication within other data sets is required in order to discern the true predictive power of the model (Whalley et al. 2006).

Subsequent studies employing various fMRI paradigms in the same high-risk cohort identified increased prefrontal activation in high-risk subjects over controls, but the data could not be utilised to predict subsequent schizophrenia as too few participants became unwell after the scan (Marjoram et al. 2006). These findings suggesting wider functional changes were further supported by a 2008 study which demonstrated functional activations in the left middle temporal gyrus that related to the emergence of psychotic symptoms in high-risk groups (Whalley et al. 2008).

Despite the relative lack of research assessing the predictive validity of fMRI within high-risk groups (Smieskova et al. 2010), there is a lot of suggestive evidence that the application of functional imaging techniques has the capability of adding to diagnostic and prognostic approaches. There is however a need for further longitudinal functional imaging studies within other cohorts, particularly in light of observed correlations with symptom profiles and clinical outcomes.

11 Connectomic Studies

Brain networks are disorganised in patients with a diagnosis of schizophrenia when compared to healthy controls (Palaniyappan 2012; Heuvel et al. 2010; Bassett et al. 2008). As such there is reason to believe that analysis of brain networks and their connectivity may offer a potential route to differentiating those who will develop psychotic disorder from those who will not. However connectivity studies have, as yet, not been successfully applied within a class prediction paradigm. In a 2014 paper utilising connectivity measures, observed differences in network structure accounted for 81% of the variance in schizotypal cognitions in first-episode populations and relatives (derived from the EHRS), whereas sMRI only accounted for 48% (Tijms et al. 2015). This finding was however unable to differentiate between subjects who subsequently transitioned to schizophrenia and those who did not. Nonetheless the finding suggests abnormalities in functional network analysis may indeed play a role in future group identification studies; this however requires replication and further investigation within similar high-risk cohorts and external controls.

12 Clinically Relevant Prediction with sMRI Models

Longitudinal cohorts have provided a wealth of data supporting the hypothesis that grey matter abnormalities are present prior to the onset of illness, more marked in those who will transition to psychotic disorder and evolve over the course of transition to full-blown schizophrenia. There are no however individual neuroanatomical abnormalities that, viewed in isolation, can reliably predict future diagnosis or prognosis on the single subject level within high-risk groups (Phillips 2012).

As such, neuroimaging data whether analysed utilising ROI, VBM or similar techniques is insufficient to predict outcome on a single-subject level without more sophisticated analysis. Early models employed parametric and non-parametric statistical techniques (see Pantelis et al. 2003; Job et al. 2006; Mechelli et al. 2011) to assess variance between high-risk individuals who transitioned to illness and those who remained well and thus designate group allocation on the basis of observed patterns in grey matter change. However, the application of various statistical approaches able to differentiate between groups has advanced in recent years with emergence of machine learning protocols (Yang et al. 2010; Shimizu et al. 2015; Yu et al. 2016). As such between group analysis, employing traditional statistical approaches has largely been superseded with more recent papers tending to employ the use of support vector machines and related pattern classification approaches.

The application of support vector machines (SVMs) employing supervised machine learning algorithms (Burges 1998; Noble 2006) to data sets has resulted in improved accuracy of classification as these methods allow for prediction of individual outcomes in the context of complex pattern recognition (Zarogianni et al. 2013, 2017a; Klöppel et al. 2012). The utility of SVMs in allocating psychiatric diagnosis and treatment response has been demonstrated in studies investigating their clinical utility in mental disorders such as Alzheimer's disease, mild cognitive impairment and depression (Fu et al. 2008; Fan et al. 2008). Given the complex patterns of grey matter abnormalities observed within high-risk populations, SVMs' ability to allocate group (diagnostic) classifications on an individual level and the demonstration of accurate group classification within other disorders, it is unsurprising that they have been readily adopted and effectively employed in predictive diagnosis research. In psychosis research, however, they are yet to be applied to predicting treatment response or outcome based upon diagnosis.

13 Diagnostic Criteria

Prior to discussion focussing to the overall diagnostic accuracy of the models employed, it is worth commenting on the heterogeneous diagnoses of psychotic disorder that have arisen in high-risk studies, illustrated in Table 3. Although all studies employ the use of validated and well-recognised diagnostic tools, there are some significant differences in how "psychosis", "psychotic disorder" or

Table 3 Diagnostic approach and operational tools employed

Paper	Cohort	HR criteria	Diagnostic tool	Transition rates		Transition definition	Diagnosis breakdown
Pantelis et al. (2003)	PACE	PACE-ARMS	SCID	23/75 (31%)		DSM-IV psychotic disorder	*SCID, DSM-IV* Schizophrenia – 8 Schizoaffective disorder – 2 Brief psychotic episode – 1 Psychosis NOS – 1 Bipolar affective disorder with psychotic symptoms – 6 Major depression with mood incongruent psychotic disorder – 4 Psychosis with affective features – 1
Job et al. (2006)	EHRS	EHRS (FHR)	PSE	8/68 (12%)		ICD-10 schizophrenia	*ICD-10* Schizophrenia – 8
Koutsouleris et al. (2009)	FETZ	BSABS PACE-ARMS	ICD-10 diagnosis	17/45 (38%)		ICD-10 schizophrenia spectrum disorder	*ICD-10* Schizophrenia – 10 Schizoaffective disorder – 4 Schizotypal personality disorder – 1
Mechelli et al. (2011)	FETZ, FePSY, PACE, OASIS	PACE-ARMS	Not defined	48/182 (26%)		Varies depending on cohort	Not specified
Koutsouleris et al. (2012a, b)	FePSY	BSIP-ARMS	PACE-ARMS (Yung et al. 1998)	16/37 (43%)		PACE-ARMS transition	Not specified
Borgwardt et al. (2013)	FePSY	BSIP-ARMS	PACE-ARMS (Yung et al. 1998)	N/A		PACE-ARMS transition	Not specified
Koutsouleris et al. (2014)	FePSY	BSIP-ARMS	BPRS PACE-ARMS (Yung et al. 1998)	43.2%	Cumulative 33/73 (45%) (CI 33.5–6.9%)	OPCRIT	*OPCRIT* Schizophrenia – 22 Schizoaffective disorder – 5 Data unavailable – 6
	FETZ	PACE ARMS + BSABS	PANSS PACE-ARMS (Yung et al. 1998)	47.2%		DSM-IV	
Zarogianni et al. (2017a)	EHRS	EHRS (Johnstone et al. 2000)	PSE	N/A		ICD-10 schizophrenia	N/A
Zarogianni et al. (2017b)	FePSY	BSIP-ARMS	PACE-ARMS (Yung et al. 1998)	16/35 (45.7%)		PACE-ARMS transition	Not specified

Personal Assessment and Crisis Evaluation defined at-risk mental state (PACE-ARMS), Structured Clinical Interview for DSM-IV (SCID), Edinburgh High-Risk Study (EHRS), Present State Examination (PSE), Bonn Scale for Assessment of Basic Symptoms (BSABS), Früherkennungs- und Therapiezentrum (FETZ, Early Detection and Intervention Centre, Ludwig Maximillian University, Munich), Outreach and Support in South London (OASIS), Fruherkennung von Psychosen (FePsy), Basel Screening Instrument for Psychosis defined at-risk mental state (BSIP-ARMS), Operational Criteria Checklist for Psychotic and Affective Illness (OPCRIT), Brief Psychiatric Rating Scale (BPRS), Positive and Negative Symptoms Scale (PANSS)

"schizophrenia" are defined. The EHRS papers employ the use of ICD-10 (International Classification of Diseases – 10th edition) (Organization W 1992) schizophrenia as the outcome of interest, diagnosed through the use of PSE (Present State Examination) (Wing et al. 2012). The Basel group employed both ICD-10 and DSM-IV (Diagnostic and Statistical Manual of Mental Disorder-IV) (American Psychiatric Association 1994) via the OPCRIT (Operational Criteria Checklist for Psychotic Illness and Affective Illness) (Bergman et al. 2014). Pantelis et al. (2003) utilised the SCID (Structured Clinical Interview for DSM-IV) (DATA D 1997) as a diagnostic tool and measured psychopathology using the BPRS (Brief Psychiatric Rating Scale) (Overall and Gorham 1962) and SANS (Scale for the Assessment of Negative Symptoms) (Andreasen 1989).

It is worth bearing this in mind as although broadly very similar there are subtle differences in the diagnostic approach adopted between research groups, and as such the outcome in question, transition to disorder, is defined in slightly different ways. In particular, the Melbourne and Basel studies tend to include patients with brief psychotic disorders and delusional disorder in their transition groups. This is not to be seen as a major limitation in the studies discussed; as mentioned all have employed well-validated and recognised diagnostic tools, although the therapeutic and prognostic implications of these different diagnoses is clearly variable. It is also a reflection that we continue to struggle to define clear syndromes in the absence of quantitative biomarkers. A more detailed discussion regarding the merits of each diagnostic approach is beyond the scope of this chapter; however for an overview, please see Sheehan et al. (1998) and Yung and McGorry (1996).

14 Accuracy

As illustrated in Table 4, the balanced accuracy (BAC) of current models ranges from 67% to 94%. This is largely independent of cohort, imaging modality, transition rates and statistical analysis techniques. Although applied within a research setting, all models apart from one relied solely upon imaging as the method of identifying patients who would transition to psychotic disorder. The strengths of using neuroimaging in isolation are multiple; it lends objectivity to the diagnostic process, reduces reliance on highly specialised healthcare services (contrast this with the relative ubiquity of imaging in industrialised healthcare systems; Taylor et al. 2012; White et al. 2002) and decreases the use of unstable and fluctuant clinical presentations to stratify risk. There is also the opportunity to expand the use of imaging into non-help-seeking individuals who would otherwise not meet high-risk criteria. Of note, recently published research has, for the first time, validated a model using data from one high risk cohort in another unrelated cohort utilising different ascertainment methods (genetic high risk vs clinical high risk). Zarogianni et al. (2017b) employed a diagnostic model derived from data obtained from the Edinburgh High-Risk Study in a cohort of patients from the Basel early detection of psychosis study. Although overall accuracy was lower (Table 4), it demonstrates the

Table 4 Classification performance

Paper	Method of statistical analysis		Sensitivity (%)	Specificity (%)	Balanced accuracy (%)	FPR (%)	PPV (%)	NPV (%)	LR+	LR−	OR
Job et al. (2006)	Univariate statistical analysis		38	96	–	–	60	92	10.69	0.65	–
Koutsouleris et al. (2009)	SVM		83	80	82	20	83	80	–	–	–
Mechelli et al. (2011)	Univariate statistical analysis		68	66	67	–	–	–	–	–	–
Koutsouleris et al. (2012a, b)	SVM	Basel	81	87.5	84.2	19.1	77.8	89.5	6.5	0.2	–
		FETZ	83	80	82	20	–	–	4.3	–	–
Borgwardt et al. (2013)	SVM	HC vs ARMS-T	75	86	80.7	25	82	80	–	–	–
		ARMS-T vs FE	91.3	68.8	80	8.7	84.6	80.8	–	–	–
Koutsouleris et al. (2014)	SVM		75.8	85	80.4	15	80.6	81	5.1	0.29	17.7
Zarogianni et al. (2017a)	SVM	Neuroimaging	76	100	88	0	100	80.9	–	–	–
		+ clinical RISC	100	88	94	2	89.4	100	–	–	–
Zarogianni et al. (2017b)	SVM		62.5	84.2	74.2		77	73	3.9	0.45	–

Likelihood ratio positive (LR+) (sensitivity/1 − specificity), likelihood ratio negative (LR−) (1 − sensitivity/specificity), positive/negative predictive value (PPV/NPV), odds ratio (OR), support vector machine (SVM), healthy control (HC), at-risk mental state-transition (ARMS-T), first episode (FE)

versatility of sMRI classification within high-risk groups and their potential application across patients presenting with different risk factors (Zarogianni et al. 2017b).

Imaging as discussed may provide a route towards the development of diagnostically useful biomarkers. The demonstration of accurate prediction algorithms in both clinical high-risk (Koutsouleris et al. 2012b, 2014) and familial high-risk cohorts (Zarogianni et al. 2017a; Job et al. 2006) certainly serves as proof of concept within high-risk cohorts. At this stage it is however not possible to generalise beyond the confines of such research cohorts. Despite the relative effectiveness of models employing sMRI as a predictive tool in isolation, accuracy has been demonstrably improved in at least one study with the inclusion of an additional clinical variable.

15 Inclusion of Additional Measures

Most of the papers discussed have confined analysis to imaging measures alone. The exception (Zarogianni et al. 2017a) includes the additional measures of schizotypal features as well as verbal memory and learning. Based on a relatively recent and developing body of work indicating the validity of sMRI used in conjunction with various clinical and genetic measures within a machine learning paradigm (Pettersson-Yeo et al. 2013; Karageorgiou et al. 2011), the team demonstrated excellent discriminative ability to correctly assign patients to appropriate diagnostic categories when using neuroimaging alone (BAC 88%) or in combination with clinical measures (BAC 94%).

The inclusion of multiple measures has echoes of more clinically focussed multivariate risk predictive algorithms (Thompson et al. 2011; Cannon et al. 2016; Dragt et al. 2011; Ruhrmann et al. 2010; Eack et al. 2008) indicating that a combined approach of selected clinical measures in conjunction with neuroimaging may provide the most reliable method of predicting onward transition to psychotic disorder. It is clear however that the development of clinically applicable models requires further research to ascertain the most reliable clinical predictors across cohorts and outcome groups.

Although not the focus of this chapter, it is worth commenting on the development and application of clinically and demographically focussed multivariate risk prediction calculators within psychosis research. In parallel to the development of imaging-based predictors, the last 15 years has witnessed the emergence of a body of multivariate risk prediction algorithms that have been developed with the goal of predicting onward transition to psychotic disorder and thus stratifying care delivery.

Often applied within similar high-risk cohorts, such predictive models usually rely upon the use of varied neuropsychological tests combined with family history, clinical presentation and demographic factors (Ruhrmann et al. 2010; Cannon et al. 2008; Shah et al. 2012). Although delivering promising results, they are limited in application out with research centres due to the specialist training required to appropriately administer the tests and the time taken (Koutsouleris et al. 2009). Even when applied as a battery with multiple additional measures, they struggle to achieve the

accuracy demonstrated in neuroimaging studies. The reliance on symptom scores and neuropsychological testing in development of these calculators is a direct reflection of the paucity of clinically meaningful biomarkers within psychiatry (Prata et al. 2014).

There is marked heterogeneity across studies both in approach and in results. It is perhaps however worth recognising that many of the calculators that have been developed have achieved excellent accuracy; most recently Cannon et al. (2016) achieved a C-index of 0.71, a result comparable to commonly utilised cardiovascular risk calculators. Whether employing a complex mixture of clinical and demographic variables or focussing solely on biomarkers, the accuracy achieved is comparable to that of many of the diagnostic tools most commonly employed within other medical specialties (Lee et al. 1999; Kattan et al. 2013; Pfeiffer et al. 2013). Despite this the translation of diagnostic tools from research setting to clinical practice has not yet been achieved; there are numerous factors that likely contribute to this. The application of complex neuropsychological testing, the limited availability of high-risk services and the ethical considerations (Haroun et al. 2006; Corcoran et al. 2005) are all relevant when considering why diagnostic risk calculators have failed to make the transition to clinical practice. Ultimately the move from the lab to the clinic will take place gradually as the evidence base, accuracy and clinical utility of diagnostic prediction tools improve. It is not inevitable that this transition will happen, but given the advances that are continually being made and our increasing confidence in results, it seems more likely now than a decade ago.

16 Genetic Predictors of Transition to Schizophrenia

Schizophrenia is highly heritable (Gottesman 1991; Gottesman and Erlenmeyer-Kimling 2001). It is estimated that 80–85% of variance observed in the population is accounted for by genetic factors (Sullivan et al. 2003; Cardno and Gottesman 2000). The disorder does not however follow a simple pattern of Mendelian inheritance; the observed genetic risk is accounted for by the interaction of many single nucleotide polymorphisms (SNPs) resulting in a cumulative risk, i.e. it is polygenic (Lee et al. 2012; Ripke et al. 2014). In recent years genome-wide association studies have increasingly informed our understanding of the complex genetic architecture of mental disorders such as schizophrenia, bipolar and depression (Ripke et al. 2014; Purcell et al. 2007; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium et al. 2012; Davies et al. 2016; Mühleisen et al. 2014). As the understanding of the genetic makeup of the disorder develops, the emergence of techniques capable of quantifying genetic risk has provided researchers with objective tools capable of providing increasingly detailed information that may inform diagnostic and prognostic approaches within high-risk groups.

At the present time, there are numerous genetic loci that contribute to the development of schizophrenia at a population level (Ripke et al. 2014). As such, in the context of a complex and heterogeneous disorder, each SNP taken in isolation lacks predictive power to contribute to overall state prediction (So et al. 2011; Maier et al.

2015; International Schizophrenia Consortium et al. 2009). The development of polygenic risk scores (PGRS) offers a quantitative method for measuring cumulative genetic risk generated through the combination of numerous SNPs (International Schizophrenia Consortium et al. 2009; Dudbridge 2013; Vassos et al. 2017). Importantly, when applied to schizophrenia, the predictive power of this technique is increasing rapidly; from being able to account for only 3% of variance in 2009 (International Schizophrenia Consortium et al. 2009), it has risen to 18% as of 2014 (Ripke et al. 2014). Given the increasing power of PGRS, it is likely that the inclusion of genetic data would contribute towards models predicting transition to psychotic disorders. There are however only a small handful of models that have attempted this approach thus far.

Although a developing field, current attempts to utilise PGRS in group classification have generated some promising early results. Utilising the EHRS cohort data, Neilson et al. (2017) tested the discriminative ability of a PGRS-SCZ (polygenic risk score for schizophrenia), derived from the most recent genome-wide association data obtained from the Psychiatric Genetics Consortium-2 (PGC-2) (Ripke et al. 2014), to predict onward transition to ICD-10 schizophrenia in a cohort of patients at genetic high risk.

The team demonstrated that high-risk subjects presenting with active symptoms had statistically significant increases in PGRS-SCZ when compared to controls and that elevated scores were associated with transition to schizophrenia. A significant effect was observed of PGRS-SCZ on prefrontal gyrification, with increased cortical folding or "hyper-gyrification" bilaterally within the frontal lobes linked to increased PGRS-SCZ. This indicates a possible relationship between increased genetic load and gyrification patterns within the prefrontal lobes which may contribute to the pathogenesis of schizophrenia and serve as a biomarker in future work (Neilson et al. 2017). Frontal hyper-gyrification has been previously demonstrated in populations with established illness as well as high-risk cohorts and unaffected family members and has been associated with measures of genetic loading and treatment outcome (Falkai et al. 2007; Palaniyappan et al. 2011, 2013). However, there is no clear consensus, and an alternative body of evidence suggests that patients with established schizophrenia have decreased gyral folding (Sallet et al. 2003; McIntosh et al. 2009). Nevertheless, the inclusion of measures of gyrification of the prefrontal lobes is novel in the context of a diagnostic study and serves to effectively illustrate the complementary nature of combining genetic risk scores with neuroimaging data.

The combination of imaging and genetic data was explored in a 2010 paper in which Yang et al. demonstrated the application of a support vector machine framework to differentiate between healthy controls and patients with established illness. Although not a predictive study, given the cross-sectional design, it provides an insight into the power of combining genetic and imaging data as being more effective than either biomarker alone. The overall model accuracy of 87% when combining imaging and genetic variables is impressive and serves to highlight the value of a multimodal approach to diagnostic classification that could prove effective if transferred to high-risk cohorts (Yang et al. 2010).

Further use of PGRS to allocate group classification has been recently demonstrated by Vassos et al. (2017). Again, utilising data derived from the PGC-2 consortium to calculate a PGRS-SCZ, in a cohort of patients presenting during FEP in South London, the group was able to account for 3.6% of the overall variance observed in the sample. This however varied depending on ethnic group. Explaining as much as 9.4% of the variance in individuals of European ancestry, it was however not predictive of group classification in individuals of African ancestry (Vassos et al. 2017). The team demonstrated that PGRS was predictive of diagnosed schizophrenia but not other psychosis in individuals of European ancestry. They also demonstrated similar results in patients with established illness but unexpectedly found that the discriminative accuracy of a PGRS method was less so in this group. The paper, despite promising findings, concludes that there is a large body of work that remains to be explored, using larger cohorts and more diverse ethnic samples (Vassos et al. 2017).

The utility of PGRS is as yet not fully realised. More information regarding the genetic risk profile of psychotic disorders will be discovered. It is however unlikely that genetic testing alone will achieve the required power to contribute meaningfully to diagnosis and prognosis (Janssens et al. 2006). Nonetheless, there is a growing body of evidence that indicates, if appropriate samples are included, that PGRS can contribute to diagnosis and group allocation in a meaningful way (So et al. 2011; Dudbridge 2013; Hartz et al. 2017). Ultimately larger cohorts providing greater diversity will allow for a more nuanced approach to PGRS, greater generalisability and perhaps, eventually, in combination with clinical and neuroimaging measures translation into clinical practice.

17 Conclusion

The discovery and application of diagnostic and prognostic biomarkers in medicine has transformed practice in the last half century (Mayeux 2004; Ludwig and Weinstein 2005; Jaffe et al. 2006). There are however no clinically useful biomarkers employed in routine psychiatric care (Venkatasubramanian and Keshavan 2016). This is not to say that the pursuit of viable biomarkers within psychiatric research is by any means a recent endeavour (Boksa 2013; Pue et al. 1969; Buck et al. 1955). Indeed, it is well recognised that biomarkers capable of aiding diagnosis, predicting transition to illness and mapping prognosis have the potential to revolutionise care and practice (Venkatasubramanian and Keshavan 2016). The emergence of multiple models focussing on the application of serum (Jha et al. 2017; Perkins et al. 2015), imaging (Chen et al. 2011; Orrù et al. 2012; Phillips and Vieta 2007) and genetic (Sokolowska et al. 2015) biomarkers speaks to the wealth of research and investment that is currently being directed towards this end.

Since the early 1990s, schizophrenia research and care has been largely characterised by two complementary pathways. The high-risk paradigm has given rise to cohorts of patients at increased risk of transitioning to psychosis, while the development of early intervention services has provided a viable and generally acceptable

vehicle for delivering care to high-risk groups. Despite advances in overall service delivery, clinicians continue to struggle to predict individuals who will transition to disorder and thus require the greatest degree of intervention and care. One proposed route towards achieving early diagnosis and improved prognostication has been the development of multivariate risk prediction algorithms, whether utilising clinical and demographic data or purely based on objective and quantifiable biomarkers the approach has met with mixed but promising results.

The development of multiple models theoretically capable of predicting transition to psychosis within high-risk groups has at least been achieved within research settings. Although overall model accuracy varies and approaches across research teams are heterogeneous, their development is in itself a promising step towards proactive, diagnosis, intervention and care in the early stages of schizophrenia. Proponents argue, supported by a wealth of data, that early intervention, decreased DUP and proactive care in those who later transition to schizophrenia improve outcomes, patient experience and engagement with services. The application of imaging and genetic biomarkers capable of assisting clinicians in the complex task of making diagnoses has the potential to add a powerful tool to the clinical armamentarium.

A wealth of data gathered from longitudinal follow-up studies has informed our understanding of the dynamic nature of brain changes that occur during the transition from health to established psychotic disorder. Decreases in grey matter volume that are present in high-risk individuals and family members prior to the onset of illness evolve over time and are predictive of future transition to diagnosed schizophrenia which offers a viable and potentially powerful method of predicting individuals who will subsequently become ill and may in turn require the greatest degree of support and care. Ultimately their value is contingent upon their potential to offer real benefits to patients and healthcare systems. Although confined to the research settings and lacking in clinical application, the field has developed substantially over the last 15 years. While the translation of findings into clinical practice is not currently viable, as our understanding of dynamic grey matter change increases, there is the potential that in the future routine, neuroimaging may indeed assist clinicians in making complex diagnostic decisions, planning treatment and offering greater clarity when discussing prognosis.

Although less well established, genetic biomarkers capable of predicting the transition to psychosis have been demonstrated to compliment the use of imaging and aid in differentiating between individuals who transition to illness and those who do not. Of particular interest is the rate at which the field of genetics is developing. Increasingly powered genome-wide association studies are shedding light on the genetic makeup of disorders as complex as schizophrenia. Recent advances are increasingly capable of accounting for the variance observed in the population, and the application of the work produced by large consortia to local data has been demonstrated to improve differentiation between schizophrenia and other psychoses as well as predict those who will transition to psychotic disorder in high-risk groups.

The findings from imaging studies and from recent studies focussing on the use of quantifiable genetic risk scores will continue to inform the developing field of diagnostic biomarkers within psychiatry. The application of modern machine learning

techniques and imaging protocols in the context of ever-increasing computational power offer novel and exciting approaches to the development of tools that will in our view likely, one day, offer clinicians a viable and acceptable method of assisting in complex diagnostic and treatment decisions. Although there are challenges inherent in translating current research from the lab to clinical practice, the potential benefits that early diagnosis and treatment can offer individuals who present with psychotic disorders are great, and in this regard it is hoped that future research, building upon our current and developing understanding, will offer tools that can improve outcomes and care for patients experiencing the emergence of schizophrenia at a critical time point in the course of the disorder.

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Immunological Processes in Schizophrenia Pathology: Potential Biomarkers?

Tina Notter

Contents

Abstract Accumulating evidence suggests that the pathophysiology or schizophrenia involves alterations in immune functions, both peripherally and centrally. Immunopsychiatric research has provided a number of candidate biomarkers that could aid estimating the risk of developing schizophrenia and/or predicting its clinical course or outcomes. This chapter summarizes the findings of immune dysfunctions along the clinical course of schizophrenia and discusses their potential value as predictive, trait or state biomarkers. Given the convergence of findings deriving from immunology, epidemiology, and genetics, the possibility of identifying immune-based biomarkers of schizophrenia seems realistic. Despite these promises, however,

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Curr Topics Behav Neurosci (2018) 40: 389–410
DOI 10.1007/7854_2018_43
Published Online: 6 April 2018

the field has realized that immune dysfunctions in schizophrenia may be as heterogeneous as the disorder itself. While challenging for psychiatric nosology, this heterogeneity offers the opportunity to define patient subgroups based on the presence or absence of distinct immune dysfunctions. This stratification may be clinically relevant for schizophrenic patients as it may help establishing personalized add-on therapies or preventive interventions with immunomodulating drugs.

Keywords Biomarkers · Cytokines · Immune mediators · Immune system · Inflammation · Schizophrenia

1 Introduction

A possible association between immune system dysfunctions and schizophrenia was postulated over a century ago (Kraepelin 1890; Menninger 1919) and has attracted the attention of clinicians and basic scientists alike ever since. With the reconceptualization of "immune privilege" in the central nervous system (CNS), research investigating this link has risen steadily in the last decades. Advances in the fields of immunology and genetics, as well as the increasing understanding of how immunological processes can influence brain development and functions (Reemst et al. 2016; Thion and Garel 2017), have also contributed to the growing recognition of immune system dysfunctions in schizophrenia. Immunological abnormalities are evident across the entire clinical course of schizophrenia, ranging from subjects at high risk of developing the disorder to patients with chronic schizophrenia.

Although there is quite substantial discrepancy between individual findings, genetic, epidemiological, and preclinical studies, all suggest that aberrant immunological processes could play a key role in the pathogenesis of schizophrenia. Firstly, genetic variations that confer increased risk of developing schizophrenia include a number of genes that play important roles in immune functioning and neurodevelopment (Consortium 2014; Shi et al. 2009). For example, a recent study by *Sekar* et al. revealed an association between common allelic variations in the complement component 4 gene and schizophrenia risk (Sekar et al. 2016). This gene encodes for the complement C4 protein, which is known to be relevant for complement- and microglia-dependent synaptic pruning (Presumey et al. 2017). The findings provided by *Sekar* et al. thus suggests that there are plausible biological mechanisms by which variations in immune-related genes could affect brain development during critical developmental periods and increase the risk for schizophrenia (Sekar et al. 2016). Secondly, epidemiological studies over the past decades have repeatedly implicated prenatal environmental factors, including maternal immune activation (MIA), in the etiology of schizophrenia and related disorder's (Brown and Derkits 2010). Maternal infectious pathogens and inflammation are plausible risk factors for these outcomes (Brown and Derkits 2010; Estes and McAllister 2016) and can interact synergistically with genetic or other environmental risk factors for schizophrenia (Abazyan et al. 2010; Clarke et al. 2009; Debost et al. 2017; Giovanoli et al. 2013). Thirdly, population-based studies found severe postnatal infections and autoimmune disorders

to be associated with the risk of schizophrenia, and that these associations appeared to be bidirectional (Benros et al. 2014; Nielsen et al. 2014). Fourthly, there is substantial evidence that at least a subset of schizophrenic patients display overt peripheral and central changes in inflammatory markers, immune cell numbers, and antibody titers (Kirkpatrick and Miller 2013; Miller and Goldsmith 2017). Finally, several clinical trials revealed superior beneficial treatment effects in a subset of patients when standard antipsychotic drugs were co-administered with anti-inflammatory compounds, as compared with treatment outcomes using antipsychotic drugs alone (Keller et al. 2013; Meyer et al. 2011; Muller et al. 2002; Nitta et al. 2013; Sommer et al. 2014). Intriguingly, baseline blood cytokine levels predicted the responses to such adjunctive anti-inflammatory treatments (Laan et al. 2010; Muller et al. 2004), supporting the idea that immune dysfunctions could influence the clinical course and outcomes of some schizophrenia cases. Thus, the stratification of patients based on immune-related biomarkers could aid the development of novel approaches for personalized therapies. The identification of immune changes during presymptomatic stages may also open new avenues for the development of possible immune-based preventive and/or early interventional strategies.

The main objective of this chapter is to review immunological dysfunctions that appear in schizophrenia and discuss their potential use as predictive, trait or state biomarkers. The first two sections provide an overview of the major components of the immune system and of the historical perspectives on immunological abnormalities in schizophrenia. Key findings of altered immunological processes across the clinical course of schizophrenia are then summarized, thereby discussing whether distinct immune changes could be used to serve as state and trait biomarkers. The last sections focus on aberrant immune processes that can be triggered by genetic and environmental risk factors, and how they might serve as potential predictive biomarkers.

2 A Brief Overview of Immune System Components

The main function of the immune system is to protect an organism from intrinsic (e.g., tissue damage or physiological stressors) or exogenous toxins such as infectious pathogens. Depending on the nature of a potentially harmful stimulus, distinct components of the immune system initiate a highly complex and coordinated immune response to assure efficient removal of the insult without inducing collateral damage to noninfected, healthy tissue (Ahmed 2011; Parkin and Cohen 2001). This process involves activation and recruitment of immune cells, vasodilatation and increased vascular permeability, production and secretion of inflammatory mediators such as pro-inflammatory cytokines and chemokines, acute phase proteins such as C-reactive protein (CRP), complement proteins, antibodies, and reactive oxygen and nitrogen species.

In higher animals, the immune system can be broadly classified into innate and adaptive immunity. These two branches are, however, highly interconnected and dependent on each other (Iwasaki and Medzhitov 2010, 2015). Innate immunity

provides a first and relatively nonspecific protection and is not dependent on a previous encounter with a pathogen. The major components of the innate immune system are biological barriers (e.g., endothelium, epidermis, and blood–brain barrier), monocytes/macrophages, dendritic cells, natural killer cells, and circulating plasma proteins. The innate immune response is the immediate reaction towards invading pathogens or compromised cells, which are often recognized by a family of receptors known as pathogen recognition receptors (Mogensen 2009). It relies on a restricted set of effectors that include expression and secretion of pro- and anti-inflammatory cytokines (interleukins (IL), interferons (IFN), colony stimulating factors, tumor necrosis factors (TNF), and various chemokines), other inflammatory mediators such as reactive oxygen and nitrogen species, the complement system, and removal of pathogens or cell debris by phagocytes (e.g., macrophages and dendritic cells) (Beutler 2004; Warrington et al. 2011). The adaptive immune system, on the other hand, is more specific and depends on the encounter and recognition of a specific antigen (Warrington et al. 2011). Contrary to the innate immune system, adaptive immunity can form remarkable immunological memory, which in turn promotes faster and more efficient immunity against a previously encountered pathogen upon second exposure. The cellular components of the adaptive immune system are B- and T-lymphocytes, which are responsible for humoral (antibody-mediated) and cellular immunity, respectively. Activation of B-cells – which in contrast to T-cells is independent of antigen presenting cells (APCs) – leads to the production and secretion of antigen-specific antibodies and the formation of memory B-cells. Upon encounter of an APC, activation of T-cells involves their differentiation into T-helper cells (Th_1, Th_2, Th_{17}, and Th_{reg}) or cytotoxic T-cells. Cytotoxic T-cells are primarily involved in the destruction of cells infected by foreign agents and pathogens. T-helper cells, on the other hand, play an important role in establishing, maximizing, and controlling the immune response by helping the activation of B-cells to secrete antibodies, and activated cytotoxic T-cells to kill infected cells, as well as supporting macrophages. These cells have no cytotoxic or phagocytic activity per se and cannot kill infected cells or clear pathogens (Warrington et al. 2011).

3 Historical Perspectives on Immune Dysfunctions in Schizophrenia

The idea that aberrant immune responses may be involved in some psychotic disorders has been the matter of discussions for more than 120 years. The French psychiatrist Jean-Etienne Esquirol is attributed to be among the first to have set the basis on the association between infectious diseases and psychoses. In his book "Mental Maladies; a Treatise on Insanity" (originally published in French in 1838), he described the epidemic appearance and character of some psychotic illnesses without specifying a pathogenic cause (Esquirol 1845). However, a direct

link between infections and psychoses was only established 45 years later by the German psychiatrist Emil Kraepelin. During the German influenza epidemic in 1889, he published his observational study "Ueber Psychosen nach Influenza" ("About Psychoses after Influenza"), where he described 11 cases who suffered from various psychotic syndromes immediately after recovering from influenza infection (Kraepelin 1890). Similarly to Kraepelin, the American psychiatrist Karl A. Menninger documented schizophrenia-like psychoses after the 1918 influenza epidemic in the USA (Menninger 1919, 1926, 1928). In his early study, Menninger observed and documented the clinical course of 80 patients admitted to the Boston Psychopathic Hospital suffering from influenza associated "mental disturbances." Out of these, there were 25 cases who were diagnosed with dementia praecox, 16 with delirium, and 23 with other forms of psychosis (Menninger 1919). Based on his follow-up studies, where he found a full recovery of approximately 2/3 of cases diagnosed with influenza-induced schizophrenia syndromes (Menninger 1926), he concluded that infectious diseases could represent one of the etiological factors relevant for a subset of schizophrenic illnesses (Menninger 1928).

Observational studies during epidemic typhus outbreaks in the second half of the nineteenth century also provided early evidence for the involvement of the immune system in psychotic disorders. Several asylums reported that psychotic patients showed an increased resistance towards infectious diseases in comparison to the guards. Furthermore, it was observed that people with psychosis, who were infected with typhus, showed marked improvements in their psychiatric symptoms upon recovery from the typhus infection (Wagner-Jauregg 1887). These early observations formed the basis of Wagner-Jauregg's "vaccination therapy" for psychotic patients, which represented the first immune-based therapeutic approach in psychiatry (Wagner-Jauregg 1926).

The first observational studies describing increased white blood cells in psychotic patients appeared as early as in 1903 (Bruce and Peebles 1903, 1904). Although total cell counts are crude measures that can be influenced by confounding factors such as stress and dehydration, these reports provided the first findings of altered cellular immunity in psychotic disorders. Likewise, the hypothesis surrounding the involvement of abnormal autoimmune processes in schizophrenia has its origin in the early twentieth century. The formulation of this hypothesis temporarily coincided with the development of a novel technique, which enabled researchers to detect brain-lipoid antibodies in the cerebrospinal fluid (CSF) (Lehmann-Facius 1937). The main rationale behind this technique, which was named after its inventor, Lehmann-Facius, was driven by the prevailing idea that patients with schizophrenia may develop autoantibodies against brain tissue, which in turn may initiate destructive processes in the CNS. The early work of Lehmann-Facius represented the starting point of the autoimmune hypothesis of schizophrenia, which still attracts considerable interests in the scientific community (Al-Diwani et al. 2017; Kirch 1993).

The discovery of the neuroleptic effects of chlorpromazine in the 1950s (Hamon et al. 1952), however, has rapidly shifted schizophrenia research from abnormal immunity to altered catecholaminergic neurotransmission. After they had largely sunken into oblivion, the various hypotheses postulating an involvement

of immune processes in schizophrenia have been reappraised in the 1970s through the work of Torrey and Peterson (1973), who suggested that latent viruses might be involved in the disorder's etiopathogenesis (Torrey and Peterson 1973). As discussed below, the field has since greatly expanded, and various infectious agents are now being considered to play an etiopathological role in schizophrenia and related disorders.

4 Overview of Immunological Dysfunctions in Schizophrenia

In schizophrenia, signs of abnormal immunological processes can be detected in various peripheral and central tissues, including blood, CSF, and brain parenchyma (Miller and Goldsmith 2017). These involve alterations in immune cell number and activity, abnormal expression of inflammatory mediators and acute phase proteins, and altered antibody titers. Some of these markers have been shown to vary according to the clinical status of patients, suggesting that certain immune-related biomarkers may be categorized into state and trait markers (Miller et al. 2011). Others may be more directly related to, or even induced by, chronic exposure to antipsychotic drugs (Al-Amin et al. 2013; Chen et al. 2011, 2013; Cotel et al. 2015), thus representing medication effects. It should be noted, however, that various immunological abnormalities have been described in antipsychotic-naïve, first episode patients (FEP), suggesting that at least some of the described associations may be independent of antipsychotics (Ezeoke et al. 2013; Fernandes et al. 2016; Goldsmith et al. 2016; Miller et al. 2011; Upthegrove et al. 2014).

4.1 Number of Peripheral and Central Immune Cells: Possible State and Trait Markers?

Various studies describe changes of peripheral lymphocyte numbers in schizophrenic patients relative to healthy controls. A meta-analysis including 16 studies investigating blood lymphocytes found increased percentages of $CD4^+$ cells (T-helper cells) and $CD56^+$ cells (natural killer cells) in acutely relapsed inpatients relative to controls (Miller et al. 2013). Furthermore, drug-naïve FEP patients were shown to have significantly increased levels of $CD3^+$ cells (entire T-cells population) and $CD4^+$ cells (Miller et al. 2013). Drug-naïve FEP patients also showed an increase in the $CD4^+/CD8^+$ ratio, suggesting that the entire T-cell population is shifted towards an abundance of $CD4^+$ cells (T-helper cells) at the expense of $CD8^+$ cells (cytotoxic T-cells). In longitudinal studies, the $CD4^+/CD8^+$ ratio appeared to be state-related, as it decreased following antipsychotic treatment for acute exacerbations of psychosis (Miller et al. 2013). By contrast, the absolute levels of $CD56^+$ cells increased following antipsychotic treatment for acute exacerbation of psychosis (Miller et al. 2013), suggesting that CD56 may serve as a trait marker of schizophrenia. Despite these promising findings,

however, more research is needed to explore possible correlations between these markers and specific clinical features, and to examine the impact of potential confounding factors (e.g., smoking and body mass index) that could influence immune cell numbers.

Contrary to those that focus on lymphocytes, studies investigating peripheral monocytes in schizophrenia are scarce. Nevertheless, there is evidence for alterations in this system as well. For example, blood monocytes isolated from schizophrenic patients were shown to display changes in cytokine production and secretion in comparison to monocytes isolated from healthy controls, both under basal condition and after stimulation with immune stimulants (Kowalski et al. 2001; Krause et al. 2012a, b; Muller et al. 2012). These alterations also involve increased pro-inflammatory gene expression (Drexhage et al. 2010) and monocytosis (Dimitrov 2011), the latter of which was shown to be associated with worsening of psychotic symptoms and to resolve after changing antipsychotic treatment (Dimitrov 2011). These findings are in line with the observed normalization of IL-1β and TNF-α secretion from isolated and stimulated monocytes of schizophrenic patients after antipsychotic treatment with the typical antipsychotic haloperidol and perazine (Kowalski et al. 2001). Taken together, there is initial evidence suggesting that monocytosis or gene expression profiles from isolated monocytes could serve as possible state markers of schizophrenia. Whether alterations in blood mononuclear cells could serve as a trait biomarker still warrants investigation.

Thus far, only two studies reported alterations in CSF leukocytes in people with schizophrenia (Nikkila et al. 1999, 2001). Over the last decades, however, numerous attempts have been made to identify possible changes in microglia and astrocytes in postmortem brains from schizophrenic patients. The findings from these studies are equivocal (Trepanier et al. 2016), which may be explained by various factors, including duration of illness, antipsychotic treatment, brain regions investigated, and cause of death. Despite this, a recent meta-analysis including 11 postmortem studies revealed a significantly increased density of microglia in the brains of people with schizophrenia in comparison to controls. While there is substantial variation in outcomes between individual studies, this increase was most consistently observed in temporal cortex (van Kesteren et al. 2017). In contrast to this, no significant changes were observed in the meta-analysis of 18 studies assessing macroglia (astrocytes and oligodendrocytes) in postmortem brains of people with schizophrenia relative to matched controls (van Kesteren et al. 2017).

While postmortem investigations allow detailed cell morphological analyses, they are less suitable for identifying biomarkers that can be used to guide clinical assessments and treatments. In vivo neuroimaging techniques can overcome these limitations by offering the possibility to measure CNS immune parameters along the clinical course of the disease. Positron emission tomography (PET) using radiolabeled ligands selective for the mitochondrial 18-kDa translocator protein (TSPO) has become the most widely used in vivo technique to assess abnormal inflammatory processes and microglia activation in schizophrenia (Notter et al. 2018b). Similar to the postmortem findings of microglia changes in schizophrenia, there is substantial heterogeneity among TSPO PET imaging studies as well (Notter et al. 2018b). There are several plausible explanations for these inconsistencies, including use of

different radiotracers and methods of their quantification, differences in the stage and/or duration of the disease, possible influence of antipsychotic drugs, and possible immune-related subtypes of schizophrenia (Holmes et al. 2016; Notter and Meyer 2017). Another consideration that has to be taken into account is the unresolved question of what altered TSPO binding actually signifies, especially under mild inflammatory conditions such as present in schizophrenia. TSPO is an evolutionary conserved transmembrane protein with varying (and still ill-defined) physiological functions and cellular expression patterns in health and disease (Notter et al. 2018b). Since TSPO is expressed by different CNS cell types (Notter et al. 2018b), changes in TSPO binding or expression could theoretically reflect pathophysiological processes that are unrelated to microglia activity; or it could signify pathophysiological processes that concomitantly occur in various CNS cell types. Support for the latter notion has recently been obtained in an infection-mediated mouse model of neurodevelopmental disruption, which is characterized by substantial preclinical relevance to schizophrenia and related psychotic disorders (Notter et al. 2018a). In this model, schizophrenia-relevant behavioral abnormalities and increased inflammatory cytokine expression were found to be associated with reduced prefrontal TSPO expression. Intriguingly, the changes in TSPO levels were not restricted to microglia but emerged in various CNS cell types, including microglia, astrocytes, and vascular endothelial cells. These findings support the notion that TSPO should not be regarded as a microglia-specific marker. The increasing popularity of TSPO in immunopsychiatric research has, however, introduced substantial confusion regarding its suitability to identify neuroinflammatory processes in general, and microglial activation in particular. It is often neglected which components of these processes are actually captured by TSPO imaging. It is very likely that the pathological meanings of altered TSPO binding or expression are disease-specific, and therefore, not easily generalizable across different neuropathologies or inflammatory conditions.

4.2 Changes in Peripheral and Central Cytokine Levels: Possible State and Trait Markers?

Cytokines are soluble factors secreted by virtually all immune (and many non-immune) cells (Parkin and Cohen 2001). They are pivotal for mediating the communication between the innate and adaptive branches of the immune system and critically help orchestrating and controlling their actions (Parkin and Cohen 2001). Upon binding to their specific receptors, they regulate the activation and recruitment of other immune cells to the site of injury. The latter requires increased blood supply and vascular permeability, induction of migration, proliferation, differentiation of immune cells, and increasing the production and secretion of other immune mediators. Cytokines can further bind to soluble cytokine receptors, which either enhance (e.g., soluble IL-6 receptor (sIL-6R)) or inhibit (e.g., sIL-2R) the biological activity of cytokines (Miller et al. 2011).

Significant cytokine abnormalities appear to exist in both drug-naïve FEP patients and acutely relapsed inpatients, as supported by a meta-analysis of 40 studies that included FEP patients, acutely relapsed inpatients, stable medicated outpatients, and treatment-resistant patients (Miller et al. 2011). The latter analysis suggests that schizophrenic patients display higher blood levels of IL-1β, IL-6, and transforming growth factor-β (TGF-β) during acute exacerbation of symptoms, which significantly decreased upon antipsychotic treatment. Hence, blood IL-1β, IL-6, and TGF-β levels may be considered potential state markers of schizophrenia (Miller et al. 2011). On the other hand, IL-12, IFN-γ, TNF-α, and sIL-2R may represent possible trait markers as they were shown to be significantly higher in FEP patients and in patients with chronic schizophrenia, including both clinically stable patients and patients with periods of symptomatic worsening compared to healthy controls (Miller et al. 2011). Furthermore, in view of the similar effect sizes found in drug-naïve FEP patients and acutely relapsed patients (Miller et al. 2011), the association between cytokine abnormalities and acute exacerbation of schizophrenia seems to be independent of antipsychotic treatment. A recent meta-analysis, which focused on FEP patients, confirmed that drug-naïve FEP show elevated blood levels of IL-1β, IL-6, sIL-2R, and TNF-α as compared to matched controls (Upthegrove et al. 2014).

In attempts to examine the specificity of changes, Goldsmith et al. (2016) have recently performed a meta-analysis focusing on blood cytokine levels in acutely ill (68 studies) and chronic patients (46 studies) suffering from three major psychiatric illnesses, including major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia (Goldsmith et al. 2016). It was found that IL-6, TNF- α, sIL-2R, and IL-1RA were significantly increased in acutely ill patients with schizophrenia, bipolar mania, and MDD compared with corresponding controls. Following treatment of the acute illness, IL-6 levels significantly decreased in both schizophrenia and MDD, whereas sIL-2R levels increased in schizophrenia and IL-1RA decreased in bipolar mania. The levels of sIL-2R and IL-6 were also significantly increased in both chronic schizophrenia and euthymic BD relative to matched controls. These findings suggest that there are similarities in the pattern of cytokine alterations in schizophrenia, BD, and MDD during acute and chronic phases of illness. Taken together, various meta-analyses suggest that schizophrenic patients appear to have robust changes in peripheral blood cytokines during different stages of the disease. Peripheral cytokines may thus have a great potential to serve as state and trait biomarkers of schizophrenia. In attempts to define and use such immune-based biomarkers, however, one needs to consider that some abnormalities are not specific to schizophrenia but can similarly exist in BD and MDD as well.

Studies assessing CSF cytokine changes in schizophrenic patients are less frequent. Nevertheless, a recent meta-analysis including 16 studies found IL-1β, IL-6, and IL-8 levels to be significantly increased in people with schizophrenia, whereas levels of sIL-2R were significantly decreased (Wang and Miller 2017). Intriguingly, there seems to be a certain degree of concordance between cytokine changes in the CSF and blood (Goldsmith et al. 2016; Wang and Miller 2017), indicating that cytokine abnormalities in the CSF could, at least in part, be mirrored by peripheral

cytokine profiles in blood samples. To what extent peripheral and central cytokine levels correlate, however, still warrants further investigation.

4.3 Presence of Autoantibodies in Schizophrenia

According to the autoimmune hypothesis of schizophrenia, autoantibodies targeting and interfering with peripheral or central tissues may be involved in the etiopathogenesis of schizophrenia (Al-Diwani et al. 2017; Kirch 1993). Support for this hypothesis stems from findings suggesting that autoimmune diseases and schizophrenia are associated with each other in a bidirectional manner. For example, in schizophrenia, patients have been shown to have an increased risk of developing autoimmune diseases (Benros et al. 2014), whereas having a personal or family history of any autoimmune disease can increase the risk for schizophrenia.

The autoimmune hypothesis of schizophrenia may also provide an opportunity to identify disease-related biomarkers. A systematic, quantitative review of 81 studies found that psychosis-related disorders are associated with increased prevalence of positive titers for 20 different autoantibodies, some of which target cellular components of the CNS (Ezeoke et al. 2013). Another meta-analysis of nine studies that focused on N-Methyl-D-aspartate receptor (NMDAR) autoantibodies revealed that patients with schizophrenia or schizoaffective disorders are three times more likely to have elevated anti-NMDAR titers compared to healthy controls (Pearlman and Najjar 2014). These findings are of particular interest as NMDAR hypofunction may play a key role in the pathophysiology of schizophrenia and related disorders (Balu 2016).

4.4 Changes in Complement System Proteins

The role of the complement system in schizophrenia has been explored since the late 1980s (Mayilyan et al. 2008). The complement system consists of approximately 35 plasma and cell-surface proteins that can recognize endogenous and foreign materials and is a major effector of the innate immune system and an adjuvant for the adaptive immunity (Parkin and Cohen 2001). It can be activated through three pathways, which are referred to as the classical, alternative, and mannan binding lectin pathways (Mayilyan et al. 2008; Parkin and Cohen 2001). Within the context of immunity, the complement system has various important roles. Firstly, it is a first-line defense against foreign pathogens and promotes opsonization, adjuvant activities, foreign cell lysis, and activation and recruitment of leukocytes. Secondly, by enhancing antibody responses, improving adaptive immune memory, and regulating T-lymphocytes, it bridges innate and adaptive branches of the immune system. Thirdly, it influences cellular homeostasis by promoting the clearance of apoptotic or necrotic cells and immune complexes.

Numerous studies have investigated the complement system in schizophrenia and found it to be aberrantly activated, although the findings are not consistent

(as summarized by Arakelyan et al. 2011; Boyajyan et al. 2010; Kucharska-Mazur et al. 2014; Mayilyan et al. 2008; Santos Soria et al. 2012; Severance et al. 2012). Hence, it appears premature to define immune-based biomarkers of schizophrenia that are based on altered expression of complement system proteins. It should be noted, however, that a recent study found schizophrenia to be associated with common allelic variations in the complement component 4 (C4) gene (Sekar et al. 2016). The associated deficits in C4 protein expression were further linked to altered synaptic pruning, which may be one of the etiopathogenetic factors in schizophrenia (Sekar et al. 2016).

4.5 Changes in C-Reactive Protein: Possible Trait Marker?

CRP is a plasma protein synthesized by the liver during the course of inflammation. It belongs to the class of acute phase proteins and is induced by cytokines, including IL-1β, IL-6, and IL-17 (Eklund 2009). CRP binds to damaged tissue, to nuclear antigens, and to certain pathogenic organisms, which activates the complement cascade and opsonizes various pathogens (Du Clos 2000). A recent meta-analysis of 26 longitudinal or cross-sectional studies revealed moderate but significant elevated CRP levels in blood from schizophrenic patients relative to levels found in control subjects (Fernandes et al. 2016). Importantly, FEP patients and patients with chronic schizophrenia show similar increase in blood CRP levels, and these increases do not seem to normalize upon initiation of antipsychotic medication (Fernandes et al. 2016). Together, these findings suggest that altered CRP in blood may serve as a valuable trait marker for schizophrenia. In further support of this notion, the amount of peripheral blood CRP levels was found to be correlative with the severity of positive (but not negative) symptoms (Fernandes et al. 2016).

Thus far, only one study assessed whether blood CRP levels are altered in subjects who are at risk for developing schizophrenia (Labad et al. 2015). This study did not reveal significant alterations in at-risk individuals who did not convert to schizophrenia (Labad et al. 2015). Interestingly, however, a tendency towards lower blood CRP levels was found for at-risk subjects who developed schizophrenia (Labad et al. 2015). In keeping with the aforementioned findings of increased blood CRP levels in schizophrenia, it appears that the nature of peripheral CRP changes is markedly influenced by the clinical course of the disorder. If confirmed by future studies, both reduced and increased blood CRP may be considered as a potential biomarker of schizophrenia, depending on whether the subjects are at high-risk stage or already developed the full-blown disorder.

5 Immunological Processes as Risk Factors for Schizophrenia: Potential Predictive Biomarkers?

5.1 Genetic Variations in Immune Genes

The contribution of genetic factors in schizophrenia has long been recognized (Henriksen et al. 2017; Sullivan et al. 2003). The current consensus is that the combination of a number of genes contributes to the heritability of schizophrenia. Thus, genetic risk of schizophrenia depends on the accumulation of many common allelic variations with small effect sizes (polygenic), rather than on single allelic variations with big effect sizes (Henriksen et al. 2017). Interestingly, genome-wide association studies (GWAS) have identified common variations in immune genes to be among the strongest risk factors. These include polymorphisms in the locus of the major histocompatibility complex (MHC) on chromosome 6 (6p22.1) (Shi et al. 2009).

Another large-scale GWAS identified 128 independent associations spanning 108 conservatively defined loci that are enriched in schizophrenic patients, among which some were enriched in genes that have important roles in immunity (Consortium 2014). The work by Sekar et al. further identified the complement component C4 gene to be the strongest signal within the MHC locus (Sekar et al. 2016).

The findings from several meta-analyses further suggest associations between schizophrenia risk and polymorphisms in cytokine genes. Significant associations have been identified for genes encoding for IL-1β (rs1143627 polymorphism: odds ratio (OR) = 1.91 (AA homozygosity), OR = 0.40 (GG homozygosity)), IL-6 (rs1800795 polymorphism: OR = 0.95 (A allele), OR = 0.65 (AA genotype)), sIL-6R (rs8192284 polymorphism: OR = 0.96 (A allele), OR = 0.72 (AA genotype)), and IL-10 (OR = 1.351 (SNP), OR = 1.761 (two haplotypes)) (Gao et al. 2014; Hudson and Miller 2016; Shibuya et al. 2014; Xu and He 2010). These polymorphisms may provide a genetic contribution to abnormalities in peripheral and central cytokine levels that have been detected in people with schizophrenia.

5.2 Infection and Cytokine Imbalances in the Prenatal Period

A plethora of epidemiological studies has implicated maternal infection as a risk factor for schizophrenia and other neuropsychiatric disorders with neurodevelopmental components (Brown 2011; Brown and Derkits 2010). While early epidemiological studies using ecological data suggested associations between influenza epidemics and increased risk of schizophrenia among offspring (Mednick et al. 1988), these findings were inconsistent (Brown 2011; Brown and Derkits 2010). These early studies have been surrogated in more recent years with birth cohort studies that utilize prospectively acquired serologic biomarkers of infection in individual pregnancies (Brown 2011; Brown and Derkits 2010). Notably, associations between prenatal infection and schizophrenia have been identified for a number of pathogens, including: (1) viral

agents such as rubella (Brown et al. 2001), measles (Torrey et al. 1988), polio (Suvisaari et al. 1999), and herpes simplex (Buka et al. 2001a), (2) bacterial pathogens that cause sinusitis, tonsillitis, and pneumonia (Sorensen et al. 2009), (3) genital and/or reproductive infections (Babulas et al. 2006), and (4) the protozoan parasite *Toxoplasma gondii* (Mortensen et al. 2007). As mentioned above, epidemiological studies have provided serological evidence for at least some of these associations, including maternal infection with influenza, rubella, and *T. gondii* infection (Brown et al. 2001, 2004a, 2005, 2009; Mortensen et al. 2007).

The myriad of different pathogens associated with schizophrenia suggests that factors common to the immune response may be critical for mediating the association between prenatal infection and schizophrenia. This has led to the hypothesis that an imbalance in pro- and anti-inflammatory cytokines induced by maternal infection could play a key role in altering neurodevelopmental trajectories, which results in an increased risk to develop schizophrenia in the offspring (Gilmore and Jarskog 1997). Support for this hypothesis also stems from a number of preclinical animal models of MIA, which use immune-activating agents that evoke cytokine-associated immune responses without exposure to live pathogens (Meyer et al. 2009). A widely used approach is based on maternal exposure to polyriboinosinic–polyribocytidilic acid (poly(I:C)), a synthetic double stranded RNA that induces an acute antiviral response. Another common model system makes use of the bacterial endotoxin lipopolysaccharide (LPS), which mimics an acute phase response to bacterial infections. Poly(I:C)- or LPS-induced MIA has repeatedly been shown to cause changes in behavior, cognition, brain morphology, and neurochemistry relevant to schizophrenia and related disorders (Meyer 2014).

The concept that an imbalance in pro- and anti-inflammatory cytokines could mediate the effect of maternal infection on the offspring is further supported by the findings that blocking the action of the pro-inflammatory cytokine IL-6 in pregnant mice through genetic or pharmacological intervention prevents the development of long-term brain and behavioral abnormalities in offspring prenatally exposed to poly (I:C) (Smith et al. 2007). In line with this, overexpression of the anti-inflammatory cytokine IL-10 was shown to prevent the emergence of multiple behavioral abnormalities in poly(I:C)-exposed offspring (Meyer et al. 2008). Recent findings further suggest an important role of IL-17a in MIA-induced brain and behavioral abnormalities (Choi et al. 2016).

Concurrent with the preclinical work are epidemiological studies demonstrating association between altered inflammatory cytokine levels during pregnancy and the risk for schizophrenia. For example, increased maternal serum levels of IL-8 during the second/third trimester of pregnancy (Brown et al. 2004b) and increased maternal serum levels of TNF-α together with a clinical report for infection during late pregnancy (Buka et al. 2001b) were both shown to increase the offspring's risk to develop schizophrenia (significant likelihood ratio of $I^2 = 5.41$ for IL-8; OR of 8.5 for TNF-α). A recent study further showed that elevated maternal serum levels of anti-inflammatory Th_2 cytokines, including IL-4, IL-5, and IL-13, are associated with decreased risk of schizophrenia in the offspring (significantly lower odds, $B = -1.096$, Wald's $I^2 = 4.612$) (Allswede et al. 2016). Together, the findings

from epidemiological and preclinical studies suggest that the measurement of maternal immune exposures, including antibody titers and cytokines, could be used for the assessment of schizophrenia risk in the offspring. In attempts to define and implement such "predictive" biomarkers, however, one needs to consider that prenatal exposure to infection and/or cytokine imbalances is not specific to schizophrenia but has been identified as a risk factor for several other psychiatric disorders, including autism spectrum disorder (ASD), BD, and MDD (Careaga et al. 2017; Estes and McAllister 2015, 2016). Nevertheless, maternal immune measures may provide general risk estimates for neurodevelopmental disorders that cross classical nosologic boundaries.

5.3 *Infection and Cytokine Imbalances in the Postnatal Period (Premorbid Stage)*

Akin to exposures taking place prenatally, infections and/or imbalances in cytokines and other mediators of inflammation during the postnatal period can also increase the risk of neuropsychiatric disorders. For example, hospital contact for infections during childhood and early adolescence has been associated with a 1.41-fold increased risk of schizophrenia (Nielsen et al. 2014). Another study found that elevated blood IL-6 levels at the age of 9 increased the risk of having psychotic experiences (OR = 1.81) and psychotic disorders (OR = 2.4) at age 18 (Khandaker et al. 2014). Intriguingly, the association between increased childhood IL-6 and development of psychosis in early adulthood was found to be dose-dependent. Although these findings warrant replication in longitudinal studies, the existing data suggest that blood levels of IL-6 during childhood could serve as an early biomarker for the estimation of whether an individual is at heightened risk of developing neuropsychiatric disorders in later life. Ideally, measures of IL-6 (and possibly of other markers of inflammation) should be obtained repeatedly, given that they can be influenced by a number of factors, including prevailing immune status, nutrition, physical activity, and stress (Rohleder et al. 2012).

5.4 *Inflammatory Mediators as Risk Factors in the Prodrome*

The increasing interest in preventive and/or interventional strategies in schizophrenia necessitates the identification of at-risk individuals. Consequently, biomarkers that could assist the assessment of the prodromal state and possibly predict the conversion to full-blown schizophrenia are of great value.

Against this background, Perkins et al. (2015) aimed at establishing a blood biomarker assay that can be used to predict the conversion to overt psychosis in individuals who display the attenuated psychosis risk syndrome. The authors identified 15 analytes that could distinguish between high-risk individuals who

subsequently developed psychosis from those who did not develop psychosis within a 2-year period. Intriguingly, the majority of analytes belong to the family of cytokines and related immune mediators (IL-1β, growth hormone, KIT ligand, IL-8, IL-7, resistin, and chemokine [c-c motif] ligand 8) or to the family of immunomodulatory proteins (Perkins et al. 2015).

Two other studies were recently conducted to assess blood cytokine levels in individuals who were identified as being in an at-risk mental state (Stojanovic et al. 2014) or at ultrahigh risk to develop psychosis (Zeni-Graiff et al. 2016). Higher blood IL-6 levels were noted in both at-risk populations (Stojanovic et al. 2014; Zeni-Graiff et al. 2016). While these findings corroborate the aforementioned association between elevated IL-6 levels in childhood and increased risk of psychosis in early adulthood (Khandaker et al. 2014), they are based on a relatively small sample size and should therefore be replicated in larger studies.

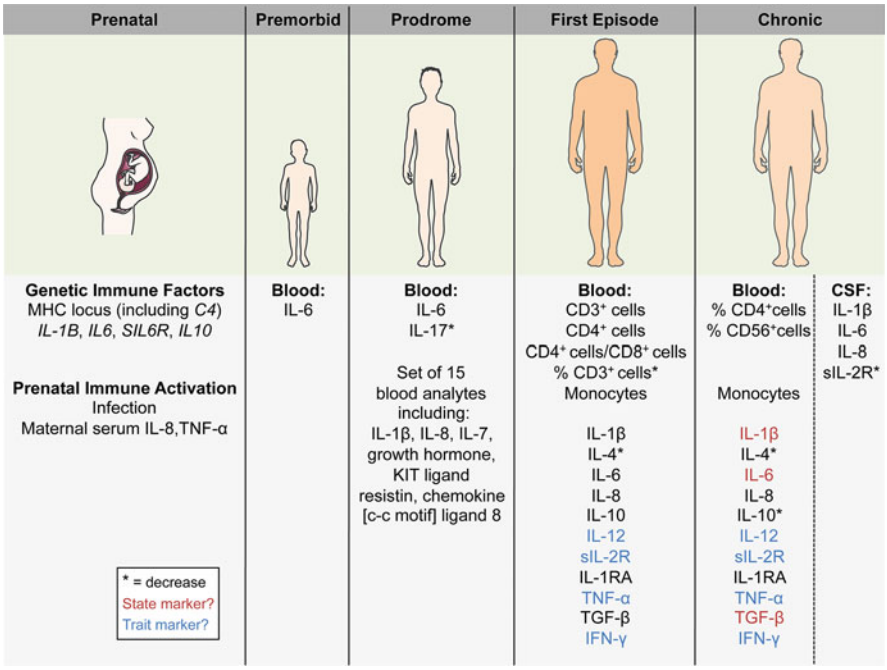

Fig. 1 Peripheral and central immune alterations along the clinical course of schizophrenia and their potential as immune-related biomarkers. Relevant immune-related markers are summarized against the background of developmental stages (potential predictive biomarkers) and the clinical course of schizophrenia. All immune markers listed signify increased levels detected in at-risk individuals or patients relative to healthy controls, except when denoted by the symbol (*) (= decrease). Putative state and trait markers are depicted in red and blue color, respectively. Abbreviations: *MHC* major histocompatibility complex, *C4* complement component 4, *IL* interleukin, *TNF* tumor necrosis factor, *CD* cluster of differentiation, *sIL-2R* soluble interleukin 2 receptor, *IL-1RA* interleukin 1 receptor antagonist, *TGF* transforming growth factor, *IFN* interferon

6 Concluding Remarks

Accumulating evidence suggests that the pathophysiology or schizophrenia involves alterations in immune functions, both peripherally and centrally. To what extent these dysfunctions contribute to the pathogenesis of the disorder is currently the subject of lively debates (Birnbaum et al. 2017; Estes and McAllister 2016; Khandaker et al. 2015). However, the fact that significant changes in various immune networks exist along the clinical course of schizophrenia, and likely even in the disorder's premorbid phases (Fig. 1), makes the possibility of identifying immune-based biomarkers of schizophrenia an achievable objective.

In keeping with the present state of knowledge, however, the prospect of identifying robust immune-based biomarkers of schizophrenia still needs to be met with caution. Indeed, even if numerous meta-analyses support the presence of immune dysfunctions in the pathophysiology of schizophrenia (Fig. 1), there is substantial heterogeneity between individual studies. There are many possible explanations for this discrepancy, including use of small sample sizes, differences in the stage and duration of the illness, confounding influences of genetic or environmental factors background, and infectious comorbidities. Moreover, there is also considerable heterogeneity within individual studies, suggesting that only a subgroup of people with schizophrenia may display overt immune abnormalities. The latter has been demonstrated both in postmortem (Fillman et al. 2013) and peripheral (Boerrigter et al. 2017; Fillman et al. 2016) tissues of cases with schizophrenia. The use of stratification strategies, which define subgroups of schizophrenia based on overt immune dysfunctions, appears relevant for the success of immune-based interventions (Laan et al. 2010; Muller et al. 2004).

Taken together, immunopsychiatric research has provided a number of candidate biomarkers that could aid estimating the risk of developing schizophrenia and/or predicting its clinical course or outcomes (Fig. 1). While challenging for psychiatric nosology, the heterogeneity of immune dysfunctions offers the opportunity to define patient subgroups based on the presence or absence of distinct immune dysfunctions. This stratification may be clinically relevant for schizophrenic patients as it may help establishing personalized add-on therapies or preventive interventions with immunomodulating drugs.

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Using Pattern Classification to Identify Brain Imaging Markers in Autism Spectrum Disorder

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Curr Topics Behav Neurosci (2018) 40: 413–436
DOI 10.1007/7854_2018_47
Published Online: 6 April 2018

Abstract Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in social interaction and communication, as well as repetitive and restrictive behaviours. The etiological and phenotypic complexity of ASD has so far hindered the development of clinically useful biomarkers for the condition. Neuroimaging studies have been valuable in establishing a biological basis for ASD. Increasingly, neuroimaging has been combined with 'machine learning'-based pattern classification methods to make individual diagnostic predictions. Moving forward, the hope is that these techniques may not only facilitate the diagnostic process but may also aid in fractionating the ASD phenotype into more biologically homogeneous sub-groups, with defined pathophysiology, predictable outcomes and/or responses to targeted treatments and/or interventions. This review chapter will first introduce 'machine learning' and pattern recognition methods in general, with a focus on their application to diagnostic classification. It will highlight why such approaches to biomarker discovery may have advantages over more conventional analytical methods. Magnetic resonance imaging (MRI) findings of atypical brain structure, function and connectivity in ASD will be briefly reviewed before we describe how pattern recognition has been applied to generate predictive models for ASD. Last, we will discuss some limitations and pitfalls of pattern recognition analyses in ASD and consider how the field can advance beyond the prediction of binary outcomes.

Keywords Autism · Imaging · Pattern recognition

1 Introduction

Across the general population, autism spectrum disorder (ASD) affects over 1 in 100 individuals. Annually, ASD costs the UK and USA more than £33 billion and $235 billion, respectively (Buescher et al. 2014). Clinically, ASD is highly heterogenous. Although its core symptoms include social and communication difficulties and repetitive behaviours (including sensory hyper- and hyposensitivities), ASD is commonly associated with co-occurring neurological conditions, such as epilepsy,

and mental health difficulties, particularly anxiety and mood disorders (World Health Organization 2004).

Diagnosis of ASD is therefore time-consuming and costly, depending upon the availability of an experienced multidisciplinary team and carefully structured behavioural observations and clinical interviews. The complexity of ASD phenotypes has hindered the development of useful diagnostic biomarkers for the condition, none of which are currently available for clinical use (Ruggeri et al. 2013). Moreover, it explains why there are currently no pharmacological treatments for the core symptoms of ASD and potentially why individuals with ASD with co-occurring conditions do not always respond to conventional treatments for those disorders. Thus, prognostic biomarkers or tools that could identify biologically responsive individuals who may benefit from specific interventions would represent an important advance.

2 Causes of Autism Spectrum Disorder

The development of ASD is now widely believed to involve complex interactions between several different environmental and genetic risk factors (Sandin et al. 2014). Genetic factors are strongly implicated in ASD; however, extensive heterogeneity exists in the genetic pathways believed to contribute to the development of the condition. For example, in ~10% of ASD cases, a specific genetic condition (e.g. fragile X syndrome, Rett syndrome, 22q-11 deletion disorder, etc.) can be established (Tammimies et al. 2015; Carter and Scherer 2013). These cases where ASD is a secondary diagnosis to a known genetic condition are referred to as 'syndromic' autism (as opposed to 'idiopathic' autism). New risk genes for ASD continue to be identified (C Yuen et al. 2017) and add to the over 850 genes currently implicated in ASD (sfari.org/resources/sfari-gene). Collectively these risk genes largely impact upon processes that affect neurogenesis and neuronal and synaptic homeostasis, such as chromatin remodelling, metabolism, mRNA translation and synaptic functioning (Huguet et al. 2013; Packer 2016). However, each of these specific genes in isolation confers only a small amount of risk for ASD and therefore accounts for only a small proportion of cases within the total population (Huguet et al. 2013).

This can also be said of environmental risk factors for ASD. For example, parental age (Gardener et al. 2009), low birth weight (Schendel and Bhasin 2008), multiple births (Croen et al. 2002), maternal infections and illness during pregnancy (Sandin et al. 2014; Lichtenstein et al. 2010; Hallmayer et al. 2011; Atladóttir et al. 2010; Lee et al. 2015) and exposure to toxins (Adams et al. 2009) have all been linked to significant but small increases in ASD risk. Importantly, despite speculation, there is no evidence to support an association between vaccinations and ASD (Taylor et al. 2014).

3 Magnetic Resonance Imaging in Autism Spectrum Disorder

Neuroimaging studies have been critical in establishing a biological basis for ASD. These methods, most notably magnetic resonance imaging (MRI), have provided substantial evidence that ASD is accompanied by atypical brain structure, function and connectivity (Ecker and Murphy 2014). Many of these findings implicate brain regions known to be involved in social processing. For example, MRI studies of the amygdala in ASD have shown hypoactiviation of the region during social processing tasks (Herrington et al. 2016), reduced connectivity of the amygdala with frontal and temporal regions (Shen et al. 2016) and atypical growth rates of the structure during early life (Nordahl et al. 2012), all of which are likely to contribute to ASD behaviours. However, although these approaches have provided valuable insights, their early promise has been constrained by replication difficulties. The results of MRI studies of anatomical and functional differences between individuals with and without ASD have been variable. This can partially be attributed to differences in cohort ages, sex and intellectual ability, as well as the utilization of a range of different imaging methods. In addition to these methodological issues, the inherent heterogeneity of ASD is also likely to contribute to variable findings in the condition.

Increasingly, 'machine learning'-based pattern classification methods are being applied to imaging data in ASD to generate diagnostic predictions for individuals (Table 1). Moving forward, the hope is that these techniques will not only facilitate the diagnostic process but also evolve to help fractionate the diversity of the autism spectrum into more biologically homogeneous sub-groups with well-defined pathophysiology, predictable outcomes and/or responses to targeted treatments and/or interventions.

This chapter will first introduce 'machine learning' and pattern recognition methods in general, with a focus on their application to diagnostic classification. It will highlight why such approaches to biomarker discovery may have advantages over more conventional analytical methods. Magnetic resonance imaging (MRI) findings of atypical brain structure, function and connectivity in ASD will be briefly reviewed before we describe how pattern recognition has been applied to generate predictive models for ASD. Last, we will discuss some limitations and pitfalls of pattern recognition analyses in ASD and consider how the field can advance beyond the prediction of binary outcomes.

4 Pattern Classification

4.1 Machine Learning

Broadly speaking, pattern classification encompasses several distinct 'machine learning' methods that aim to make class label predictions based on multivariate

Table 1 Pattern classification studies of autism spectrum disorder

Modality	Features	Classifier	Testing group	Number of participants	Biological sex	ASD age	Reference
sMRI	Volumetric measures	Discriminant function analysis	None	TDC = 15, ASD = 52	Male	3.4 ± 0.7	Akshoomoff et al. (2004)
sMRI	VBM GM and WM volume maps	SVM	k = 2 CV	TDC = 22, ASD = 22	Male	27.0 ± 7.0	Ecker et al. (2010)
sMRI	Cortical thickness	Logistic model trees	k = 10 CV	TDC = 16, ASD = 22	32 male and 6 female	9.2 ± 2.1	Jiao et al. (2010)
sMRI	Cortical thickness, surface area, sulcal depth, curvature, metric distortion	SVM	k = 2 CV	TDC = 20, ASD = 20	Male	33.0 ± 11.0	Ecker et al. (2010)
sMRI	VBM GM and WM volume maps	SVM	k = 10 CV	TDC = 24, ASD = 24	44 male and 4 female	13.2 ± 0.6	Uddin et al. (2011)
rsMRI	ROI based functional connectivity	Thresholding	k = 1 CV	TDC = 40, ASD = 40	Male	22.7 ± 7.4	Anderson et al. (2011)
dMRI	FA and MD based ROI	SVM	k = 1 CV	TDC = 30, ASD = 45	56 male and 18 female	10.5 ± 2.5	Ingalhalikar et al. (2011)
sMRI	VBM GM volume maps	SVM	k = 2 CV	TDC = 38, ASD = 30	Female	4.4 ± 1.5	Calderoni et al. (2012)
fMRI	Language and theory of mind task based functional connectivity	Logistic regression	k = 1 CV	TDC = 14, ASD = 13	Male	21.4 ± 3.9	Murdaugh et al. (2012)
rsMRI	rsfMRI ICA components	Logistic regression	CV	TDC = 20, ASD = 20	32 male and 8 female	9.9 ± 1.5	Uddin et al. (2013)
fMRI and dMRI	Functional connectivity and FA values	SVM	k = 10% CV	TDC = 15, ASD = 15	Not reported	21.1 ± 0.9	Deshpande et al. (2013)
sMRI	Cortical thickness and volumetric ROIs	SVM	k = 20% CV & IND	TDC = 325, ASD = 325	576 male and 74 female	17.8 ± 7.4	Sabuncu et al. (2014)
sMRI	Cortical thickness and volumetric ROIs	Multi-kernel SVM	k = 50% CV	TDC = 59, ASD = 58	85 male and 26 female	10.8 ± 4.0	Wee et al. (2014)

(continued)

Table 1 (continued)

Modality	Features	Classifier	Testing group	Number of participants	Biological sex	ASD age	Reference
sMRI	GM volume map	SVM	k = 10% CV	TDC = 40, ASD = 52	67 male and 65 female	14.4 ± 1.7	Segovia et al. (2014)
fMRI	Activation of selected voxels during social interaction task	Gaussian naïve Bayes	k = 1 CV	TDC = 17, ASD = 17	32 male and 2 female	25.6 ± 6.7	Just et al. (2014)
sMRI and rsMRI	rsfMRI, cortical thickness and volume based graph theory metrics	Random forest	k = 2–10 or 10–90% CV	TDC = 153, ASD = 127	211 male and 69 female	13.5 ± 6.0	Zhou et al. (2014)
sMRI	VBM and SBM based ROI	SVM	k = 2 CV	TDC = 20, ASD = 21	Male	4.1 ± 0.8	Gori et al. (2015)
rsMRI	ROI based functional connectivity	SVM & random forest	IND	TDC = 126, ASD = 126	206 male and 35 female	14.8 ± 1.6	Chen et al. (2015)
rsMRI	ROI based functional connectivity	Logistic regression and SVM	k = 1 CV	TDC = 59, ASD = 59	Male	17.66 ± 2.7	Plitt et al. (2015)
rsMRI	ROI based functional connectivity	Probabilistic neural network	k = 1 CV	TDC = 328, ASD = 312	540 male and 100 female	13.2 ± 3.1	Iidaka (2015)
sMRI, dMRI and MRS	Cortical thickness, FA and neurochemical concentration	Decision tree	k = 1 CV	TDC = 18, ASD = 19	29 male and 8 female	27.1 ± 1.3	Libero et al. (2015)
rsMRI	Functional connectivity	SVM	k = 1 CV	TDC = 128, ASD = 112	205 male and 35 female	14.8 ± 1.7	Chen et al. (2016)
fMRI	Activation of selected voxels during social interaction task	SVM	k = 1 CV	TDC = 14, ASD = 15	25 male and 4 female	28.6 ± 1.8	Chanel et al. (2016)
rsMRI	Functional connectivity	Sparse logistic regression	k = 1 CV	TDC = 107, ASD = 74	50 female, 131 male	31.43 ± 8.5	Yahata et al. (2016)
rsMRI	Functional connectivity	SVM	k = 1 CV	HRN = 48, ASD = 11	18 female, 41 male	24 months	Emerson et al. (2017)
sMRI	Age, sex, volume, surface area, and cortical thickness ROIs	Neural network	k = 10% CV	HRN = 145, ASD = 34	114 male and 66 female	6 and 12 months	Hazlett et al. (2017)

sMRI	Extra-axial cerebrospinal fluid	Decision tree	k = 4% CV & IND	HRN = 174, ASD = 47	137 male and 84 female	6 months	Shen et al. (2017)
rsMRI	Functional connectivity	Neural network	None	TDC = 55, ASD = 55	84 male and 26 female	12.7 ± 2.4	Guo et al. (2017)
sMRI	Cortical thickness, surface area, and volumetric ROIs	SVM, random forest, naïve Bayes	None & k = 33% CV	DD = 39, ASD = 46	75 males and 10 female	2.25 ± 0.3	Xiao et al. (2017)

Note: Modality denotes type of magnetic resonance imaging data utilized: structural MRI (sMRI), diffusion MRI (dMRI), functional MRI (fMRI), resting state fMRI (rsMRI), magnetic resonance spectroscopy (MRS). Feature denotes type of MRI data used for classification: voxel based morphometry (VBM), grey matter (GM), white matter (WM) ,region of interest (ROI), fractional anisotropy (FA), mean diffusivity (MD), independent component analysis (ICA). Classifier: support vector machine (SVM). Testing group denotes type of validation method used: k = number or percent of total sample (if specified) left out for each cross validation (CV) fold, IND = independent sample, none = whole training set used as testing set, typically developing control (TDC), autism spectrum disorder (ASD), high risk for autism – negative (HRN), developmental delay (DD)

patterns within data. Within the 'machine learning' framework, an algorithm is said to 'learn' from experience *E* with respect to some class of tasks *T* and performance measure *P*. Learning occurs when performance in task *T* improves with experience *E* as measured by performance *P* (Mitchell 1997). For example, a pattern classification model could learn to predict diagnostic categories (*T*), based on MRI scans of the brains of patients and controls (*E*), with performance (*P*) being measured by the accuracy of the model's diagnostic predictions. This classification example represents a common 'supervised learning' problem in which examples from a training set of empirical data (i.e. brain scans) are mapped to a known class label (i.e. diagnostic category). Once a classification model has been trained, it can be tested on individuals not included in the training set to determine the model's ability to identify cases from controls. This ability to make predictions at the level of an individual is a key strength of these methods over traditional analytical techniques that identify mean differences in measures only at group level.

4.2 Model Training and Testing

In order for a pattern classification model's predictions to be of real value, they must be generalizable. A model can be said to be generalizable when it is able to make accurate predictions for cases not included in the initial training data (e.g. the broader population). During training, it is possible for a model to become 'overfit' to the limited amount of examples used as training data. Overfitting often results in a biased model with limited generalizability, which can lead to overestimations of model performance. Accordingly, it is critical that the cases used for testing a classification model have been used in the training of that model. Independence of training and testing data helps assure that established prediction models are not overfit to training data by providing more accurate estimations of their generalizability to newly introduced test cases. However, within the field of psychiatric neuroimaging, data from independent cohorts may not always be easily available. MRI is expensive and there may be particular challenges in recruiting large number of patients from a particular population; and data collected in multiple centres or using different imaging sequences may not be directly comparable.

To address these practical constraints, cross validation paradigms have become the standard for estimating classification model performance within a single cohort. For example, in *k*-fold cross validation, *k* number of cases are 'left out' of model training, and predictions are then tested using the excluded samples. The cross validation loop is then repeated *n* times, such that all individual's data in turn are left out from the training phase and are used to test the model. Subsequently, by comparing the predicted class labels following either independent or cross validation to the known labels, it is possible to estimate a pattern recognition model's accuracy (i.e. percentage of test individuals assigned the correct class label).

In addition to evaluating a model's overall accuracy, the sensitivity (i.e. true positive rate), specificity (true negative rate), positive predictive value and/or

negative predictive value can also be calculated. In clinical settings, a model's sensitivity or specificity may be a more valuable metric of performance than overall accuracy alone. For example, in some instances, a highly sensitive test – one which captures most positive cases but wrongly assigns some negative cases (a false positive diagnosis) – may be more useful than a highly specific test, one which is rarely wrong when a positive classification is made but fails to identify the majority of positive cases. Once a model is established, metrics such as positive and negative predictive value can be used to determine the probability that individuals with a positive (or negative) classification actually have (or don't have) a diagnosis.

4.3 Pattern Classification Models

Several different pattern classification algorithms have been applied to neuroimaging data in psychiatric and neurodevelopmental conditions (Wolfers et al. 2015; Arbabshirani et al. 2016). Of these, the support vector machine (SVM) has been the most widely implemented. This can largely be attributed to the SVM's ability to handle large dimensionality data in a computationally efficient manner, making it an effective method for neuroimaging applications (Orrú et al. 2012). Other algorithms that have been reported in ASD studies include logistic regression, Gaussian processes, random forest decision trees, neural networks and others. Those few studies that have directly tested the performance of different classification algorithms in the prediction of ASD suggest that there is no single optimal choice (Sabuncu and Konukoglu 2015; Xiao et al. 2017). Instead, preference of one particular algorithm over another (even if significant) is likely to be less important than the identification and selection of high-quality training data (i.e. imaging modalities/features) that is relevant to the learning problem at hand (i.e. class labels).

4.4 Feature Extraction and Selection

In pattern recognition/classification, the data used to train a classification model is known as a 'feature'. Feature extraction is the process of calculating a particular feature from the raw data set. Within MRI, feature extraction could involve the segmentation of images into different tissue types, the calculation of cortical morphometric indices, diffusion maps, functional or resting state MRI time courses or connectivity matrices. Therefore, MRI-based data used for classification can potentially contain several thousand features (e.g. voxels, vertices, nodes). Accordingly, it is often desired to limit the number of features used to train a classifier to increase the amplitude of the signal relating to the class labels. This 'feature selection' can be performed in two ways, (1) through prior knowledge and expertise (e.g. a hypothesis-driven selection of features believed to be relevant to a condition) or (2) via an automated feature selection algorithm (Mwangi et al. 2014). However, while feature selection – when properly implemented – can be a valuable tool to

increase model performance, it may also introduce the risk of 'overfitting' a predictive model to the training set, thereby limiting its generalizability to new test examples. Consequently, it is critical that feature selection is performed in a non-biased manner, either based on a priori (i.e. manual) feature selection or by assuring that automated feature selection parameters are tuned without input from test cases. A variety of these techniques have been increasingly applied in classification models of ASD across a wide range of neuroimaging features.

4.5 Evaluating Performance

When evaluating pattern classification results, it is critical that all aspects of the study are carefully considered. Each of the factors discussed above including study sample size, cohort demographics, validation methods (e.g. independent or cross validation) and the use of feature selection can significantly influence estimates of model performance. The diversity of ASD cohorts and classification methods that have been reported, therefore, makes direct comparisons of these studies based solely on performance metrics difficult. In other words, metrics such as 'overall accuracy' can be highly study specific and not generalizable to the wider population or across the autism spectrum and thus should be interpreted with a certain degree of caution.

5 Magnetic Resonance Imaging and Pattern Classification in Autism Spectrum Disorder

Please see Table 1 for a summary of pattern classification studies in ASD.

5.1 Structural Features

The suggestion that ASD is accompanied by atypical brain structure first arose from observations made by Kanner (1943) who noted greater head circumference among individuals with the condition. This finding was later supported by early MRI studies that reported larger brain volumes in individuals with ASD (Piven et al. 1995) particularly affecting the temporal, parietal and occipital lobes (Piven et al. 1996). However, more recent studies have suggested that greater brain volumes may be present in a subset, and not all, individuals with ASD (Libero et al. 2016). Accordingly, the first studies employing pattern classification in ASD utilized global cerebral and cerebellar grey and white matter volumes to identify the condition among a sample of preschool-aged children (Akshoomoff et al. 2004).

Subsequently, volumetric studies at the level of the voxel ('volume unit'), i.e. voxel-based morphometry (VBM) (Ashburner and Friston 2000), have ascribed highly variable regional volumetric differences to the condition (Ecker et al. 2012; McAlonan et al. 2008, 2009; Nickl-Jockschat et al. 2012). Possible explanations for the inconsistencies among VBM studies in ASD include small sample sizes, dynamic non-linear changes in brain structure across development (Ecker et al. 2014; Courchesne et al. 2011a) and phenotypic heterogeneity within the autism spectrum.

Nevertheless, SVM studies using grey matter VBM feature sets to discriminate children, adolescents and adults with ASD from unaffected controls have achieved promising accuracies (77–92%) (Segovia et al. 2014; Ecker et al. 2010b; Uddin et al. 2011). With the caveat that most of these studies recruited relatively small, well-defined research samples, thus potentially limiting the generalizability of these classification models (Segovia et al. 2014; Ecker et al. 2010b; Uddin et al. 2011). Furthermore, compared to traditional univariate analytical techniques, these multivariate methods are more sensitive to the diffuse pathology dispersed across brain networks in ASD. For example, voxels contributing most to the classification of ASD in these VBM studies are located in brain regions that have previously implicated in the condition (Amaral et al. 2008), including the medial temporal (amygdala) and posterior parietal regions as well as the inferior frontal gyrus, cingulate gyrus, hippocampus and cerebellum (Segovia et al. 2014; Ecker et al. 2010b; Uddin et al. 2011). However, the patterns informing classification in these studies did not always include brain regions with significant mean differences between groups, meaning that standard VBM approaches would not necessarily have identified their link to ASD.

Although resolution at the level of the voxel is a strength of VBM-driven classification approaches, further model refinement is now possible on the basis that cortical volume is, by definition, a product of cortical thickness (CT) and surface area (SA) (e.g. Ecker et al. 2010a). This is important as CT and SA represent distinct aspects of cortical architecture (Rakic 1995) with different developmental trajectories (Ecker et al. 2014) and genetic determinants (Panizzon et al. 2009). Therefore, these measures may not only comprise more sensitive features for classification models but may also be more closely related to specific causal mechanisms underlying ASD. In line with this, preliminary evidence seems to suggest that models based on CT may show higher overall classification accuracies compared to those trained using volumetric features (Jiao et al. 2010; Sabuncu and Konukoglu 2015; Xiao et al. 2017), though to date, relatively few studies have reported classification of ASD using SA measures. One such study compared the predictive value of five different morphological features, including CT, SA and measures of cortical folding (mean curvature, sulcal depth, metric distortion) in ASD (Ecker et al. 2010a). The authors found that, of all these features, CT measures in the left hemisphere had the highest overall accuracy, sensitivity and specificity for the classification of ASD (Ecker et al. 2010a). This suggests that identifying the specific biological underpinnings of atypical CT in ASD may be etiologically meaningful. For example, it has been proposed that factors including increased numbers of neurons

(Courchesne et al. 2011b), dysregulated dendritic arborization (Tang et al. 2014) and/or atypical myelination (Sowell et al. 2004) may influence CT and consequently contribute to ASD phenotypes.

The wide distribution of grey matter pathology revealed by both conventional and pattern classification studies of ASD has been taken to imply a network level disruption in the condition. Diffusion weighted imaging (DWI) studies of white matter tracts – and particularly long-range connections between disparate brain regions – are consistent with this proposal. Of these, the corpus callosum has been the most widely studied, with reports of decreased volume (Frazier and Hardan 2009), lower fractional anisotropy and higher radial diffusivity (Alexander et al. 2007; Barnea-Goraly et al. 2004; Shukla et al. 2010) in ASD individuals relative to controls. These findings are believed to reflect underlying changes in myelination, density and size of axons within tracts (Hong et al. 2011) and, at a behavioural level, lead to deficits in information integration (i.e. weak 'central coherence') (Geschwind and Levitt 2007; Frith 2004; Just et al. 2012). Classification studies using SVMs trained on white matter VBM data have been successful in separating individuals with and without ASD, though they tend to have lower accuracies compared to SVMs trained on grey matter VBM features (Uddin et al. 2011; Ecker et al. 2010b). However, other studies that have utilized more specific diffusion measures of white matter tract microstructure, namely, fractional anisotropy (FA) and mean diffusivity (MD), have reported classification accuracies in line with the best performing grey matter features (i.e. 80–90%) (Ingalhalikar et al. 2010, 2011). These studies support the role of the corpus callosum in ASD but have also found that diffusion measures within tracts such as the superior longitudinal fasciculus and temporal white matter are highly discriminative in ASD.

5.2 Functional and Connectivity Features

Beyond the use of structural features, a number of groups have examined whether measures of brain function can classify individuals with ASD. These studies have explored the use of both task-based and so-called 'resting state' functional MRI (fMRI). In task-based paradigms, fMRI signal is acquired while individuals are presented with sets of stimuli or cognitive tasks. Conventional task-based fMRI studies have identified atypicalities in ASD during a wide variety of paradigms designed to probe executive functioning, visual processing, auditory and language perception and social cognition (for review, see Philip et al. 2012). As task-based studies are designed to probe the cognitive underpinnings of specific clinical symptoms or behaviours in ASD, the expectation is that task-based fMRI data would have particular strengths for the classification of ASD. Indeed, tasks based on theory of mind (Deshpande et al. 2013) and social processing (Just et al. 2014), albeit in small research samples (n = 15–17 ASD participants), have reported high accuracies in classifying individuals with ASD.

In contrast to task-based fMRI, 'resting state' fMRI (rsMRI) seeks to identify intrinsic synchronous activity across brain networks while individuals are awake but not engaged in a particular cognitive task. This approach helps avoid the possibility that the cognitive or performance demands of a task non-specifically confound interpretation (e.g. in individuals with low IQ). A growing body of rsMRI literature in ASD indicates that, although there are alterations in neural connectivity in the condition, these are complex. Initially, it was proposed that ASD could be described as a condition of local over connectivity and global under connectivity. However, there has been challenges in replicating these findings (for review, see Hull et al. 2016). Within ASD, the default mode network (DMN) has received particular attention. This global network is active when individuals are awake and alert but not engaged in a particular cognitive task or goal-oriented behaviour and is 'deactivated' during active cognitive processing (Raichle et al. 2001). The DMN has been linked to theory of mind and social processing tasks (Andrews-Hanna 2012) and has been reported to be underconnected in ASD (Jung et al. 2014; Murdaugh et al. 2012; Starck et al. 2013; Wiggins et al. 2011). Indeed, some studies have identified a relationship between the degree of DMN underconnectivity and the severity of ASD symptoms (Weng et al. 2010; Assaf et al. 2010). Similarly, rsMRI under connectivity in ASD has been reported within the salience network (Ebisch et al. 2011; von dem Hagen et al. 2012), which incorporates brain regions implicated in self-awareness (i.e. the insula), as well as language networks (Verly et al. 2014). In contrast, overconnectivity of subcortical regions such as the striatum and thalamus has been reported in ASD (Di Martino et al. 2011; Cerliani et al. 2015) and linked to sensory abnormalities that are core to the condition. These differences in intrinsic rsMRI network connectivity have been used to distinguish individuals with ASD from unaffected controls with varying degrees of success (overall classification accuracy 76–96%).

By and large, these studies suggest that connectivity within the default mode and salience networks may be the most informative for ASD (Plitt et al. 2015; Murdaugh et al. 2012; Anderson et al. 2011; Uddin et al. 2013). Most recently, ASD classification using rsMRI data has been tested within the Autism Brain Imaging Database Exchange (ABIDE), with some promising findings emerging within this large publicly available multicentre data set (Chen et al. 2016; Iidaka 2015; Guo et al. 2017). However, reports of connectivity differences in default mode and salience networks are not restricted to ASD. For example, aberrant function within these networks has also been linked to schizophrenia and depression (Wang et al. 2015; Kaiser et al. 2015). This is consistent with the increasing appreciation that the diagnostic categories between psychiatric disorders can be 'blurred'. Thus, genetic and environmental risk factors for ASD overlap with risk factors for schizophrenia (Carroll and Owen 2009; Gandal et al. 2018), and both conditions increase the risk of mental illness such as depression (Buckley et al. 2008). Whether classification approaches targeting resting networks would also separate ASD from related neurodevelopmental disorders and common comorbid conditions has not been examined in depth; this is further discussed in the limitations section below.

5.3 Multimodal Classifiers

To date, classification studies have shown that multiple structural and functional imaging features can provide discriminative information for ASD. However, no single approach has been shown to significantly outperform all others. As a result, there is emerging interest in combining different imaging modalities together in single classification models. For example, decision tree algorithms have been used to include diffusion, morphometric and MR spectroscopy features as inputs for a single ASD classification model (Libero et al. 2015). This strategy not only showed significant discriminative accuracy for ASD but also suggested the possibility that classification methods could contribute to a better understanding of ASD risk and protective mechanisms. For example, high fractional anisotropy measures within the forceps minor, a white matter tract believed to be important for inter-hemispheric connectivity in the prefrontal cortex, was found to be associated with a lower chance of being classified as having ASD (Libero et al. 2015). Still other multimodal studies have managed to discriminate sub-groups within the spectrum, in one instance showing that diffusion MRI plus auditory task-based magnetoencephalography (MEG) data can not only classify ASD cases and controls but also ASD individuals with and without learning disability (Ingalhalikar et al. 2014).

5.4 Diagnostic Prediction in Infants

The classification of individuals with an existing diagnosis of ASD based on brain imaging measures has shown promise in the research setting. However, the participants in the studies reviewed thus far were preselected and already diagnosed according to gold-standard research criterion. It remains uncertain if these classifiers are generalizable to individuals whose diagnosis of ASD is not already known, i.e. whether the classification approach translates to 'real-world' clinical settings and to the general population. Arguably even more valuable would be a classification tool that can predict a clinical outcome which is not possible to determine at the time of the scan, i.e. where there is true diagnostic uncertainty. This is now a major focus in the field, which is working to deliver a model that can identify individuals with ASD before a clinical diagnosis can reliably be obtained via current gold-standard diagnostic tools (i.e. ~2 years of age). The overarching aim of such studies is for early identification to guide early interventions and improve childhood outcomes.

To address this goal, three recent studies have used MRI pattern classification of data acquired in the first year of life to predict an ASD diagnosis at 24 months. The first of these found that SA expansion rates in individuals with ASD from 6 to 12 months predicted later volumetric overgrowth that was related to the development and severity of social deficits (Hazlett et al. 2017). Furthermore, using a 'deep learning' neural network approach that mainly utilized SA measures, this study was able to classify infants who went on to receive a ASD diagnosis with 88%

sensitivity within the training set (Hazlett et al. 2017). A second SVM study of rsMRI in high-risk infants showed similar capacity to identify individuals who went on to receive an ASD diagnosis at 24 months based on connectivity patterns acquired at 6 months (Emerson et al. 2017). Last, measures of extra-axial spinal fluid at 6 months have been shown to predicative of later ASD diagnosis (Shen et al. 2017). Importantly, this later study reported a significant overall classification accuracy of 69% when applied to an independent testing sample of infants at elevated risk for developing ASD (Shen et al. 2017).

6 Limitations and Pitfalls of Pattern Recognition

While the studies reviewed here provide proof of concept that brain-imaging data can be used to identify individuals with ASD, their results and their translational value must be interpreted with caution. To date, the vast majority of classification studies in ASD have trained and tested predictive models within relatively small research samples using cross validation methods. Accordingly, a high degree of variance in model performance is to be expected, with negative results being potentially excluded from the literature due to a bias towards reporting positive results, i.e. the 'file drawer problem'. Within psychiatric and neurodevelopmental conditions, a trend towards decreasing model performance with increasing training sample sizes has been observed (Wolfers et al. 2015). This is likely largely attributable to the increased heterogeneity in clinical phenotypes within these large samples. Furthermore, in supervised learning problems (e.g. binary classification), training data is (ideally) composed of individuals that are very well characterized clinically (e.g. via expert clinical teams). Thus, all cases included in the training set meet strict gold-standard diagnostic criteria with non-case data derived from well-matched but unaffected controls. However, within the autistic spectrum, several overlapping symptoms and high rates of comorbidities introduce a risk for misdiagnoses in patients or the possibility that controls have subclinical ASD behaviours that may hinder model performance.

For example, individuals with ASD will generally be excluded from research if they (1) have comorbid conditions, (2) are taking certain medications or (3) fail to reach cut-offs on behavioural metrics such as the autism diagnostic interview (ADI) (Lord et al. 1994) and/or autism diagnostic observation schedule (ADOS) (Lord et al. 2000). In addition, individuals with severe ASD behaviours are often not included in imaging research as they may not tolerate MRI without sedation. The clinical ASD phenotype is highly heterogeneous and up to 70% of individuals with ASD also meet criteria for the diagnosis of a comorbid condition (i.e. major depressive disorder, generalized anxiety disorder, social anxiety, obsessive compulsive disorder, attention deficit/hyperactivity disorder) (Ashwood et al. 2016). Not only are the research cohorts with ASD used in classification studies unrepresentative of this heterogeneity and comorbidity rates, but control samples are also almost entirely free of the common mental health difficulties, which affect both ASD and the general

population. Some groups have attempted to address this and shown that ASD classifiers can distinguish the condition from related conditions such as ADHD (Ecker et al. 2010a; Lim et al. 2013), but no one has examined individuals with multiple co-occurring conditions. This is challenging but if addressed could be of great practical value.

The development of a classification model for ASD based on neuroimaging measures is also potentially impacted by the complex non-linear changes in volumetric (Courchesne et al. 2011a), morphometric (Ecker et al. 2014), diffusion (Lebel et al. 2012) and connectivity (Betzel et al. 2014) measures across the lifespan. Changes in imaging features across development are therefore likely to limit the efficacy of ASD classifiers to the particular stage of development in which they were initially trained. Additionally, there is growing evidence that factors related to biological sex may modulate risk for ASD (Ecker et al. 2017; Werling et al. 2016; Werling 2016) and contribute to the observed ~2–5:1 male bias in diagnosis of the condition (Lai et al. 2015). Given potential sex differences in the ASD phenotype, the development of sex-specific ASD classifiers may be warranted. To date most ASD classification models have been trained in male (or majority male) samples. To the best of our knowledge, only one study to date has trained and tested an ASD classifier in an all-female sample (Calderoni et al. 2012). Thus it is debatable whether future ASD classifiers should be sex-specific or mixed sex.

7 Future Directions

Classification studies in ASD have so far have attempted to distinguish mostly male individuals with relatively high-functioning, well-defined ASD from typically developing individuals without comorbid psychiatric (or other) conditions in the research setting. However, pattern recognition methods that can parse heterogeneity across the autism spectrum and effectively stratify individuals into biologically homogenous subgroups would not only aid in understanding the complex aetiology of the condition but potentially lead to more informed diagnoses and personalized targeting of interventions.

Methodological frameworks to achieve this are evolving (Marquand et al. 2016; Mourão-Miranda et al. 2011). These new techniques seek to identify individuals who significantly deviate from the normative population in particular symptom domains and to map the biological patterns associated with these deviations. A particular strength of these normative modelling approaches is that they are not limited to traditional diagnostic categories but can accommodate the complexities of an ASD population that is likely to contain heterogenous phenotypes, misdiagnosed individuals and related comorbidities (Marquand et al. 2016). Biological patterns associated with significant deviations from the normative range could be particularly informative for stratifying or clustering individuals with ASD into more homogenous sub-groups.

Recognising the dearth of pharmacological treatments for ASD, a further hope is that, in time, applications of normative modelling may improve targeting of treatments for the core symptoms and commonly co-occurring mental health difficulties associated with ASD. This approach has not yet been examined in ASD but is starting to show utility in other conditions. For example, in a study of depressed individuals, higher rates of treatment response were observed among patients who did not significantly deviated from a normative model based on fMRI responses to sad face stimuli (Mourão-Miranda et al. 2011). While several crucial issues remain to be addressed in the future, these techniques provide hope for better, more personalized intervention for mental illnesses that increase the burden for individuals with ASD and their families (Chang et al. 2012; Sukhodolsky et al. 2008).

8 Conclusion

As a complex and behaviourally diagnosed condition, autism spectrum disorder (ASD) presents several challenges to both clinicians and researchers seeking to understand the biological underpinnings of the condition. Neuroimaging studies have reported atypical measures of brain structure, function and connectivity that have been valuable in establishing a biological basis for ASD. More recently, several proof of concept pattern classification studies have shown that patterns in brain imaging data can be used to identify individuals with ASD, potentially even before a diagnosis for the condition is possible. While these studies are promising, the complexity of ASD and differences between clinical and research cohorts mean additional work remains to realize clinically useful imaging tools for ASD. Recent applications of normative models to clinical populations are particularly promising as they provide an analytical framework to address the large degree of phenotypic heterogeneity observed within ASD. These methods may be particularly valuable for stratifying the condition and delivering more personalized interventions for particularly burdensome symptoms.

Acknowledgements The authors would like to acknowledge all the participants, families and researchers who contributed to the studies reviewed in this chapter. The authors acknowledge support from the Innovative Medicines Initiative Joint Undertaking under grant agreement no 115300, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies' in kind contribution. DA and GM are partly funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. DA acknowledges support from the National Institutes of Health (NIH) grants MH103371 and MH104438. CE acknowledges support by grants EC480/1-1 and EC480/2-1 from the German Research Foundation under the Heisenberg Programme. AFM gratefully acknowledges support from the Dutch Organisation for Scientific Research (NWO), under a Vernieuwingsimpuls 'VIDI' Fellowship (grant number 016.156.415).

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Correction to: Imaging and Genetic Approaches to Inform Biomarkers for Anxiety Disorders, Obsessive–Compulsive Disorders, and PSTD

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Correction to:
Chapter "Imaging and Genetic Approaches to Inform Biomarkers for Anxiety Disorders, Obsessive–Compulsive Disorders, and PSTD" in: E. Maron et al., Curr Topics Behav Neurosci, DOI 10.1007/7854_2018_49

This chapter was inadvertently published with Fig. 1 which do not belong to this chapter and hence Fig. 1 is deleted from this chapter later.

The updated online version of this chapter can be found at DOI 10.1007/7854_2018_49

Curr Topics Behav Neurosci (2018) 40: 437–438
DOI 10.1007/7854_2018_64
Published Online: 24 October 2018