Innovations in Cognitive Neuroscience
Series Editor: Vinoth Jagaroo

Vinoth Jagaroo
Susan L. Santangelo *Editors*

Neurophenotypes

Advancing Psychiatry and Neuropsychology in the "OMICS" Era

Springer

Innovations in Cognitive Neuroscience

Series editor

Vinoth Jagaroo, Boston, Massachusetts, USA

More information about this series at http://www.springer.com/series/8817

Vinoth Jagaroo · Susan L. Santangelo
Editors

Neurophenotypes

Advancing Psychiatry and Neuropsychology in the "OMICS" Era

Springer

Editors
Vinoth Jagaroo
Department of Communication Sciences
and Disorders
Emerson College
Boston, MA
USA

and

Behavioral Neuroscience Program
Boston University School
of Medicine
Boston, MA
USA

Susan L. Santangelo
Department of Psychiatry
Tufts University School of Medicine
Boston, MA
USA

and

Maine Medical Center Research Institute
Maine Medical Center
Portland, ME
USA

and

Department of Epidemiology
Harvard T. H. Chan School
of Public Health
Boston, MA
USA

ISSN 2509-730X ISSN 2509-7318 (electronic)
Innovations in Cognitive Neuroscience
ISBN 978-1-4614-3845-8 ISBN 978-1-4614-3846-5 (eBook)
DOI 10.1007/978-1-4614-3846-5

Library of Congress Control Number: 2016958982

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Science+Business Media LLC
The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Preface

In recent decades, biology and medicine have seen developments that differ uniquely from the research contexts of the past. If there is a single term that captures these developments and the new landscape that they shape, it is 'omics.' It represents an approach to describing a biological entity or system using detailed, multi-scaled, multi-dimensional data and equally complex analyses of the data, both made possible by bioinformatics. 'Omics' is synonymous with systems biology, which deals with the relational understanding of complex, collective systems of organisms. So widespread and intense have been the proliferation of omics disciplines that it has prompted the expression in jest, 'Who needs another omics discipline?'

To the brain-behavioral sciences, omics is a welcome and much needed approach. Unraveling the complexity of the brain and the intricacies of interactions between the genome, the brain, and the environment demands an approach commensurate in its sophistication. Powerfully emerging omics approaches applied to the brain are moving brain science into a new era. Numerous genetic loci are showing statistically significant associations with schizophrenia in genomic studies involving tens of thousands of cases. Brain circuits are being linked to gene modules via transcriptomic studies of brain tissue. Genome-to-phenome mapping has inspired the discipline of cognitive phenomics. Connectomics signals the prospect of dense and detailed mapping of neurons. And the US National Institutes of Mental Health has set in motion Research Domain Criteria (RDoC), an initiative toward a brain-based nosology of mental disorders where neural circuits and related phenotypic markers form the units of analysis.

These developments translate into various breakthrough achievements. Though remaining far from fully understood, it has long been recognized that a multitude of variables are orchestrated in brain development and in brain-behavioral relationships. Even a 'simpler' question such as the adaptation of a neural circuit to a new stimulus requires the study of numerous elements and variables. With the omics scale of data volume, data specification, data quantification, and complex mappings between multi-level data sets, the functionality and methods are provided to investigate complex questions such as follows: What might be the

polygenic nature of a mental disorder and how might this be expressed at subcellular and synaptic levels or at the levels of neural circuits? How do the permutations of multiple brain systems result in specific patterns in cognitive functional domains? and How can the spectral nature of many cognitive and psychiatric disorders be understood in terms of the differential expression of neural systems? Such questions, as this volume illustrates, are no longer lofty and solely theoretical. And they are beginning to compel major course changes in the clinical neurosciences. The development of RDoC is evidence enough of the near certainty that description and diagnosis of cognitive and psychiatric disorders will shift from categorical approaches to dimensional approaches—where discrete, separable cognitive, and neural features along various continua converge to form a diagnostic profile.

There are many ways by which psychiatry and neuropsychology can engage with this new research environment. This volume is about one all-important step. To both serve and benefit from a meaningful integration with the omics approach to the brain, cognitive and neural features need to be described in a standardized, scientific format. For the cognitive and neural phenome to be systematically linked to the genome and to other shaping or modulatory factors, and for this to be carried out in an omics/informatics environment, the units of analysis are critically important. They need to be precise and they need to have relational utility so that they can be tied to all their shaping mechanisms and developmental precedents. The term 'neurophenotype' is used in this volume as a general term to describe this kind of neural or cognitive feature. The neurophenotype approach to brain-behavioral associations and clinical diagnoses relies on precise cognitive and neural markers. It differs from approaches that are phenomenological-descriptive and detached from brain science (psychiatric diagnostic manuals), or approaches that compound many cognitive processes into a poorly operationalized amalgam (a subtest in a neuropsychological battery) and which, at best, can only be tied to the brain at a gross anatomical level. The neurophenotype approach facilitates the understanding of a profile of cognitive and neural features of an individual, the coexpression or variable expression of a common set of features across different diagnostic groups, and the biological mechanisms that may mediate the features.

The neurophenotype approach is, however, in its infancy. Neurophenotypes are currently not specified in a uniform or organized manner. Some of this has to with the difficulty of circumscribing processes or neural systems that may constitute neurophenotypes. If neuronal, circuit, or neuroanatomic phenotypes are viewed primarily in terms of genetic precedents, the possible impact of non-genetic factors can obviously be raised. If circuit neurophenotypes are viewed as central mediators of cognitive processes, then a host of intrinsic and extrinsic circuit modulatory variables complicate the picture, and the question of just what is the circuit, arises. There are many putative neurophenotypes. Many neural systems and cognitive processes have been cast into working definitions as neurophenotypes. All of these can be debated. Neurophenotypes and all their formalisms are evolving, but as a force. The current stage of this development and its associated topics, especially as applied to the clinical neurosciences, are discussed in this volume.

The volume was motivated by the authors' interests in cognitive neuroscience and neuroinformatics (Jagaroo) and cognitive and psychiatric genetics and bioinformatics (Santangelo). The vibrant intersections of neuroscience and genomics contextualized within a genome-to-phenome landscape can be felt throughout the research literature. It is hoped that capturing these developments and organizing the themes using the format of a composed volume will help better engage the clinical neurosciences in the discourse.

Boston, MA, USA Vinoth Jagaroo
Portland, ME, USA/Boston, MA, USA Susan L. Santangelo

Acknowledgements

To our many colleagues who generously submitted chapters to this volume, we are thankful for their contributions and for their patience that we no doubt tested. Thanks also to Janice Stern and Christina Turballes at Springer for their cooperation and guidance. Special acknowledgment goes out to our former and current graduate assistants who worked so diligently on the logistics, production, formatting, and proofing of the volume: Melissa Marois whose assistance helped set the volume in motion; Yukiha Maruyama, who played a pivotal role in the middle phase of the project; and Kim Wang, for her invaluable assistance with the completion of the project.

Contents

Part I
Research and Conceptual Developments

Chapter 1
Introduction and Structure of the Volume

Vinoth Jagaroo and Susan L. Santangelo

Biomedical research has over the past few decades been dominated by the revolution of molecular biology and genetics. Featuring prominently during this time has been the notion of "biomarkers." The very ubiquity of the term signifies the utility and promise of a strategy that identifies genetic, molecular, neurophysiologic, neuroanatomic, and neurocognitive features as indices of disease. The interest in biomarkers has been a part and parcel of the rise of molecular biology—certainly the mapping of the human genome which was driven in part by the goal of mapping genes to diseases (International Human Genome Sequencing Consortium 2001) was a major catalyst event. Biomarkers have been cast as objectively measured characteristics that signal a pathogenic condition or aid in predicting treatment efficacy and prognosis (Biomarkers Definitions Working Group 2001).[1] They may indicate disease presence, type, stage, etc., but may also aid in the subtyping of the

[1]The Biomarkers Definition Working Group was convened by the National Institutes of Health.

V. Jagaroo (✉)
Department of Communication Sciences and Disorders, Emerson College, Boston, MA, USA
e-mail: jagaroo@bu.edu

V. Jagaroo
Behavioral Neuroscience Program, Boston University School of Medicine, Boston, MA, USA

S.L. Santangelo
Department of Psychiatry, Tufts University School of Medicine, Boston, MA, USA
e-mail: ssantangel@mmc.org

S.L. Santangelo
Maine Medical Center Research Institute, Maine Medical Center, Portland, ME, USA

S.L. Santangelo
Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA, USA

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_1

normal phenotypes, and a biomarker may have stand-alone predictive power or may be useful when seen in specific combination with other markers.

Advances in molecular biology have been intertwined with technological advances enabling large, complex data sets to be captured, analyzed, and deciphered using automated procedures at high speed and relatively low cost. Biomarker developments have been closely tied with the "omics" revolution. "Omics" in terms such as *genomics*, *transcriptomics*, *proteomics*, and *phenomics* marks two notable features: (a) A massive scale of data sets or analytical variables processed via automated, "high-throughput" procedures; and (b) that which enables the former—computerized tools, databases, knowledge discovery/datamining algorithms, etc., encompassed by the field of *bioinformatics*. Over the last two-and-a-half decades, biomedical sciences have been marked by the "omics revolution." In the omics era, biomarker discovery has made great strides, which has sweeping implications for all biomedical disciplines.

Over the past decade and half, there has also been extensive discussion of markers in the context of the behavioral neurosciences. This surge of interest has been tied in part to major advances in genetic analysis, especially genome-wide association studies (GWAS)—high-throughput scans of the common variation in the entire genome that identify single-nucleotide polymorphisms (SNPs) associated with diseases. Such developments have given significant impetus to the idea of neurocognitive markers in the context of neuropsychology and neuropsychiatry. In these domains, specific cognitive and neural phenotypes or features have come to be viewed as constituent or putative markers—markers framed around the constructs of cognitive and neural systems. Discussion of neurocognitive markers went through a phase when it was heavily anchored around the very influential construct of the endophenotype (reviewed in other chapters). However, in the short span of the last ten years, the concept of neurocognitive markers has found itself in a new theoretical landscape, one marked by a confluence of a few major and inter-related developments. Altogether, these developments have been making for a greater push toward refined neurobehavioral descriptors. These developments are described below.

Genome-to-Phenome Mapping and Phenomics: The proliferation of the omics disciplines can also be viewed as the result of the greater force of "systems biology," the approach in biology that seeks to quantify genes, their molecular and protein products and regulatory functions, as well as the complex interactions between these elements. The mapping of an entire biological system involves the mapping of genes (the genome) to their products and functions—phenotypes or the "phenome." In between the genome and the most visible phenotype level, lies a myriad of phenotypic strata (proteins, cells, tissues, etc.). Many complex interactions occur between these "intermediate" phenotypes. The mappings between the genome and the phenome, intricate as they may be, are now rendered tractable with the advances in systems biology and information technology. However, while there have been considerable gains in profiling the genomic end of the genome-phenome spectrum, the phenomics end, especially in terms of neurobehavioral features, has not seen a commensurate level of analysis. For genomic data to have

greater utility and meaning, it needs to interface with similarly specified phenomic data. This calls for a finer specification of the phenome—"high-dimensional" phenomic data, described along a format that enables meaningful mappings with lower level phenomic data, and ultimately with the genome level. *Phenomics* is the discipline that seeks to specify and quantify the phenotype in such a manner as to enable the systematic understanding of the phenotype in the context of genomics, that is, to bring a systems-level analysis to the phenotype. With reference to neural systems and neurocognitive disorders, the phenomics approach can be framed around questions such as: How can the brain and brain-mediated illness be informed by the context of molecular biology, genetics, and the neurobiological systems that they shape? How can neuropsychiatry and neuropsychology reap the benefits of systems biology and integrative neuroscience?

Connectomics: Large-scale initiatives aimed at creating a detailed map of the structural connections of the brain have gotten under way in recent years. Known as "connectomics," these initiatives seek to understand neuronal and glial connectivity patterns in the entire brain. The envisaged map, "the connectome," can be described at many scales. They range from the cellular/microscale end to the white matter projection systems/macroscopic end. Microscale connectomics relies mainly on the tools of automated electron microscopy combined with artificial (computer) vision algorithms—images of tissue slices are integrated into 3D volumetric representations of a sample of brain tissue, showing cell structure, synaptic and subcellular detail. Macroscale connectomics relies mainly on fMRI (especially resting-state fMRI)—white matter fiber systems can be traced, and distributed functional brain networks can be mapped dynamically. The trajectory of connectomics has not been tied per se to the general initiative of phenomics; it has had a separate course. It just so happens to be a well-specified example of an initiative that meets the call of phenomics since it amounts to a rendering of the neural phenome. A number of issues have arisen around connectomics—questions such as the optimal scale (level of detail) at which the connectome should be specified, the utility of detailed maps. By any account though, connectomics is on the fast lane, and with the prospect of detailed neural mapping comes an array of challenges to behavioral neuroscience. If neural circuitry can be finely mapped, how are functional data to be overlaid on well-specified circuits of all scales? Transposing the problem to neuropsychology and psychiatry implies, again, that cognitive and behavioral constructs need be specified in a form that can be rendered compatible with emerging neural detail at the physical level. The development, described below, is even more explicit on this point.

Research Domain Criteria (RDoC): In 2008, the US National Institute of Mental Health laid out an initiative to describe and define mental disorders based on neural features that can be tied to the biology of the brain, that is, an initiative toward a nosology for mental disorders that is aligned with neuroscience. Diagnostic categories of mental disorders based on symptom clusters have faced some classic shortcomings, among them being the lack of representation of heterogeneity within diagnostic groups, ill-suitability to understanding comorbidity across diagnostic groups, and having a profound incompatibility with the current

era of biological and brain sciences. If what is termed a syndrome in a conventional classification system is comprised of a combination of discrete neural features, and if each feature can be mapped to specific genetic abnormalities, then it is theoretically possible to plot the genome-phenome matrix for each feature. With this type of mapping, the polygenic nature of mental disorders can be better elucidated as can the spectral nature of disorders and the complex permutations of a shared neural matrix that mediates the disorders. To enable such possibilities, RDoC adopts a dimensional view of a trait—viewing it along a continuum. It also postulates that dysregulation of "neural circuits" (variation in circuit phenotypes) accounts for disorders. In the genome-phenome matrix, RDoC is pitched at the level of the "neural circuit." While RDoC has been heavily debated since its inception, and remains at early stages of development, it marks a turning point in the study and classification of neurocognitive and neuropsychiatric disorders. It attempts to lay the groundwork for a neuroscience-based description of normal and disordered perception, cognition, and emotion as well as a neuroscience-centered nosology of mental disorders. This fundamentally changes the language and methods of classification.

Bioinformatics and Knowledge Discovery through Data Mining: Well established over the course of more than three decades, the discipline of bioinformatics needs little introduction. It is widely recognized for its highly specialized application of computer science, information science, and mathematics to the research context defined by the biomedical sciences. Specifically, it is geared to challenges around data cataloging, data visualization, and data mining—for patterns and comparisons among intricate and/or large data sets, and the drawing of conceptual frameworks for complex biological systems. Bioinformatics has developed in parallel with molecular biology and has gained prominence in the process. The application of bioinformatics in the neurosciences is often referred as *neuroinformatics*—exemplified by the Human Brain Project—that involved a host of neuroimaging tools, and a range of organism-specific databases on neural structure. In neuropsychology, there has been a slow but steadily growing call for a reformatting of the discipline to make it informatics-compatible.

Biomarker discovery is inseparable from bioinformatics. Analytic variables and data on a massive scale, as often seen in the omics disciplines, require automated data handling, extraction and databasing. High-dimensional data sets are manageable only with compatible forms of databases. And most significantly, pattern extraction across the data is algorithm-driven. The discernment of meaningful patterns in the data via data mining algorithms alone has come to be termed *knowledge discovery through databases* (KDD). It has emerged as a new ("fourth dimension") dimension of research and has come to be termed *discovery science*. That is, with the inordinate amount of research data available, discoveries can be made "in silica" (through bioinformatics and data mining methods)—discoveries made possible only with large or complex, and often pooled data sets, and which lie well outside the scope of single experiments or the capabilities of individual scientists. In contrast to traditional hypothesis-driven research, discovery science is generally hypothesis-generating.

In the omics environment, the achievement of bioinformatics-driven discovery hinges on a central operating principle: The data are coded and classified using a common format, thus enabling comparison between or across multiple strata in the genome-phenome matrix. Common ontological formats have been established to the point where one researcher's data set can be compared to another's using common descriptors fed into a computer. However, the glaring exception to this critical adaptation happens to be in the realm of neurobehavioral descriptors—neurocognitive- and neuropsychiatric-related processes, concepts, etc. And this problem is crucially tied in with the mission call of RDoC and phenomics. Further, integration across the G-P matrix is entirely dependent on informatics platforms. And if the processes of perception, cognition, and emotion, lying at one end of the G-P matrix are to be meaningfully integrated with other levels of analysis, these processes have to be spelled out in a language that is both compatible with a systems-level format and an informatics-driven integrative platform. Psychiatry and neuropsychology, hence, will need to face radical adjustments or realignments. The biomarker approach, fitting in with systems-level, informatics-geared analyses, is a logical strategy in aiding this transition.

The collective force of these developments has made for an environment where the biomarker approach to neurocognitive processes, in view of its sweeping significance must be engaged with. It is a strategy that is compelled by current technical advancements that show promise in the linkage of biology and behavior. In essence, the biomarker approach to brain and behavior is driven by developments around fundamental imperatives—the mapping of the biological matrix of the brain, from genes through to the neural circuits they shape; how behavior is an emergent property mediated by neural systems; and the parsing out of neural and cognitive features which will in turn aid in the understanding of their normal and abnormal variations and permutations. It is about the specification of neural systems and dynamic neural processes, and a descriptive framework for cognition and emotion that is commensurate with the emerging neural delineations. Clinical imperatives are in turn served by the biomarker approach. These markers may offer accurate predictive and diagnostic features, may serve to monitor disease state and progression, define clinical end points, and gauge clinical efficacy. The cataloging of brain-related biomarkers and their analyses through novel computational techniques and big data sets is fundamentally changing the way the brain and brain-related disorders are being approached.

The discourse on the biomarker approach in clinical neuroscience has been generally affirmative. Certainly arguments for the utility in defining intermediate phenotypes, the calls for phenomics, and the calls for an RDoC-based model for psychiatry have been passionate and explicit. However, the marker-phenotype approach has also met with critical examination: Exactly what defines neural and cognitive markers? What is the optimal level of definition when dealing with neural systems and the brain—the gene-, cell-, circuit-, or some other level? What kinds of markers, intermediate or otherwise, have relevance to neuropsychology and psychiatry? When dealing with cognition and emotion, mediated by multiple neural systems, how are discrete features to be parcellated? What

about environmental variables and the epigenome—how do they factor into a G-P matrix? Can the complexities of behavior and its mediating neural systems be neatly refracted using the G-P model? And is the nature of behavior and clinical practice such that a certain amount of (multifactorial/multivariate) fuzziness will always remain? These are just some examples of the many issues that can be raised in critique of the biomarker (neurocognitive marker) approach.

Yet, by any account of the current trends in systems biology, especially systems neuroscience, genomics, and phenomics, by any account of the overall discussion of RDoC (let alone the very compelling fact that the initiative has already been established), and by any account of the new informatics-driven research environment defined by "big data" and discovery science, it is abundantly clear that the biomarker and systems neuroscience approach in psychiatry and neuropsychology is not a passing trend. It is here to stay and will sooner than later change the entire playing field.

Within this context, this volume explores the domain of neural and cognitive markers in neuropsychiatry and neuropsychology. It outlines the factors that compel the biomarker approach. It relates some of the many processes seen as constituting markers in the neurobehavioral domains. It also highlights the theoretical complications arising when trying to define cognitive and neural systems as markers in the realm of cognition and emotion. The volume clearly takes the perspective that neurocognitive markers make for a fitting approach by which the clinical and cognitive neurosciences can strive toward greater connection with systems biology and genome-to-phenome integrative models. The motivation behind the volume was to organize and present this very significant topic to a greater professional audience—to take it beyond the relatively small and specialized research/academic clusters where different facets of the topic have been comfortably lodged. The topic of the volume is pertinent to both the clinical and research domains in neuropsychology, psychiatry, neurology, cognitive neuroscience, and allied disciplines. Current, cutting-edge developments in the brain sciences and systems biology call for the structure and processes of perception, cognition, emotion, motivation, mood, personality, etc., to be delineated in a new fashion. This structure is far more sophisticated than conventional psychometric quantification and phenomenological, syndromal-based clinical descriptions. This volume serves to outline this new operating environment. It serves to embrace the initiative of reformatting the clinical neurosciences so that they can better serve research and clinical imperatives. And, quite importantly, the volume also serves to highlight a multitude of issues that arise as psychiatry and neuropsychology find themselves in a new and arguably unprecedented, "disrupting," technological-scientific environment. But the volume neither attempts nor presumes to constitute an exhaustive rendering of the subject—which in this age of rapid research and informational shifts would be unrealistic. The volume simply offers a synopsis to serve as a basis for discourse in the clinical neurosciences, as prompted by compelling scientific shifts.

1.1 Coming to Terms: "Neurophenotype"

The concept of a neurocognitive marker does not fit a static or neatly circumscribed definition. Specification of the concept has been generally poor, hinging heavily on the endophenotype concept. And markers in the domains of neuroscience and cognition are inevitably shadowed by biomarker concepts that have a strong clinical orientation—the Biomarkers Definitions Working Group (2001) placed emphasis on biomarker utility in clinical applications—disease diagnosis, staging, etc. Certainly, the lack of consensus around the term "behavioral phenotype" has long been acknowledged (see Skuse 2000), while an attempted consensus-based working definition refers broadly to features and characteristics of cognitive and motor patterns that may have genetic associations (see Society for the Study of Behavioural Phenotypes, www.ssbp.co.uk). Definitions and conceptions of neurocognitive markers are likely to evolve dynamically, directed by new research gains and new analytic approaches. Yet, an operating definition of neural and cognitive markers at this early point in the volume is called for, as is a simple and expedient umbrella term to cover the expanse of potential neural and cognitive processes and patterns. We adopt the term "neurophenotype" for its conciseness and its embrace of neural systems and the sensory, cognitive, and emotional processes that they mediate.[2] Depending on the specificity of the application, parts of the volume may apply the terms "neural" or "cognitive" phenotypes. Our usage of the term "neurophenotype" (NP) rests on the following operating definition:

a. Neurophenotypes may be inclusive of all sensory, motor, cognitive, and emotive processes, and their neural correlates, ranging from subcellular processes, to all scales of circuitry, to neuroanatomic features, and including dynamic neural activation patterns (electrophysiological, functional imaging, etc.). However, it should be representative of the complexity or functional mechanism of the particular level/s in the phenotypic matrix in which it is situated.
b. A neurophenotype need not be associated exclusively with a disease state but must constitute, either singularly or in combination with other NPs, a reliable marker—differentially expressing in subgroups of the normal population, as well as in disease populations when compared to a normal population. However, what defines a "reliable" or even a "useful" marker is not a question we presume to resolve—but certainly entertain through the discourse of the volume. Notions of reliability and usefulness will in part be dictated by evolving research data.
c. A neurophenotype should ideally have an integrated fit, or have the potential to fit, within a larger associative, developmental, or physiological network. Examples of these are gene-regulatory networks (perhaps the best known

[2]The term was used by Sörös and Stanton (2012) in a discussion on a revised approach to studying auditory brain function, factoring in genomics and neuroimaging. The term has also been embraced by Craddock et al. (2013) in the context of neuroimaging-related phenotypes.

> Bakare et al. (2012) example), epigenetic-neurodevelopmental interactive networks; neurohormonal modulatory networks; and bio-electrically driven, gap-junction (synaptic)-mediated regulatory networks. In such associative networks, the NP may be part of a gene-linked causal chain, and may in some instances mark causality, but this is not a criterion. Certainly in this definition, the principle that a marker be tied via phenome-to-genome dissection to a genotype is not a requirement, and the rationale for this is summarized below and elaborated in Chap. 15.

Neural systems and hence the processes they mediate may be causally linked to deeper levels of the phenomic strata (e.g., proteins, cells), but their physical or functional patterns may also be significantly determined by (a) external, environmental, and epigenetic factors, (b) by intrinsic circuit dynamics that involve factors such as resting potentials, bioelelectric voltage gradients, and long-term potentiation, and (c) chemically based gradients and modulatory networks. The intrinsic dynamics of complex physiological networks can manifest patterns strong enough to instruct neural or cognitive phenotypes such that in these instances, the phenotypes are independent of gene-regulatory networks. This is a factor that is substantiated in Chap. 3 and shapes our working definition of NPs. All levels of neural and cognitive phenomic space are accommodated. And while these complex systems can in turn be conceived as interacting with the total phenomic makeup of the organism, such consideration is well beyond the scope of our focus. Wide accommodation of features runs the risk that any random feature, trait, or test result can be cast as a NP. We mitigate this seeming pitfall by applying the framework of an associated or linkage network within which a NP should ideally be contextualized. However, we also emphasize that regulatory networks that causally and scientifically frame NPs are not limited to gene networks. Further, in the context of phenomics and data-driven neuroscience, NPs may also be derived through informatics-driven discovery and may take novel forms; examples of this will be covered in later chapters.

1.2 Structure

The volume is structured in three parts. The first part of the volume (Chaps. 1–3) is introductory—presenting various research and conceptual developments in the neurosciences and biomedical arena that are directing changes in neuropsychology and psychiatry. It affirms the new omics environment while also highlighting the complications around NPs in the genome-to-phenome framework. In Chap. 2, we, Susan Santangelo and Vinoth Jagaroo, elaborate on some of the developments outlined in this introduction, developments that propel the NP approach. The focus on phenomics, connectomics, and RDoC details the landscape that compels the NP approach, especially NPs that are described at the level of "neural circuit." Chapter 3, by Vinoth Jagaroo, William Bosl, and Susan Santangelo, delves into the notion

of neural circuits. It appraises "circuit-centered" NPs by raising a number of factors that complicate circuit phenotypes, and also by addressing the value of circuit-centered NPs.

Part 2 of the volume (Chaps. 4–6) provides a review of the endophenotype (EP) concept. The currency it wields in the very subject of this volume necessitates some revisiting of the concept—the imperative in raising it has to do with the discourse on the broader concept of NPs. While NPs have evolved in ways far divergent from the EP concept, this concept has been a major influence in the NP/marker approach in neuropsychology and psychiatry. (A theme that is raised in the volume is that as much as the EP concept has facilitated a marker-based approach in the behavioral neurosciences; its inertia has also hindered a broader exploration of neural markers in all their complexity.)

In Chap. 4, a systematic review of the EP concept is given by Carrie Bearden, Anderson Winkler, Katherine Karlsgodt, and Robert Bilder. How EPs are differentiated from other markers and the criteria by which they are defined are laid out. Chapter 5, by Ellen Quillen, David Glahn, and Laura Almasy, further probes the strategy and utility of the EP approach but with special attention to the genetic and etiological heterogeneity of psychiatric diseases. As will be apparent to the reader, complications tied to the EP concept as seen through Chaps. 4 and 5, to varying degrees extend to NPs. Chapter 6, by Amy Vashlishan-Murray, provides a critique of the EP concept in the form expressed within an idealized notion of a genome-to-phenome framework. It examines assumptions made about heritability in GWAS studies, heritability of complex traits, and what they imply about the reliability and validity of genome-phenome situated EPs.

The third part of the volume (Chaps. 7–14) samples various neural and cognitive processes that have been or may be explored as NPs or cognitive EPs. Each chapter in this section describes a neural system or cognitive process and then explicitly examines how it may constitute an EP or NP. Because the extensive literature on cognitive EPs provided a common reference point for most of these chapters, they refer more frequently to the EP concept. The question of whether the cognitive or neural operation under discussion constitutes an EP or NP is also carried implicitly. It is to be judged by the reader against the backdrop of themes covered in the preceding parts of the volume.

Cognitive and neural phenotypes are still emerging concepts. It is infeasible that any single volume at this point can capture an optimally representative set of NPs. Nor can there presently be a finite set of questions and issues around NPs. The selection of putative or suggested NPs described in this second part of the volume was made through informal consultations with colleagues doing work on the subject and guided by surveys of the literature at the time the volume was conceived. It was also influenced by very practical constraints, namely aiming for a concise volume (fitting in with the Springer series of which this is part), and the availability of those invited to submit chapters. Completely different sets of topics in this second part of the volume could just as well serve the purpose of the section. The selection was configured so as to reflect a wide-ranging set of questions around the concept of the NP, not a wide-ranging assortment of possible NPs. (We

fully acknowledge that many kinds of NPs, including some that are prominent in the research literature, may not be represented in Part 3. The group of NPs constituted by functional magnetic imagining profiles is a case in point—a topic so extensive that it is better suited to a dedicated volume.)

Part 3 is arranged as follows: Chap. 7, by Kei Mochizuki and Shintaro Funahashi, deals with *response inhibition* and its related prefrontal circuitry. The authors then consider response inhibition as an EP with reference to attention deficit hyperactivity disorder. In Chap. 8, Bronwyn Graham and Mohammed Milad tackle *fear conditioning,* including neurobiological models of fear conditioning and extinction. Discussion of fear conditioning as an EP is also discussed in the context of anxiety disorders. *Error Processing* is the topic of Chap. 9 where Dara Manoach and Yigal Agam cover its behavioral hallmarks and its neural mechanisms. This is followed by a discussion of error processing as an EP through its manifestation in schizophrenia, obsessive compulsive disorder, and autism spectrum disorder. In Chap. 10, Marlene Oscar Berman and Kenneth Blum detail the neural network for *reward reinforcement*, with emphasis on the dopamine D2 receptor system. This is contextualized by a discussion of the evolutionary genetics of dopamine, followed by a discussion of reward dependence and deficiency as EPs, and how this plays out in addiction, impulsivity, and compulsivity.

Face Perception as an EP, the topic of Chap. 11, is discussed by Jennifer Richler and Isabel Gauthier. Concisely covered are the neurocognitve mechanisms of face perception, which is then examined as an EP in consideration of distinct functions of the Fusiform Face Area, and also against the complexity of face perception as cognitive–perceptual specialization. Chapter 12, on *Language Phenoptypes*, varies the general thematic structure of chapters in Part 3 in that it samples not a single EP specific to a functional domain or neural system but numerous EPs within a functional domain. Here, Mabel Rice and Helen Tager-Flusberg give attention to language EPs that have emerged in the realm of developmental language disorders and which can be examined in relation to typical language acquisition. Chapters 13 and 14 take us into the realm of elecrophysiological markers. In Chap. 13, Mei-Hua Hall discusses *event-related potentials* (ERPs) as EPs. Six ERPs are selectively profiled to demonstrate their utility in neuropsychiatric diagnosis and brain-behavior investigations. Chapter 14 by William Bosl relates to *encephalographic (EEG) data,* but the chapter offers a novel perspective on EEG data that is quite unlike the conventional interpretation of the data. Viewing the brain through the frame of dynamical systems theory ("chaos theory" in the mathematical and physical sciences), the chapter describes how subtle yet highly dynamic data are reflected in EEG, and how such data can be exploited in detecting brain disorders and in monitoring the brain over the life span. The chapter also brings forth the utility of and power of NPs in a data-driven context—describing how machine learning algorithms combined with portable EEG systems and big data platforms can be leveraged in the context of global mental health initiatives.

In Chap. 15, the concluding chapter, Susan Santangelo and Vinoth Jagaroo consider various implications for neuropsychology and psychiatry brought on by the

need for NP specification in the context of the omics operating environment. The chapter raises a few conceptual and programmatic adjustments from which these disciplines could benefit. They include as follows: Some constraints on the default (inertial) application of the EP concept in order that emerging concepts that better fit contemporary network models in neuroscience and genetics can be appreciated; refinement of cognitive and behavioral constructs in the form of NPs that are compatible with genome-phenome or other scientifically based causal-associative matrices; and the use of NPs as the currency by which these disciplines can partake in a data-driven knowledge environment via the tools of bioinformatics.

References

Bakare MO, Munir KM, Bello-Mojeed M (2012) Public health and research funding for childhood neurodevelopmental disorders in Sub-Saharan Africa: a time to balance priorities. Healthc Low-Resour Settings 2014(2):1559

Biomarkers Definition Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69, 89–95 (Paper written by Biomarkers Definition Working Group, convened by the National Institutes of Health)

Craddock RC, Jbabdi S, Yan CG et al (2013) Imaging human connectomes at the macroscale. Nat Methods 10(6):524–539

International Human Genome Sequencing Consortium (2001) Human genome: initial sequencing and analysis of the human genome. Nature 409:860–921

Skuse DH (2000) Behavioural phenotypes: what do they teach us? Arch Dis Child 82:222–225

Sörös P, Stanton SG (2012) On variability and genes: inter-individual differences in auditory brain function. Front Hum Neurosci 6(150):1–3

Chapter 2
Brain and Cognition in the "Omics" Era

Susan L. Santangelo and Vinoth Jagaroo

The strategy of neural and cognitive markers as outlined in the introduction to the volume has been reinforced by some major research and theoretical developments. This chapter gives further consideration to these developments and includes some critical review. While the topics are greatly intertwined, they are described under specific subheadings below for ease of organization and explanation.

2.1 Genome-to-Phenome Mapping and Phenomics

Since the discovery of the structure of DNA, cell biology has been fundamentally organized around the now universal principal of DNA to RNA to proteins. How genes code for proteins, which in turn build cellular elements/cells, which form tissue types that then form organ systems, etc., has long been a central structural

S.L. Santangelo (✉)
Department of Psychiatry, Tufts University School of Medicine, Boston, MA, USA
e-mail: ssantangel@mmc.org

S.L. Santangelo
Maine Medical Center Research Institute, Maine Medical Center, Portland, ME, USA

S.L. Santangelo
Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA, USA

V. Jagaroo
Department of Communication Sciences and Disorders, Emerson College, Boston, MA, USA
e-mail: jagaroo@bu.edu

V. Jagaroo
Behavioral Neuroscience Program, Boston University School of Medicine, Boston, MA, USA

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_2

systems model in biology. Understandably then, the mapping of pathways by which genes exert their influence to build and modulate successive biological layers—genome-to-phenome ("gene-phene" or G-P) mapping—has been among the major goals of genomics (Bork et al. 1998; Korbel et al. 2005). With advances in molecular biology and with the advent of bioinformatics, the complex mappings between the genome and the phenome become tractable and feasible. G-P frameworks represent levels of analysis that describe and link the multi-level parameters in a complex biological matrix. And the mapping of these relationships hence becomes an all-important yet difficult challenge for genomics. The G-P framework is also an organizing model for systems biology " … that endeavors to quantify all of the molecular elements of a biological system to assess their interactions and to integrate that information into graphical network models … that serve as predictive hypotheses to explain emergent behaviors" (Hood et al. 2004).

In the complex equation of the G-P matrix, a thorough rendering of the picture at the phenotype level is a logical complement: If the expression of genes is to be traced to molecules, cells, tissue, organ systems, and behavior, then these characteristics, observable in different forms, are called to be systematically profiled. That is, characterization of the phenotype is a necessary complement to the progress in gene identification. Serving this agenda is the relatively new and flourishing discipline of *phenomics*. Schork (1997) made an early call for the discipline of phenomics (or "phenometrics" as he then suggested) which would seek to "unravel biochemical and physiological hierarchies leading from genes to clinical endpoints," a strategy that could be particularly useful in unraveling disease complexity.

> One could call the delineation of connections among various genes, gene products, intermediate phenotypes, and clinical endpoints "phenomics or "phenometrics" to match "genomics" and "biometrics" associated with aspects of pure genetic research. Such a science could proceed quite naturally by mapping genes involved in very low-level phenotypes and activities such as gene product variation and hormone amounts … and then attempt to link the phenotypes studied with higher-level phenotypes. (Schork, S107)

Figure 2.1 is an adaptation of Schork's schematic diagram representing a simplified "linear" relationship between a gene and its phenotypic product, via an expressed pathway. Many variations of such G-P schematics have since been rendered (e.g., Hunter and Borg 2003; Linden 2012), but Fig. 2.1 which is derived from the succinct version rendered by the Consortium for Neuropsychiatric Phenomics at UCLA (http://www.phenomics.ucla.edu/) has come to symbolize the phenomics strategy. Figure 2.2 is a more elaborate version and attempts to convey some of the hidden complexity in the model.

2.1.1 Phenomics as a Strategy and an Imperative

The case for phenomics, the systematic mapping of the entire phenome, has been cogently put forth in a series of articles by the UCLA group that has been leading

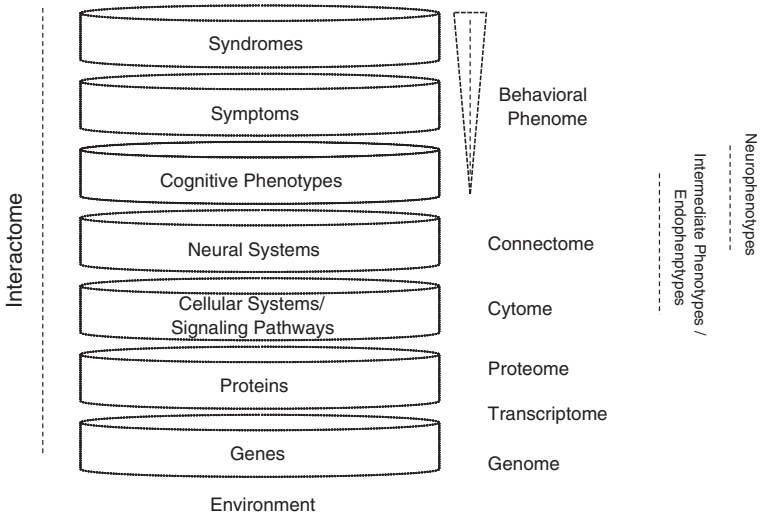

Fig. 2.1 Genome-to-phenome (G-P) framework. G-P frameworks may vary in the level of complexity spelled out and in the mappings described or hypothesized between the levels. The molecular levels typically described are genes (genome), elements, and processes of gene transcription (the transcriptome), and the resulting proteins (the proteome). Cellular levels characterize intracellular organelles, a host of intracellular processes, and cell types, altogether making a cellular phenotype (the cytome). Brain-related cellular organizational patterns and networks (the connectome) define phenotypes at a circuit level or in terms of morphologic or neuroanatomic features. Neurocognitive processes mediated by these brain systems may cluster into larger behavioral features or symptoms, and specific permutations of these may define a syndrome. Altogether, the behavioral elements comprise the behavioral phenome. Intermediate phenotypes or endophenoptypes are conceived as hidden (non-outward) phenotypes and more tractable to the genome. Neurophenotypes embrace a diversity of neural and cognitive systems and may overlap with cognitive endophenotypes. Interactions within a stratum or across the G-P strata can also be mapped (the interactome)

many initiatives in cognitive and neuropsychiatric phenomics (Bilder 2008; Bilder et al. 2009a, b; Freimer and Sabatti 2003). A central point made is that the explosion of genomics has given rise to a scenario where the large amounts of high-dimensional genomic data are unmatched by current phenomic dimensions. Finer levels of granularity and precision need to be brought to codifying the phenome so that a meaningful relational interface with the genome is facilitated. Phenotype descriptions that are incompatible with the linkage served by a G-P framework and genomics can hold back genotyping explorations (Freimer and Sabatti 2003) and has aptly been referred to as a "rate-limiting" step in terms of reaping the gains of genomic discovery (Bilder et al. 2009b). In making the case for the systematic cataloging of phenotypes, Freimer and Sabatti have called for a "Human Phenome Project," which would necessarily involve centrally coordinated and funded large-scale efforts toward objectively defined, refined, and standardized phenotypes. They also stipulated that such a strategy for phenotype discovery has to be enabled

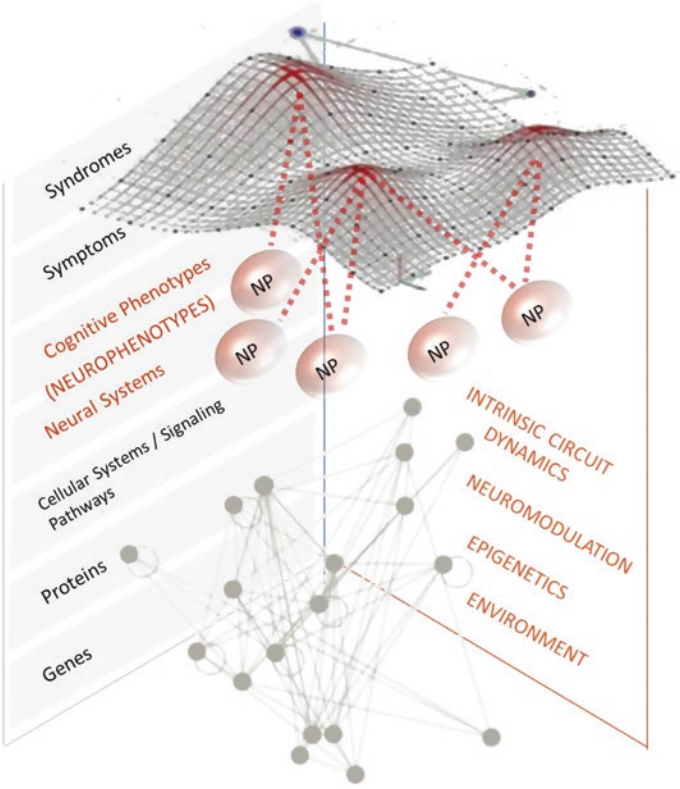

Fig. 2.2 Three-dimensional schematic of G-P space with highlights on the relational position of neurophenotypes. Multiple genes can have convergent effects on one or more NPs via intervening molecular and cellular systems (not detailed). One or more NPs may converge to produce a behavioral phenomic feature (symptom) of a disorder, and multiple features may define the disorder. The differential expression and permutations of phenomic features (manifesting as variations of a disorder) are represented in the figure by a multivariate Gaussian distribution. The *left wall* in the figure represents the G-P strata. The *right wall* represents environment, epigenetic, neuromodulatory, and other variables that are not driven by the genome but that may shape NPs (detailed in Chap. 3)

by novel methods of discovery with high-throughput analysis, which in turn will require a sophisticated informatics platform. And therein is a key aspect of phenomics—that the delineation of the phenome on scales compatible with a systems biology interface is necessarily informatics-driven. The strategy of phenomics as "the systematic study of phenotypes on a genome-wide scale" (Bilder 2008) "aims to capitalize on novel high-throughput computation and informatics technologies to derive genome-wide molecular networks of genotype-phenotype associations, or "phenomic associations" …" (Lussier and Liu 2007). Large-scale, coordinated efforts to this effect have already begun. While many phenomics consortia

centered on plants, mice, fish, and other non-human species have emerged, the leading consortium centered on (human) brain-related phenomics is the Center for Cognitive and Neuropsychiatric Phenomics (CNP) at UCLA (http://www.phenomics.ucla.edu/). This initiative is now well known for its investigations of working memory and response inhibition, from molecular to cognitive levels, using the case examples of schizophrenia and bipolar disorder.

2.1.2 Phenomics, Candidate Gene Studies, and GWAS

In the context of neuropsychiatry, the impetus for phenomics—serving the G-P associative framework—has been strengthened by the lack of meaningful findings both from candidate gene studies and, to a lesser extent, from GWAS: It has long been realized in psychiatric genetics that the candidate gene approach applied in the effort to seek genetic risk factors in psychiatric illness has not been particularly useful, in part because it involves a certain gamble that the investigator has chosen the correct genes to investigate, which is difficult, given the lack of empirical data on the underlying biology of psychiatric illness (McCarroll et al. 2014). For this and other reasons, including inadequate sample size and low statistical power, most positive associations between specific SNPs and diseases from candidate gene studies in psychiatry have not been replicated (Farrell et al. 2015; O'Donovan and Owen 1999; Sher 2002). The heterogeneity of psychiatric phenotypes, that is, their neural and behavioral permutations and overlaps, and conditions under which they present, may be best explained through combinatorial models that involve many genetic variants, epistasis, differential pathway expression, and a whole range of environmental variables that are seldom measured or modeled in genetic investigations. Hence, with such heterogeneity across the phenotypes, the effects of individual gene variants in the shaping of a particular phenotype are blurred (Congdon et al. 2010). (Section 2.3.2 references some of the often cited candidate genes in the context of neuropsychiatry.)

Until very recently, GWAS has not fared much better. While genome-wide association studies have had wide success in identifying SNPs tied to various types of medical conditions such as Crohn's disease, and Type I and Type II diabetes (Billings and Florez 2010; Franke et al. 2010; Liu and Anderson 2014), attempts at finding genes associated with neuropsychiatric conditions have lagged behind. It has long been argued that this has been due in large part to insufficient sample sizes and insufficient power of individual psychiatric GWAS to date (see Bloss et al. 2010; Congdon et al. 2010). In fact, recent meta-analyses of psychiatric GWAS data carried out by the Psychiatric Genomics Consortium (PGC) have lent support to this argument by demonstrating a clear correlation between the number of patient genomes interrogated and the number of significant associations found (in analyses of studies carried out between 2009 and 2014). Perhaps the most exciting demonstration of this was published in 2014 by the Schizophrenia Working Group of the PGC, which identified 108 statistically significant loci

associated with schizophrenia with a combined sample of nearly 37,000 cases and over 113,000 controls (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Although 83 of the 108 loci identified in the PGC schizophrenia GWAS meta-analysis were novel, the most strongly associated locus in this and some previous GWAS was the major histocompatibility complex locus (MHC). The MHC contains genes involved in immune response, and the significant association with schizophrenia which was first identified in 1979 (McGuffin) was for a long time thought to be an artifact until a recent study, published in January 2016: Sekar et al. confirmed that not only is the association real, but that a common variant of a gene in the MHC locus—C4—produces proteins—C4-A and C4-B—that influence the rate of synaptic pruning. By analyzing the genomes of over 64,000 people, and then confirming this in studies of knockout mice, this study showed that an overabundance of C4-A leads to over pruning of synapses in the prefrontal cortex during critical periods of development. This paper, which followed up on a significant GWAS finding, was the first to demonstrate a clear biological mechanism for the development of schizophrenia. So one might plausibly argue that as sample sizes increase to be comparable to those used successfully in other chronic diseases such as Crohn's disease and diabetes, GWAS will be just as successful in identifying common variants (whose effect sizes are necessarily quite small) for psychiatric illness as it has been in these other disorders.

Nonetheless, the initially posited reasons for the difficulty in linking genes to neuropsychiatric conditions via GWAS (see Bloss et al. 2010; Freimer and Sabatti 2003) still have some relevance. They have to with (a) the heterogeneity of neurophenotypes; (b) the "dispersion" or scaling down of genetic effects across a phenome due to gene–gene and gene–environment interactions, and (c) the ambiguous, imprecise manner by which neural and cognitive functional systems have traditionally been described. Neuropsychiatric and neurocognitive illnesses are most often complex in terms of their symptom and neural systems profiles (phenotypic complexity), and the genetic components may be just as multifaceted (genetic complexity). A systematic dissection of brain-mediated illnesses requires a systematic rendering of physiological systems and mental operations to which genes and gene expression pathways can be tied.

2.1.3 Aligning Gene Networks with Phenotypic Elements

That the architecture between the genome and phenome is complex, making for enormous etiological complexity of neurocognitive and neuropsychiatric conditions, is generally not underestimated. However, giving significant boost to the G-P/phenomics agenda is the increasing evidence that this complexity can be unraveled with new, sophisticated research models and methods applied to the problem. Consider the following illustrative examples: The modular view of genes posits that genes work together or co-express within discrete biological modules (Oti and Brunner 2007; Wu et al. 2009): Drawing a modular organization among

genes and phenotypic features helps recognize G-P associations by reducing the complexity in G-P maps. This perspective is ultimately concerned with aligning gene networks with phenotype clusters. It highlights that a module (phenotype), for example, a cell type of an organelle or a protein complex, which then presents as a disease phenotype, can be tapped for a more tractable linkage down into the gene level. Further, the differential clustering of a common set of modules may help to map the relationships between a set of genes and their expression in syndromes with overlapping features. Diseases that share common phenotypic modules may share common gene modules. As Wu et al. (2009) have described, the disease phenome can be depicted as overlapping networks of disease features. "Similarly, the interactome is a network of genes linked by physical interactions between their protein products. The two networks are further linked by gene-phenotype associations … the proximity between disease genes in the gene network could explain the phenotypic overlap of diseases … [suggesting] a global concordance of topology between the phenotype network and the gene network" (p. 98). Franke et al. (2006) have well extolled the prospect of gene networks mapped to phenotype networks—where the functional relationships between gene modules can be mapped differentially to the overlying symptom clusters that present as disease. And a proof of concept that phenotypic overlap signals genotypic overlap has been systematically demonstrated (Wu et al. 2009).

Oldham et al. (2008) examined gene transcriptional patterns (the transcriptome) in cells taken from the cerebral cortex, the caudate nucleus, and the cerebellum of the human brain. Their analysis of gene co-expression in these cells revealed modules of co-expressed genes, each corresponding to unique cellular makeup of the brain regions analyzed. What was also remarkable about the study, aside from providing the first views into an organization of the transcriptome of the brain, was that the transcriptome modules were filtered out through the application of a bioinformatics/systems biology-based method of network analysis and correlation patterns[1] (ft. WGCNA). In this study, conducted "in silico," the results were gained "without making any a priori assumptions regarding the cellular constitution of the tissue analyzed …" (Oldham et al. p. 1279).

The study of the modular organization of genes—how they co-express in gene modules and how such modules and genetic programs govern the development of larger-scale neuroanatomic circuits—is at the cutting edge of developmental neurobiology and neurogenetics (see Geschwind and Rakic 2013; Oldham et al. 2008; Parikshak et al. 2015). "[C]omparative genomics provides a powerful platform for identifying the genes and adaptive regulatory changes involved in cerebral cortex expansion, arealization, and other human-specific cellular or connectivity phenotypes." (Geschwind and Rakic 2013, p. 637). For there to be a more meaningful analysis of a surface-topological phenotype landscape, one where symptom clusters (phenotype networks) can be tied to gene networks (see Fig. 2.1),

[1]Weighted Gene Co-expression Network Analysis (WGCNA) is a software package used to map gene correlation and cluster patterns from microarray drawn samples.

the necessity of a uniform and structured definition of phenotypes, is once again emphasized (Oti and Brunner 2007).

Daunting as genome-to-phenome integration may seem, and as impractical as the goal of mapping NPs to all the lower levels in the G-P space may appear, novel solutions are matched to the complexity of the task. The studies of modules of co-expressed genes and transcriptome modules cited above were made possible through the application of network science (a branch of mathematics and bioinformatics)—using network methods to parcellate genes and their transcripts within a broader molecular matrix: "Gene network methods are now being applied to integrate genetics with transcriptomics, epigenomics, and proteomics to identify causal molecular drivers of cellular, circuit-level, and brain-wide pathology in disease" (Parikshak et al. 2015). Such integration has been generating novel insights into autism spectrum disorder (Geschwind 2011; Pinto et al. 2014; Sanders et al. 2015) and brain degenerative diseases (Chen et al. 2015; Miller et al. 2013), as well as the evolution of the brain as relates to cognition (Geschwind and Rakic 2013; Konopka and Geschwind 2010). That very systems-level understanding that phenomics strives for in the interest of a fine-tuned, neuroscience-compatible understanding of neuropsychiatric conditions has certainly begun.

2.2 Connectomics

The "connectome" refers to an envisaged, detailed map of the structural connections of the brain on all scales, from the microscale cellular level to the macroscale of white matter fiber systems. Hence, *connectomics* is the omics-driven initiative toward mapping the connectome. It is concerned specifically with the structural arrangements and connectivity patterns of neurons and glial cells in the matrix of the brain, while recognizing the emergence of functional circuits via organized connections (Behrens and Sporns 2012; Sporns 2011, 2012; Sporns et al. 2005). Connectomics is, by definition, informatics-heavy. In view of the complexity of neural architecture and the scale of data volume generated by its mapping, connectomics relies on numerous novel tools for high-throughput image acquisition of micro- and macrocircuitry, and visualization of circuitry on a meta-scale through image integration (see Helmstaedter 2013; Marcus et al. 2011; Shibata et al. 2015).

Connectomics as an initiative has had a separable trajectory in relation to the general calls for phenomics. However, the connectomics and phenomics initiatives happen to coincide. The connectomics agenda neatly fits in with the mission of phenomics, and both of these developments are occurring at the same point in time. For all practical purposes, connectomics can be seen as a major avenue in brain science that happens to serve the phenomics agenda well. Many of the questions and issues seen within the connectomics initiative apply equally fittingly to phenomics at large. The issues provide a remarkable window into the challenges of phenomics as it relates to neural circuitry (and this is discussed later in this chapter).

Lying at opposite ends in terms of an anatomic-physiological scale are the two major branches of connectomics: (a) connectome mapping through fMRI (hence in vivo) methods, also known as MR connectomics, and (b) connectome mapping via predominantly in vitro methods, e.g., tissue slices viewed with microscopy and assembled with 3D visualization.[2] A review of either of these branches falls far beyond the scope and purpose of this chapter. Instead, we provide a few key points below, as they relate to the discussion of phenomics. (Various images in Fig. 2.3 (from Leergaard et al. 2012) correspond to the themes of this subsection.)

2.2.1 Connectomics on the Macroscale

MR connectomics has been extensively discussed in recent years (see, e.g., Behrens and Sporns 2012; Craddock et al. 2013; Kelly et al. 2012; Snyder and Raichle 2012; Van Essen et al. 2012; Zuo et al. 2010). MR connectomics explores white matter fiber systems and tracts by employing diffusion tractography (diffusion MRI) and, increasingly, with the resting-state fMRI (R-fMRI) paradigm. (In diffusion tractography, the paths of white matter bundles are inferred on a millimeter scale—based on the selective pattern and speed by which water molecules diffuse along and within myelinated axons. In R-fMRI, intrinsic brain connectivity is inferred based on co-activation of two or more cortical areas: Fluctuating activation patterns across spatially separated brain regions are correlated in terms of spontaneous co-activation patterns. The robustness and consistency of these statistical correlations are considered indicative of a structural network that functionally links the regions. Such functional connectivity is used to map out a "functional connectome.")

R-fMRI, also known as intrinsic functional connectivity (iFC), has grown explosively over the short span of the past ten years. Central to the R-fMRI approach is the value placed on endogenous activity across a neural network seen when the brain is "at rest," meaning, not engaged in evoked activity. This is in contrast to conventional task-dependent fMRI where only those response patterns phase-synchronous with the experimental task are of interest. In R-fMRI, the interest is in functional interactions between loci in circuitry while "at rest"—referred to as resting-state functional connectivity (RSFC). However, since networks identified through R-fMRI can also be identified with task-dependent activity, some have suggested that the term "task-free MRI" (TF-MRI) be used instead (Jones et al. 2012). Nonetheless, the method sheds light on intrinsic networks and modules of the brain, the spatial organization and temporal interaction

[2]The term "connectome" has come to stand for all scales of neural mapping, from microscopic (cell/synaptic) arrangements to macroscopic (white matter) projection systems, though it has been suggested that "connectome" better references microscale connections, and that the term "projectome" better represents macroscale connections (see Kasthuri and Lichtman 2007).

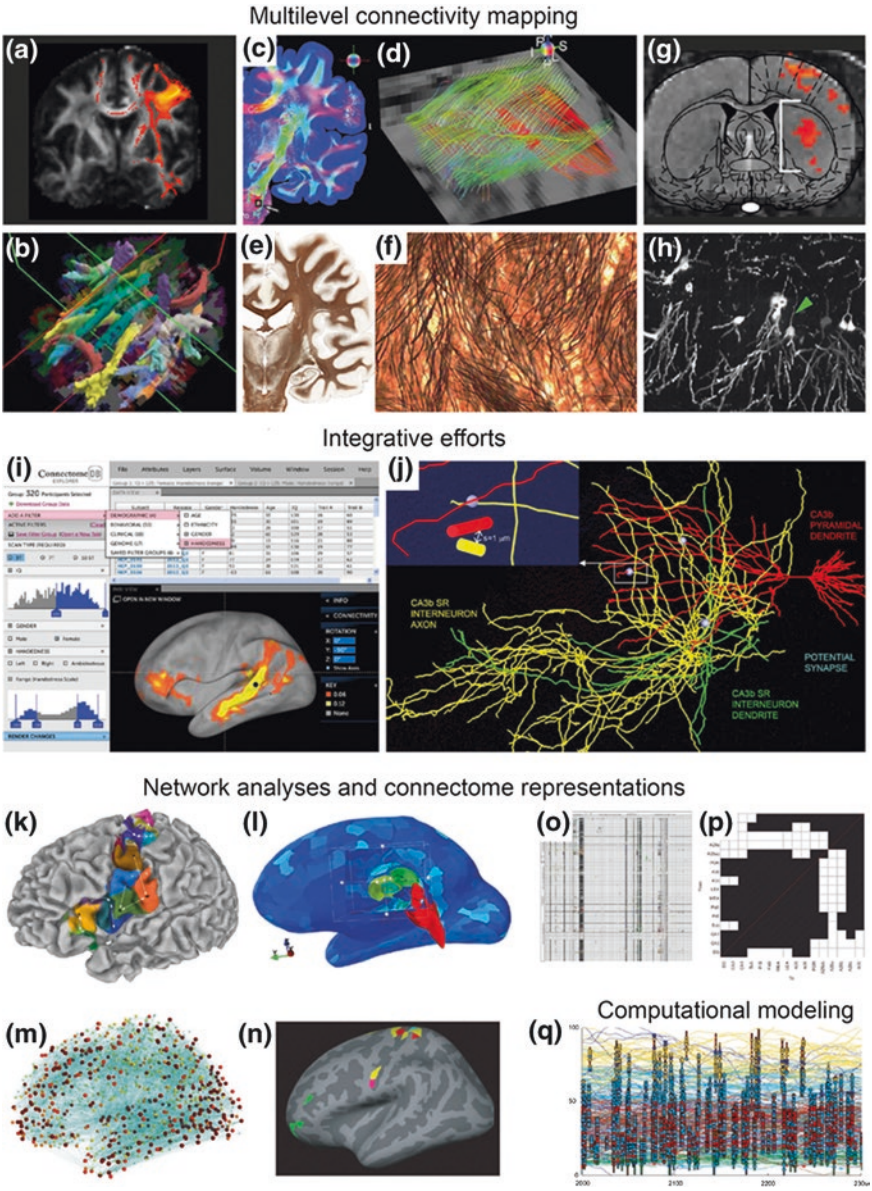

Fig. 2.3 Adapted and reproduced from the open source journal, Frontiers in Neuroinformatics (2012) 6:14. Leergaard TB, Hilgetag CC and Sporns O. *Mapping the connectome: multi-level analysis of brain connectivity*. Figure 1 in its original source served as a summary illustration of various forms of connectivity data/various types of connectomes (human and non-human): MRI tractography and related mapping (**a**–**f**); combined optogenetic and fMRI mapping (**g**); histological imaging (**h**); informatics tools for the aggregation and integration of connectivity data (**i** and **j**); brain network analysis—connectivity-based cortical parcellations and network motifs (**k**–**n**); connectome matrix representations from large-scale data mining efforts (**o**–**q**). Figure 1 serves equally well to represent multiple scales of circuitry and multiple forms of neurophenotypes—drawn from functional imaging parcellations or connectivity networks; histological and in vivo mapping data; and informatics-driven computational platforms

of these networks in the normal brain, and how they may be disrupted in neurocognitive and neuropsychiatric conditions, which is of key interest in R-fMRI.

Employing both diffusion fMRI and R-fMRI, the Human Connectome Project (HCP)[3] seeks to create a detailed, macroscale map of the "typical" connectivity in the normal adult human brain" (Barch et al. 2013; Smith et al. 2013).

2.2.2 MR Connectomics and Neurophenotypes

Nodal, regional, or dynamic permutative disruptions to functional or topological organization of large-scale brain networks, identified via MR connectomics, may constitute phenotypes at the regional or macrocircuit level (see Fornito and Bullmore 2012). R-fMRI is surpassing task-dependent MRI in terms of its utility in identifying NPs (Castellanos et al. 2013). Interpretation and modeling of the functional connectome rests on the critical tools of graph theory, graph statistics, and network science (branches of mathematics and statistics) that describe the principles of by which the nodes of complex systems interact.[4] They may reveal network organization—modular/nodal architecture, centrality in a network, nodal changes, and functional efficiency of the network. A picture of network dynamics is generated and may include, for example, specific patterns of temporal dependencies across nodes that are otherwise hidden in the network architecture. Brain networks derived from MR data are cast as annotated graphs. (The nodes drawn from fMRI studies are interpreted to represent distinct cortical, subcortical, or cerebellar nodes, though an "optimal" parcellation scheme is debatable.) Graph theory is then applied to understand the dynamics of brain network topology—a very new and emergent subspecialty in MR connectomics—that seeks to apply computational modeling to connectomics data in order to understand brain network dynamics as they manifest in neuropsychiatric disorders (see Cabral et al. 2014; Deco and Kringelbach 2014; Fornito et al. 2015; Xia et al. 2016, for excellent renderings of this topic). This area of computational connectomics is particularly focused on the following questions: What are the mechanisms by which aberrant network dynamics manifest in brain disorders? What is the network topological permutation (signature pattern) for each of various brain-related disorders? How can the functional dynamics of networks garnered through connectomics describe

[3]The HCP, run by the US National Institutes of Health, went into effect in 2010—funding research projects using noninvasive (fMRI) methods to begin the ambitious agenda of mapping out the connectome. See http://www.neuroscienceblueprint.nih.gov/connectome/.
The HCP has also been the subject of vigorous debate, facing questions about feasibility, viability, and utility. See Nature Neuroscience Editorial (2010) for a synopsis of the debate. Some of the issues are also touched upon in this chapter.

[4]"Graph" or "graph layout" in mathematical graph theory generally refers to the connectivity pattern in a network.

and predict maladaptive or pathological brain states? MR connectomics hence presents novel forms of NPs based on pathoconnectomic patterns or spatiotemporal dynamics across brain regions.

Potential NPs derived from MR connectomics, and centered on brain network connectivity patterns or functional dynamics, have been described across the spectrum of neuropsychiatric and neurocognitive disorders (for reviews, see Deco and Kringelbach 2014; Di Martino et al. 2014; Xia and He 2011). Further, such studies have also demonstrated that different brain disorders with overlapping symptomatology can be explained in terms of permutative profiles of large-scale canonical brain networks (Crossley et al. 2014; Fornito et al. 2015). Even though many refinements are still needed in the functional connectome initiative, it is a central player in image-based neurophenotypes and is especially compelling in the context of RDoC and the big data/knowledge discovery environment (Castellanos et al. 2013).

2.2.3 Connectomics on a Microscale

The second major branch of connectomics explores neural microcircuitry at the cellular and synaptic level—cellular-resolution connectomics. It seeks to catalog the brain in terms of neuronal and synaptic arrangement.

The resolution at which single cells, neurites, or synaptic structure are described is in the nanometer range. Microscale connectomics is integrally tied with high-throughput electron microscopy—which, by virtue of its power and its limitations, fundamentally shapes the initiative. A common data collection/analysis method used is the automated microtome that produces serial slices of neural tissue, each then passed on a conveyor belt to an automated electron microscope that generates a serial image set. A block of sequenced images is then analyzed manually and/or with the aid of computational vision (analysis) technology in order to align contiguous tissue elements and hence trace the neural structures (see Helmstaedter 2013; Shibata et al. 2015). The assembled 3D image block renders a cubic section of tissue volume (shown in Fig. 3.1d in Chap. 3). A saturated (comprehensive) connectomic reconstruction of a tiny sample (1500 μm^3) of mouse neocortical tissue analyzed with the above-described procedure was found to contain about 1407 axons and 1700 synaptic connections and immense synaptic redundancies (Kasthuri et al. 2015). With the currently estimated 86 billion neurons in the human brain (Azevedo et al. 2009), the prospect of high-density mapping of the entire brain is daunting even with automated microscopy.

Nonetheless, the connectomics initiative at the cellular level has particular instructive and informative value for the agenda of neural phenomics and RDoC: Aside from being aimed at the very goal of mapping phenomic structure at the neural level (and hence mapping circuits), the exploration of connectomics at this level leads to a host of questions and issues. These happen to impact the putative notions of circuits in RDoC and in circuit-centered neurophenotypes.

A vigorous debate about the need, utility, and practicality of creating a detailed map of the brain has ensued over the past decade (see: A critical look at connectomics 2010; Markram 2012; Marx 2013; Morgan and Lichtman 2013). There remains no consensus about the primary goals of connectomics, nor is there agreement about a set of standardized mapping techniques (Lichtman et al. 2014). Expressed definitions of potential scope varies from representational-probabilistic maps to detailed structural and connection mapping at the cellular scale (i.e., every neuron and synapse) to a wiring diagram that could also shed light on molecular and synaptic variations (see Lichtman et al. 2008; Morgan and Lichtman 2013).

Mapping the brain at such extremely fine levels of detail inevitably gives rise to the following questions: Is there an optimal level of resolution that best serves the understanding of the brain? and How will a mapped connectome account for dynamic changes (e.g., dendritic arborization and synaptic variables) that change with experience, maturation, and intrinsic modulatory factors? Some of those leading the efforts in microscale connectomics are careful to acknowledge that issues such as density scale and circuit stability may never be met with commonly agreed upon formula, but nonetheless have argued that a comprehensive, high-quality map of the brain is necessary if brain functions are to be understood (Kasthuri and Lichtman 2007; Lichtman and Sanes 2008; Morgan and Lichtman 2013): Having a detailed structural correlate against which biological and behavioral functions can be understood is better than a coarsely described correlate. Certain fundamental or canonical characteristics of brain networks may only be understood by mapping the connectivity patterns. Again, it is the challenges of microscale connectomics as thus far rendered through the actual initiative that translates into cautionary lessons and complications for neural phenomics and RDoC. This is laid out in Chap. 3 in a larger appraisal of neural circuits as neurophenotypes.

2.3 Research Domain Criteria (RDoC) and Related Developments

The old and difficult question of how to conceptualize and classify mental disorders took a very new turn in 2008. The US National Institute of Mental Health implemented a strategic five-year plan aimed at transforming the understanding and treatment of mental illnesses via a systematic application of scientific research. It laid down the following as the first objective among four in the strategic plan: "… in order for research on mental disorders to more fully harness the scientific power of brain-behavior science, sound efforts must be made to redefine mental disorders into dimensions or components of observable behaviors that are more closely aligned with the biology of the brain. Such an effort will result in a research-based description of key elements of mental disorders, providing even greater traction on the potential mechanisms that can cause mental suffering and

targets for more effective preemption and treatment" (National Institute of Mental Health 2008; www.nimh.hih.gov/about/strategic-planning-reports/index.shtml).

This initiative, called *Research Domain Criteria* (RDoC), has since been extensively discussed.

2.3.1 RDoC Structure and Rationale

The impetus for RDoC, and its structure and rationale, are given summary focus here—drawn from the NIMH Strategic Plan (2008), Berenbaum (2013), Cuthbert and Insel (2010a–c), Insel and Cuthbert (2009), Morris and Cuthbert (2012), Morris et al. (2014), Sanislow et al. (2010), Simmons and Quinn (2014).

RDoC prioritizes the identification and integration of biomarkers as they aggregate and constellate in mental disorders. The emphasis on a brain-based or evidence-based nosology is in contrast to conventional diagnostic systems such as the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) or the *International Classification of Diseases* (ICD). These well-known classification systems rely on qualitative interpretations of behavior which are then matched to some degree to symptom clusters; they are phenomenologically based. Such cluster-based aggregations of signs and symptoms exclude other valuable phenotypic information and do not necessarily reflect scientific constructs of psychopathology.

A great deal of heterogeneity exists within and across clinical populations described by conventional diagnostic categories. This heterogeneity and comorbidity across psychopathologic categories can be described in large part as complex functional permutations of a broad yet common set of neural systems and genes. Mental disorders are polygenic. Yet conventional diagnostic systems are ill suited to profiling differential patterns of expression arising from common genes and neural systems. Discrete categories are forced and have artificial and "fuzzy" boundaries. Symptom cluster-based diagnostic systems do not lend themselves to a scientific bridging with the biological systems that mediate the behavioral symptoms. They offer no interface for biologically based research initiatives—where mental disorders can be deconstructed along domains of perceptual, cognitive, and emotional processes, mediated by complex neural systems. The overlapping symptoms in the clusters given by conventional classification may share common neural systems driven by common gene modules. And while such insights are progressing in neuroscience and genetics, the current diagnostic systems are not complementary to the scientific or evidence-based models. In contrast to neural systems and gene networks, the diagnostic systems are not informed by nor do they serve the understanding of phenotypic heterogeneity and clustering.

In response to these shortcomings, RDoC is geared to a formulation of a new system by which psychopathology is described. It proposes a system that is a based on a biologically informed conceptual model of the brain and brain-mediated disorders that is supported by empirical data (Fig. 2.4).

	Units of Analysis							
	Genes	Molecules	Cells	Circuits	Physiology	Behavior	Self-Report	Paradigms
Negative Valence Systems								
Active Threat ("fear")								
Potential threat ("anxiety")								
Sustained threat								
Loss								
Frustrativenon-reward								
Positive Valence Systems								
Approach motivation								
Initial responsiveness to reward								
Sustained responsiveness to reward								
Reward learning								
Habit								
Cognitive Systems								
Attention								
Perception								
Working Memory								
Declarative Memory								
Language behavior								
Cognitive (effortful) control								
Systems for Social Processes								
Affiliation and Attachment								
Social Communication								
Perception and understanding of self								
Perception and understanding of others								
Arousal/Regulatory Systems								
Default mode network								
Sleep/Wakefulness								
Biological Systems								
Arousal								

Fig. 2.4 Summary of RDoC's matrix-based research framework. The *rows* in the matrix describe constructs or dimensions that represent the basic units of analysis. Related constructs are grouped as functional domains of behavior (*bold headings*). The *columns* represent the multiple perspectives or analytic variables (genome–phenome) that can be applied in describing a construct

The RDoC strategy also firmly embraces a brain-based marker approach. This compelling initiative has re-energized and reframed the utility of the NP approach in the behavioral and clinical neurosciences. The NIMH plan set forth various strategic objectives, among them (paraphrased from pp. 6–8, www.nimh.hih.gov/about/strategic-planning-reports/index.shtml):

- Development of an integrative understanding of basic brain–behavior processes that provide the foundation for understanding mental disorders;
- Identification and integration of biological markers and behavioral indicators associated with mental disorders; and
- Development, for research purposes, of new ways of classifying mental disorders based on dimensions of observable behavior and neurobiological mechanisms.

To this effect, the RDoC framework is based on discrete dimensions of behavior and on neurobiological systems that can be measured. As a starting point, the guiding model for the RDoC classification framework specifies "constructs" or dimensions of behavior, such as *Approach Motivation* and *Working Memory*. Each

construct may have numerous subconstructs. Constructs fall under larger behavioral domains. RDoC incorporates the theme of multiple levels of analysis—a construct might be analyzed at the levels of genes, molecules, cells, circuits, physiological systems, behavior, etc. However, the RDoC system centers itself around the "neural circuit" level of analysis (detailed further below).

RDoC constitutes an initial instantiation of a model that details how scientific constructs in the biological sciences and behavioral neurosciences can be integrated to promote the discovery of a scientifically based description of behavior. RDoC applies a matrix-based research framework: The rows in the matrix correspond to the constructs or dimensions representing basic units of analysis, and these are subject to refinements with emerging research. Related constructs group together to form functional domains of behavior. An example of a domain is *Negative Valence Systems*, and it includes the constructs of *fear* and *potential threat*. A construct can be described from multiple perspectives, that is, various units of analysis (the variables). These analytic variables—genes, molecules, cells, circuits, physiological systems, behavior, and self-reports—are each represented by the columns of the matrix. The neural circuit level of analysis is the reference point around which the other levels of analysis are organized. RDoC describes cognitive and neural features along a continuum—normal traits with variations in "dimension." Neurophenotypes (stable markers) of these traits would therefore hold much more utility.

In theory, this leads to the possibility of a diverse set of NPs with numerous permutations, where combinatorial associations of neurophenotypes make for particular multivariate patterns that may more accurately reflect mental disorders. What if the areas of overlap and the areas of distinction in symptomatology between different psychotic disorders, or across each of the spectral patterns seen in autism or ADHD, could be described in terms of the common or specific permutations in the genetic-neurodevelopment-neural systems matrix? Lofty as this ideal may sound, it is a central, transformative idea behind RDoC. "RDoC is an attempt to create a new type of taxonomy for mental disorders by bringing the power of modern research approaches in genetics, neuroscience, and behavioral science to the problem of mental illness. … RDoC is a new, comprehensive effort to redefine the research agenda for mental illness" (Insel and Lieberman 2013). Simmons and Quinn (2014) have described RDoC as representing a potentially new classification system for research on mental illness. RDoC is clearly a working model, dynamically structured, and fully open to modifications. Constructs and domains can be reorganized and refined, units of analysis can be added, and the criteria for construct definition can be revisited.

As is the case with any multi-leveled data integration project in biology and medicine (see Chap. 15), critical to the RDoC initiative is a data sharing and data integration platform. Bioinformatics tools and infrastructure are central to this agenda—searching for patterns among diverse sets of data and integrating data so as to make data-driven discoveries. RDoC's data platforms are necessarily federated data repositories, and these are elaborated in Sect. 15.1.

2.3.2 RDoC's Circuit-Level Pitch

As an operational model, RDoC necessarily rests on a few postulates and assumptions about the nature of mental illnesses. A central assumption is that these illnesses are rooted in dysregulation of brain circuitry (Cuthbert and Insel 2010a; Morris and Cuthbert 2012). This then provides the basis for other assumptions or hypotheses as follows: Variations of a circuit phenotype can account for variations of a disorder; developmental and environmental effects on the brain can be inferred at the circuit level in that they modify the circuit phenotype; neuroscience methods such as functional imaging and electrophysiological assessment can be used to profile the circuitry; and, intervention and treatment can target the circuit-expressed mechanism. Since the same set of cognitive or emotional processes may differentially play out in related disorders (accounting for overlapping symptomatology), studying the circuit representation of a process may also give insights into underlying circuit variations in a subset of disorders. For example, the "fear circuit" expressing fear potentiation may have distinct signatures for OCD and generalized anxiety.

As a practical, strategic constraint, a manageable reference point centering the approach at the circuit level enables bidirectional data integration—drilling downward to cellular and molecular levels or upward toward behavioral manifestations. Having a central organizing point of reference makes for a simpler integrative strategy as opposed to the specification of all possible neural/cellular constructs, which could be proliferative and unwieldy.

RDoC does not delve explicitly into notions or conceptions of neural circuits, but certain notions are implicit in the discussions in RDoC. Circuits as currently conceptualized in RDoC can be traced to a few familiar influences: The 2008 NIMH Strategic Plan Statement on RDoC references a few developments such as optogenetics and MR tractography that were emerging at that time tied to neuron labeling and white matter tracing, respectively. The NIMH Draft Statement on RDoC (version 3.1, June 2011) indicates that ' "Circuits" can refer to measurements of particular circuits as studied by neuroimaging techniques, and/or other measures validated by animal models or functional imaging (e.g., emotion-modulated startle, event-related potentials)–.' At a NIMH workshop on cognitive systems (October 23–25, 2011), convened to clarify constructs in RDoC's Cognitive Systems Domain, elaboration was also given to the various units of analysis. The workshop proceedings (revised May 2012) describe working models of circuits for a number of broad cognitive domains (e.g., attention, perception, and memory). The corresponding circuits listed reflect the contemporary influence of cognitive neuroscience and functional imaging. The referenced circuits are for the most part large-scale neuroanatomic projection systems or cortical parcellations. They are systems such as the "dorsal attentional network (superior parietal lobe, frontal eye fields, DLPFC)"; sensory projection pathways such as the magnocellular and parvocellular systems in vision; major sensory association systems such as the ventral and dorsal extra-striate projections in vision ("what" and "where" pathways); the

tri-synaptic loop of the hippocampus; and various well-documented cortical nodes (e.g., the frontal eye fields, the nucleus accumbens.) Bearing in mind that this represents an initial instantiation of a working model that is yet to be elaborated, some of the postulated circuits still lean heavily toward a particular neuroanatomic system when more than one candidate system exists. For example, the cognitive processes of response selection, inhibition, or suppression were associated by the workgroup overwhelmingly with cortical (prefrontal and posterior parietal) areas and with minimal reference to the striatum.

Another shaping force of the circuit-level pitch in RDoC is the candidate gene approach to phenotypes which has aided in the linking of genes to circuits to cognition in the G-P explanatory matrix (see Insel and Cuthbert 2009). Among the many known examples of genes that have been linked to cognition via factors expressed at the neuronal/synaptic level are those described below (see Craddock et al. 2006; Owen et al. 2004; Sabb et al. 2009). And while the strength of these G-P associations, especially the links from circuits to cognition, are generally weak or unclear, they may signal firmer associations: (a) The association of the val158met polymorphism with significant increases in catechol-*O*-methyltransferase (COMT) activity (dopamine catabolism) in the dorsolateral prefrontal cortex in patients with schizophrenia, and subsequent effects of neuropsychological tests of set switching and other aspects of "executive" function. (b) The association of various risk haplotypes with dystrobrevin-binding protein 1 (DTNBP1 or "dysbindin"), and presynaptic reductions of dysbindin glutamatergic neurons in cortical and hippocampal sites in schizophrenia with a range of effects on cognitive tasks. (c) The association of a variant of the Taq1 allele with the dopamine D2 receptor (DRD2), reduced D2 binding in all areas of the striatum, and possible effects on cognitive measures.[5]

In view of such developments, RDoC has reasonably set the "neural circuit" as the central point of reference for describing cognitive processes and mental disorders. Implicit in RDoC's circuit-level pitch are certain models of circuits—some being large-scale cortico-cortical or cortico-subcortical projection systems, some operationalized as neural/cortical nodes of the kind presented by functional imaging, and some defined at the synaptic level. RDoC clearly also makes allowance for finer elaboration of neural circuits as may be relevant to the cognitive processes set forth by RDoC. Nevertheless, there is a difficult line to be straddled in making both accommodations—purveying some particular notions of circuits while also trying to be open to other renditions of circuits. The assumption that RDoC makes that psychopathology can largely be traced to biology, spelled out in terms of neural circuitry, has been well critiqued in the research literature on RDoC (see, e.g., Nesse and Stein 2012). And any a priori assumption about

[5]In most such studies attempting to link a neural phenotype to a cognitive phenotype, major confounds arise with the use of established neuropsychological tests and batteries, as these measures compound numerous neural processes and cannot be parsed neatly to neural circuits; ironically, this problem, writ large, provided the impetus for this book. See also Chap. 15.

the nature of mental disorders inevitably shapes the kinds of research questions and models designed to study the disorders (Berenbaum 2013). In the same vein, assumptions about neural circuits, their form, size, functional properties, genetic drivers, etc., and assumptions shaped heavily by popular ideas in cognitive neuroscience, will give focus to a mere sliver of circuit forms and features in the immensely broad spectrum of circuit definitions in neuroscience.

References

A critical look at connectomics (2010) Nat Neurosci 13(12):1441

Azevedo FA, Carvalho LR, Grinberg LT et al (2009) Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comp Neurol 513(5):532–541

Barch DM, Burgess GC, Harms MP et al (2013) Function in the human connectome: task-fMRI and individual differences in behavior. Neuroimage 80:169–189

Behrens TE, Sporns O (2012) Human connectomics. Curr Opin Neurobiol 22(1):144–153

Berenbaum H (2013) Classification and psychopathology research. J Abnorm Psychol 122(3):894–901

Bilder RM (2008) Phenomics: building scaffolds for biological hypotheses in the post-genomic era. Biol Psychiatry 63(5):439–440

Bilder RM, Poldrack RA, Stott PD et al (2009a) Cognitive phenomics. The neuropsychology of mental illness. Cambridge University Press, Cambridge, pp 271–284

Bilder RM, Sabb FW, Parker DS et al (2009b) Cognitive ontologies for neuropsychiatric phenomics research. Cogn Neuropsychiatry 14(4–5):419–450

Billings LK, Florez JC (2010) The genetics of type 2 diabetes: what have we learned from GWAS? Ann NY Acad Sci 1212:59–77

Biological insights from 108 schizophrenia-associated genetic loci (2014) Nature 511(7510):421–427

Bloss CS, Schiabor KM, Schork NJ (2010) Human behavioral informatics in genetic studies of neuropsychiatric disease: multivariate profile-based analysis. Brain Res Bull 83(3–4):177–188

Bork P, Dandekar T, Diaz-Lazcoz Y et al (1998) Predicting function: from genes to genomes and back. J Mol Biol 283(4):707–725

Cabral J, Kringelbach ML, Deco G (2014) Exploring the network dynamics underlying brain activity during rest. Prog Neurobiol 114:102–131

Castellanos FX, Di Martino A, Craddock RC et al (2013) Clinical applications of the functional connectome. Neuroimage 80:527–540

Chen JA, Wang Q, Davis-Turak J et al (2015) A multiancestral genome-wide exome array study of Alzheimer disease, frontotemporal dementia, and progressive supranuclear palsy. JAMA Neurol 72(4):414–422

Cognitive systems: workshop proceedings. In: Research domain criteria (RDoC), National Institute of Mental Health, Rockville, MD, 23–25 Oct 2011

Congdon E, Poldrack RA, Freimer NB (2010) Neurocognitive phenotypes and genetic dissection of disorders of brain and behavior. Neuron 68(2):218–230

Craddock N, O'Donovan MC, Owen MJ (2006) Genes for schizophrenia and bipolar disorder? Implications for psychiatric nosology. Schizophr Bull 32(1):9–16

Craddock RC, Jbabdi S, Yan CG et al (2013) Imaging human connectomes at the macroscale. Nat Methods 10(6):524–539

Crossley NA, Mechelli A, Scott J et al (2014) The hubs of the human connectome are generally implicated in the anatomy of brain disorders. Brain 137(Pt 8):2382–2395

Cuthbert B, Insel T (2010a) Classification issues in women's mental health: clinical utility and etiological mechanisms. Arch Womens Ment Health 13(1):57–59
Cuthbert B, Insel T (2010b) The data of diagnosis: new approaches to psychiatric classification. Psychiatry 73(4):311–314
Cuthbert BN, Insel TR (2010c) Toward new approaches to psychotic disorders: the NIMH research domain criteria project. Schizophr Bull 36(6):1061–1062
Deco G, Kringelbach ML (2014) Great expectations: using whole-brain computational connectomics for understanding neuropsychiatric disorders. Neuron 84(5):892–905
Di Martino A, Fair DA, Kelly C et al (2014) Unraveling the miswired connectome: a developmental perspective. Neuron 83(6):1335–1353
Farrell MS, Werge T, Sklar P et al (2015) Evaluating historical candidate genes for schizophrenia. Mol Psychiatry 20(5):555–562
Fornito A, Bullmore ET (2012) Connectomic intermediate phenotypes for psychiatric disorders. Front Psychiatry 3(32):1–15
Fornito A, Zalesky A, Breakspear M (2015) The connectomics of brain disorders. Nat Rev Neurosci 16(3):159–172
Franke L, van Bakel H, Fokkens L et al (2006) Reconstruction of a functional human gene network, with an application for prioritizing positional candidate genes. Am J Hum Genet 78(6):1011–1025
Franke A, McGovern DP, Barrett JC et al (2010) Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet 42(12):1118–1125
Freimer N, Sabatti C (2003) The human phenome project. Nat Genet 34(1):15–21
Geschwind DH (2011) Genetics of autism spectrum disorders. Trends Cogn Sci 15(9):409–416
Geschwind DH, Rakic P (2013) Cortical evolution: judge the brain by its cover. Neuron 80(3):633–647
Helmstaedter M (2013) Cellular-resolution connectomics: challenges of dense neural circuit reconstruction. Nat Methods 10(6):501–507
Hood L, Heath JR, Phelps ME et al (2004) Systems biology and new technologies enable predictive and preventative medicine. Science 306(5696):640–643
Hunter PJ, Borg TK (2003) Integration from proteins to organs: the physiome project. Nat Rev Mol Cell Biol 4(3):237–243
Insel TR, Cuthbert BN (2009) Endophenotypes: bridging genomic complexity and disorder heterogeneity. Biol Psychiatry 66(11):988–989
Insel TR, Lieberman JA (2013) DSM-5 and RDoC: shared interests. National Institute of Mental Health, Bethesda, MD
Jones DT, Vemuri P, Murphy MC et al (2012) Non-stationarity in the "resting brain's" modular architecture. PLoS ONE 7(6):e39731
Kasthuri N, Lichtman JW (2007) The rise of the 'projectome'. Nat Methods 4(4):307–308
Kasthuri N, Hayworth KJ, Berger DR et al (2015) Saturated reconstruction of a volume of neocortex. Cell 162(3):648–661
Kelly C, Biswal BB, Craddock RC et al (2012) Characterizing variation in the functional connectome: promise and pitfalls. Trends Cogn Sci 16(3):181–188
Konopka G, Geschwind DH (2010) Human brain evolution: harnessing the genomics (r)evolution to link genes, cognition, and behavior. Neuron 68(2):231–244
Korbel JO, Doerks T, Jensen LJ et al (2005) Systematic association of genes to phenotypes by genome and literature mining. PLoS Biol 3(5):e134
Lichtman JW, Sanes JR (2008) Ome sweet ome: what can the genome tell us about the connectome? Curr Opin Neurobiol 18(3):346–353
Lichtman JW, Livet J, Sanes JR (2008) A technicolour approach to the connectome. Nat Rev Neurosci 9(6):417–422
Lichtman JW, Pfister H, Shavit N (2014) The big data challenges of connectomics. Nat Neurosci 17(11):1448–1454

Linden DE (2012) The challenges and promise of neuroimaging in psychiatry. Neuron 73(1):8–22
Liu JZ, Anderson CA (2014) Genetic studies of Crohn's disease: past, present and future. Best Pract Res Clin Gastroenterol 28(3):373–386
Lussier YA, Liu Y (2007) Computational approaches to phenotyping: high-throughput phenomics. Proc Am Thorac Soc 4(1):18–25
Marcus DS, Harwell J, Olsen T et al (2011) Informatics and data mining tools and strategies for the human connectome project. Front Neuroinform 5:4
Markram H (2012) The human brain project. Sci Am 306(6):50–55
Marx V (2013) Neurobiology: brain mapping in high resolution. Nature 503(7474):147–152
McCarroll SA, Feng G, Hyman SE (2014) Genome-scale neurogenetics: methodology and meaning. Nat Neurosci 17(6):756–763
McGuffin P (1979) Is schizophrenia an HLA-associated disease? Psychol Med 9(4):721–728
Miller JA, Woltjer RL, Goodenbour JM et al (2013) Genes and pathways underlying regional and cell type changes in Alzheimer's disease. Genome Med 5(5):48
Morgan JL, Lichtman JW (2013) Why not connectomics? Nat Methods 10(6):494–500
Morris SE, Cuthbert BN (2012) Research domain criteria: cognitive systems, neural circuits, and dimensions of behavior. Dialogues Clin Neurosci 14(1):29–37
Morris SE, Rumsey JM, Cuthbert BN (2014) Rethinking mental disorders: the role of learning and brain plasticity. Restor Neurol Neurosci 32(1):5–23
National Institute of Mental Health (2008) The national institute of mental health strategic plan. http://www.nimh.nih.gov/about/strategic-planning-reports/index.shtml. Accessed 10 Oct 2014
National Institute of Mental Health (2011) NIMH research domain criteria (RDoC). http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml. Accessed 29 Apr 2014
Nature Neuroscience Editorial (2010) A critical look at connectomics. Nat Neurosci 13:1441
Nesse RM, Stein DJ (2012) Towards a genuinely medical model for psychiatric nosology. BMC Med 10:5
O'Donovan MC, Owen MJ (1999) Candidate-gene association studies of schizophrenia. Am J Hum Genet 65(3):587–592
Oldham MC, Konopka G, Iwamoto K et al (2008) Functional organization of the transcriptome in human brain. Nat Neurosci 11(11):1271–1282
Oti M, Brunner HG (2007) The modular nature of genetic diseases. Clin Genet 71(1):1–11
Owen MJ, Williams NM, O'Donovan MC (2004) Dysbindin-1 and schizophrenia: from genetics to neuropathology. J Clin Invest 113(9):1255–1257
Parikshak NN, Gandal MJ, Geschwind DH (2015) Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders. Nat Rev Genet 16(8):441–458
Pinto D, Delaby E, Merico D et al (2014) Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. Am J Hum Genet 94(5):677–694
Sabb FW, Burggren AC, Higier RG et al (2009) Challenges in phenotype definition in the whole-genome era: multivariate models of memory and intelligence. Neuroscience 164(1):88–107
Sanders SJ, He X, Willsey AJ et al (2015) Insights into autism spectrum disorder genomic architecture and biology from 71 risk loci. Neuron 87(6):1215–1233
Sanislow CA, Pine DS, Quinn KJ et al (2010) Developing constructs for psychopathology research: research domain criteria. J Abnorm Psychol 119(4):631–639
Schizophrenia Working Group of the Psychiatric Genetics Consortium (2014) Biological Insights from 108 schizophrenia-associated genetic loci. Nature 511(7510):421–427.
Schork NJ (1997) Genetics of complex disease: approaches, problems, and solutions. Am J Respir Crit Care Med 156(4 Pt 2):S103–S109
Sekar A, Bialas AR, de Rivera H et al (2016) Schizophrenia risk from complex variation of complement component 4. Nature 530(7589):177–183
Sher LM (2002) Current methodological issues in candidate gene association studies in psychiatric disorders. Jefferson J Psychiatry 15(1)

Shibata S, Komaki Y, Seki F et al (2015) Connectomics: comprehensive approaches for whole-brain mapping. Microscopy (Oxf) 64(1):57–67
Simmons JM, Quinn KJ (2014) The NIMH research domain criteria (RDoC) project: implications for genetics research. Mamm Genome 25(1–2):23–31
Smith SM, Beckmann CF, Andersson J et al (2013) Resting-state fMRI in the human connectome project. Neuroimage 80:144–168
Snyder AZ, Raichle ME (2012) A brief history of the resting state: the Washington University perspective. Neuroimage 62(2):902–910
Sporns O (2011) The human connectome: a complex network. Ann NY Acad Sci 1224:109–125
Sporns O (2012) Discovering the human connectome. The MIT Press
Sporns O, Tononi G, Kotter R (2005) The human connectome: a structural description of the human brain. PLoS Comput Biol 1(4):e42
Van Essen DC, Ugurbil K, Auerbach E et al (2012) The human connectome project: a data acquisition perspective. Neuroimage 62(4):2222–2231
Wu X, Liu Q, Jiang R (2009) Align human interactome with phenome to identify causative genes and networks underlying disease families. Bioinformatics 25(1):98–104
Xia M, He Y (2011) Magnetic resonance imaging and graph theoretical analysis of complex brain networks in neuropsychiatric disorders. Brain Connect 1(5):349–365
Xia M, Lin Q, Bi Y, He Y (2016) connectomic insights into topologically centralized network edges and relevant motifs in the human brain. Front Hum Neuroscience 10(158):1–16
Zuo XN, Di Martino A, Kelly C et al (2010) The oscillating brain: complex and reliable. Neuroimage 49(2):1432–1445

Chapter 3
Appraising Circuit-Centered Neurophenotypes

Vinoth Jagaroo, William Bosl and Susan L. Santangelo

The Research Domain Criteria (RDoC) strategy places the "neural circuit" as the central functional unit around which the understanding of brain-behavioral relationships can be mapped (see Chap. 2). This is premised to a good degree on the G–P framework—the guiding scientific model in which the neural circuits can be relationally embedded. And through this associative framework, circuit abnormalities can be traced via gene-driven precursors to genes. NPs can then be described in terms of circuit structure and function.

RDoC also recognizes non-genetic mediators in circuit functions. Although not explicitly laid down in the in the representational/analytic matrix, RDoC recognizes the importance of developmental and environmental factors in understanding brain-mediated illnesses (www.nimh.hih.gov/about/strategic-planning-reports/

V. Jagaroo (✉)
Department of Communication Sciences and Disorders, Emerson College, Boston, MA, USA
e-mail: jagaroo@bu.edu

V. Jagaroo
Behavioral Neuroscience Program, Boston University School of Medicine, Boston, MA, USA

W. Bosl
Department of Healthcare Informatics, Benioff Children's Hospital Oakland Research Institute, University of San Francisco, San Francisco, CA, USA
e-mail: wjbosl@usfca.edu

S.L. Santangelo
Department of Psychiatry, Tufts University School of Medicine, Boston, MA, USA
e-mail: ssantangel@mmc.org

S.L. Santangelo
Maine Medical Center Research Institute, Maine Medical Center, Portland, ME, USA

S.L. Santangelo
Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA, USA

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_3

index.shtml). At the current time, RDoC does not afford the systematic integration of such factors into its matrix. These factors are also conceived as "orthogonal dimensions that should inform hypotheses and conclusions derived from the RDoC organizational structure" (Morris and Cuthbert 2012, p. 33).

The emphasis on circuits in RDoC, again, is to mark a manageable level of representation that can be given systematic focus. Notwithstanding, in light of revelations from neuroscience about complex circuit dynamics and the process of actually understanding circuits, the notion of a "circuit-level" framework in RDoC runs into complications. The neat demarcation of circuits in the nervous system is a confounding prospect let alone demarcations of circuits drawn for pragmatic reasons. And simplification and essential linear causality in any conceptualization where a neural circuit and its function are framed heavily within a G–P matrix make for a particular quagmire in the context of circuit dynamics.

Circuit functional dynamics, the multiple determinants of synaptic variables, and the complexities of neuromodulation add an array of challenges to the notion of circuit-based NPs. These have been expressed along many levels. General discourse on RDoC and circuit NPs has included questions about the appropriateness of trying to anchor complex clinical/behavioral manifestations around neural circuits. Commentary from neuropsychiatric genetics has pointed to instances of nonlinear associations between genes and receptors. However, the more serious complications faced by both G–P-driven notions of neural circuits and RDoC's conceptualization have to do with questions of use-dependent plasticity, circuit definition and scale, and complex neuromodulation. The topic of epigenetic neuromodulation in particular, while already being brought to bear on the burgeoning initiative of cellular connectomics, has been relatively unrecognized in the discourse on circuit-based NPs. Further, the push for circuit-based NPs rests in part on phenomics (see earlier sections). The technical challenges of mapping neurons, synapses, and circuits at the cellular level has been playing out aloud in cellular connectomics, and interestingly, may give sobering pause to notions of neat demarcations of neural circuits at the microscale. These issues are described below.

3.1 Circuit Phenotypes: Commentary from Psychiatry

Commentary on neurophenotyping in psychiatry and commentary on RDoC have been plentiful and continue vigorously.[1] Quite expectedly, this has come largely from the perspectives of clinical psychology and psychiatry, and psychiatric genetics. Much of this has taken place within discussions of RDoC—its constructs, its circuit-level pitch, its seemingly extreme turn to a medical/biological model of

[1]See for example the Special Issue of the *Journal of Abnormal Psychology* (2013), Vol. 122, No. 3.

brain and behavior, etc.—have been critiqued, as has been the difficulty of matching psychiatric phenotypes with gene variants. While the commentary has breadth of topical coverage—nosology for psychiatric disorders; genetics and complex behavior; notion of neural circuits; etc.—the criticism sampled below has also been unsurprising.

That cellular or circuit phenotypes can be the manifestations of a number of gene-expressed pathways, each bearing the combined influence of genetic and environmental predispositions is a reminder often given (for example, by Linden 2012). And this can account for the complications in some of the findings where genes are associated with a receptor subtype in neuropsychiatric or neurocognitive disorders, for example, in bipolar disorder, a strong association has been made with SNPs on the genes coding for the GABA-A receptor beta 1 subunit, yet protein sequence is unaffected, and it is unclear which of many possibilities underlie the association (Craddock et al. 2010). NMDA receptor complexes that are implicated in schizophrenia may be a convergent effect of CNVs on many genes (Kirov et al. 2012). Commenting on the spectral nature of many complex mental disorders and the discordant fit of highly specified DSM boundaries, Hyman (2002) highlighted the relevance of the notion of phenotypes along a continuum. It was suggested that quantitative phenotypes would better represent the scenario where multiple interacting gene variants (as opposed to rare mutations) instruct a range of possible neurophenotypes, the variations of which may account for the spectrum of behavioral symptoms within common clusters. Commenting specifically on the neural circuit-level descriptors as possible classifiers of neuropsychiatric disorders, Nesse and Stein (2012) remarked on the non-discrete nature of neural circuits: Shaped by evolution, the brain's information processing systems are characterized by distributed circuit architecture and redundancy, making for indistinct boundaries. This is in contrast to a more tractable type of circuit typical of "human-designed circuits" in which connections can be circumscribed and modules can be marked.

Views on circuit NPs in psychiatry and clinical psychology are contained by two opposing perspectives. One is the RDoC position that a brain-based (circuit-centered) nosology of behavioral dysfunction is a much needed step. The other is tempered by the fuzzy, non-Euclidean nature of both neural systems and clinical expression, a view well captured by Nesse and Stein (2012): "… some complaints about comorbidity and heterogeneity of DSM diagnosis may arise from unrealistic expectations. There is no reason to expect that syndromes arising from dysregulated systems will have specific causes or sharp boundaries, and no reason to expect that a brain-based diagnostic system will ever be able to categorize them adequately. The comorbidity, heterogeneity and blurry boundaries of many DSM categories may accurately reflect clinical reality." (p. 6).

Circuit functional dynamics and their modulatory variables are rather nonlinear for the most part. The most serious complications faced by both G–P-driven notions of neural circuits and RDoC's conceptualization have to do with questions of circuit definition, circuit scale, use-dependent plasticity, and complex neuromodulation. These complications do not by default render inviable the prospect of

a "circuit-based" system of describing behavior but they do highlight (a) the difficulty of circumscription when it comes to neural circuits, and (b) the potential for illusion when viewing circuits mainly as gene-designed entities.

3.2 The Scale of the Circuit: Minimal Circuit Definition

What defines "circuits" when describing NPs at the "circuit-level?" Are they discrete circuits defined by neurons with closely apposed synapses; are they circuits that include wide-ranging projections; or both forms including all in-between scales? At the microscale, the problem of circuit definition has been playing out finely in connectomics—where the question of "minimal circuit volume that fulfills the criterion of sufficient completeness for a sufficient number of relevant neurons" (Helmstaeder 2013, p. 502) is grappled with. And one approach that is used in connectomics is to demarcate the presence or absence of synapses in a given spatial extent of "relevant neurons" (Helmstaeder). Such an approach may work well in the case of the retinal connectome, other sensory system connectomes, or any connectome where relatively fixed connectivity rules or motifs apply. For example, in the retina, *X* number of photoreceptors can be mapped to *X* number of bipolar cells, mapped in turn to a single ganglion cell—here the criterion for circuit definition is neatly bracketed in terms of start and end points. In this instance, the connectivity scheme is easily matched to a functional scheme. Minimal circuit definition may also be easier applied to cortical–subcortical circuits that have been well established through neural tracing and other means, for example, the direct and indirect pathways to (and within) the basal ganglia.

With very different cytoarchitectonic systems, such as that of association cortices, the rubric or motif for discrete circuit definition is currently unclear. Should it be intralaminar and intracolumnar neuronal (areal) synaptic trees centered around a given (*X*) number of pyramidal cells, or *X* number of cortical columns or minicolumns? With numerous possible definitions of the core unit of the circuit comes numerous possibilities in terms of the notion of *minimal circuit dimension*, an operating principle that circuit-based NPs must address. When excitatory–inhibitory dynamics of interneurons and microcircuits are factored in, minimal circuit dimension becomes even more difficult. See Fig. 3.1.

This is further complicated by large-circuit architecture (see earlier discussion on MR connectomics). Synchronous or dynamic patterns of activity across widespread nodes may amount to the key functional property of relevance. Spatial and temporal associations across these "whole-brain" networks, mapped during resting states or task-driven states, may constitute novel neuropsychiatric and neuropsychological NPs (Castellanos et al. 2013; Deco and Kringelbach 2014; Fornito and Bullmore 2012; Fornito et al. 2015). Functional networks of this type have also long been suggested through EEG studies—neural oscillations can be phase locked across widely spread cortical regions (see Mathalon and Sohal 2015 for a recent synopsis), and the phenomenon has been interpreted as indicating

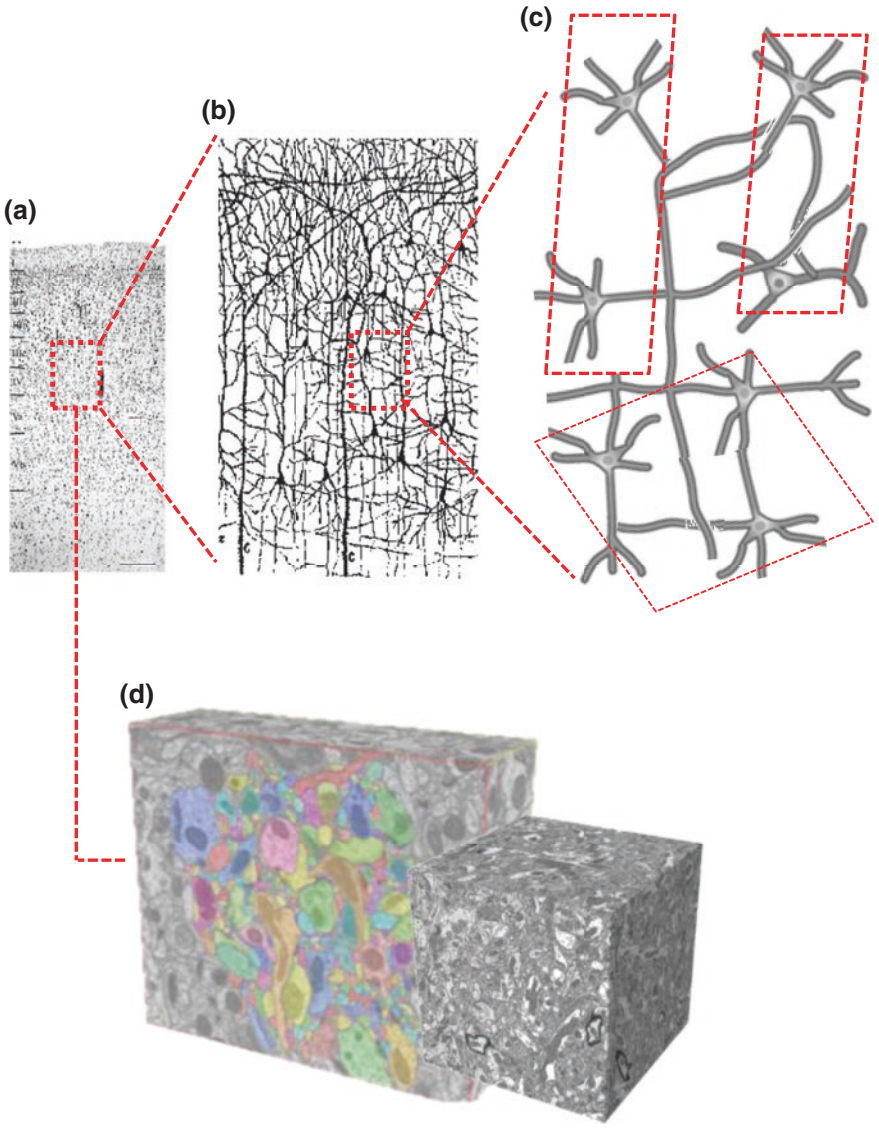

Fig. 3.1 A simplified schematic illustrating the problem of minimal circuit dimension using the example of intra-cortical circuits. Cortical laminae are depicted by **a** and **b**. In **c** that expands a small section of cortical tissue, one neuron in the figure may represent a few hundred or a few thousand neurons. The collective microcircuit may have a certain emergent property, and in theory, the role of each neuron can be weighed. If distinct neural computations at the cortical level make for NPs, what are the corresponding neural scales of circuit dimensions? Dense reconstruction of cortical tissue (serial block EM connectomics) depicted in **d** only highlights the difficulty of demarcating circuits at the microscale

distributed functional networks (Singer 1999; Fries 2005). How and whether the principle of minimal circuit definition should apply to distributed circuits cast as NPs is a wide open question. In general, a restricted connectivity scheme may be applicable for smaller scales of neural circuits but not for larger networks (Getting 1989).

If the tractability of gene-to-cell synthetic processes is an argument made within the G–P framework, then it would seem that cells within close spatial dependence (cell aggregations) would be the first level of interest in terms of circuits—from cells to local circuits to larger circuits, brain regions, etc. However, if gene expression patterns can also be mapped in terms of instructing large-scale circuits (see Parikshak et al. 2015), or cell types (see Kim et al. 2008) that may be distributed but functionally associated, the notions of circuits constructed within the G–P framework is greatly expanded.

At one level, therefore, the non-discrete nature of large-scale circuitry may pose a complication to G–P models premised on the outward neurophenotype as a discrete and tractable circuit. Similarly for RDoC schema centered on a "neural circuit," the less discrete the circuit, the more problematic the formulization becomes. On another level, gene modules linked to distributed brain circuits (see Oldham et al. 2008) hold promise in identifying large-scale circuitry that may constitute neurophenotypes and which can be framed within the G–P model. Transcriptomic analysis, for example, of tissue from perisylvian cortical areas, has revealed that related speech areas distributed across frontal, parietal, and temporal cortices share a common set of genes, implicating a shared developmental program (Johnson et al. 2009).

In summary, neural networks ranging in topology from nodular to large-scale/whole-brain distributions bear functional properties that may constitute useful NPs. However, circuit-pitched NPs will require additional development in terms of models and nomenclature that further specify the scale of the circuit. Model circuit systems vary widely in type, with spatial extent being one of many variables. The scale will be dependent on the sensory or brain system being characterized or the neural module in question: These systems may range from a few neurons in a sensory system relay to hundreds of millions of neurons in distributed circuits mediating complex cognitive processes.

3.3 Circuit Architecture and Functional Modulation via "Non-gene-Regulated" Factors

3.3.1 Synaptic Plasticity

That synaptic dynamics and neural circuit architecture can be affected by induced repetitive use or behavioral conditioning which has long been recognized in neuroscience. And the classic model of Hebbian Conditioning supported by the

mechanism of Long-term Potentiation (LTP) (Bliss and Lomo 1973) is commonly invoked when referencing synaptic and circuit modifiers, plasticity, and associative learning. That is, repetitive stimulation of a neural pathway leads to an increased probability of the pathway being activated due to metabolic or physical changes at the synaptic level (the Hebbian synapse), and after repetitive pre-synaptic stimulation, a relatively weak presynaptic input can produce a strong and lasting excitatory post-synaptic potential (LTP). In the rodent model, a host of neuronal changes have been documented in relation to exposure to different external environments (Sirevaag and Greenough 1988). Further, the physical (molecular) changes at the synaptic level resulting from convergent activity of neurons, as a basis of learning and memory (Kandel 2007), as well as changes to the number and size of sensory synapses following habituation (Miniaci et al. 2008) as demonstrated in the Aplysia model, have become landmark references of synaptic modification resulting from exogenous stimuli. These mechanisms make for the common reference points in terms of "non-gene-regulated" processes by which neural circuits can be physically modified—though mechanisms of long-term potentiation and long-term depression have been elaborated to include, for example, the temporal order of synaptic input and back propagation of action potentials (see Paulsen and Sejnowski 2000). Through the processes of Hebbian conditioning, synaptic dynamics in nodes of a circuit can be altered to the point where the large-scale circuit dynamics are re-organized. This phenomenon is backed up by theoretical (neural network) modeling, demonstrating that "the network forgets about its initial synaptic structure and is rewired by Hebbian learning into a new synaptic structure that emerges with learning and that depends on *the whole history of the neuron dynamics*." (Siri et al. 2007). It is also worth noting that when studying synapses and circuits at the cellular level, while numerous synaptic measures mark plasticity-related changes, it is experimentally difficult to establish baseline levels of synaptic function (Zucker and Regehr 2002).

Establishing a baseline is particularly problematic when developmental processes from infancy are considered. It is well established that neural circuits cannot be entirely genetically determined for the simple reason that the complexity of the brain exceeds the complexity of the genetic code (Hassan and Hiesinger 2015). Developmental or evolutionary algorithms have been invoked as a possible mechanism to describe how a few thousand genes can create the enormous complexity of the brain. This approach explains how complex neural structures can be generated through simple pattern formation rules by clarifying the difference between developmental rules versus and the mechanisms of individual molecules that execute those rules (Berardi and Maffei 2015; Hassan and Hiesinger 2015).

"Use-dependent plasticity" adds a complication to interpreting neural circuits as NPs—a hidden shaping force. This complication is now being recognized in MR connectomics where NPs are gleaned in the functional connectome. Fornito et al. (2015) have given detailed review to processes such as diaschisis, transneuronal degeneration, de-afferentation, and dedifferentiation—how they can produce pathological perturbations within large-scale connectomes. Fornito et al. highlight the phenomenon of *diaschisis*, the well-known effect where one region of insult or

dysfuntion results in an effect distal to initial lesion (Monakow 1969). They also substantiate that diaschisis and related process tend to manifest across distally separated neural nodes that are dynamically linked by strong functional circuitry.

If the physical structure and functional dynamics of a given neural circuit can be re-organized under the influence of an external stimulus, this raises the obvious question of the index-value of a neural circuit, both in the G–P context at well as RDoC. It can still be argued that the pattern of a "normal circuit" can be easily identified for a particular neurophenotype by extracting an average circuit architecture or functional pattern from many normal subjects (supporting the value of identifying circuit-based neurophenotypes). However, it is when the G–P framework is laid on as the dominant scientific, associative framework for circuit reference—where circuit design and activity are considered manifest products of gene-transcriptional networks and biochemical gradients—that a particular complication arises. This problem is especially well exposed against the body of theory on (a) extrasynaptic modulation of neural circuitry and (b) the role of bioelectric dynamics on circuit morphology. Neural circuits can be significantly modulated or radically reconfigured by epigenetic factors—and some of these factors are constituted by intrinsic cell dynamics that arise from the complex biochemical interactions that occur between DNA and neuroanatomical structure.

3.3.2 Extra-Synaptic Neuromodulation

Neurobiologists studying the complexity of neuromodulation have long cautioned against the perspective that the essential functional characteristics of a neural circuit be viewed primarily in terms of a fixed, characteristic neural architecture centered on synaptic connectivity patterns (Getting 1989; Marder and Thirumalai 2002; Marder et al. 2014). The essential message of these studies is that in addition to the neurotransmitter associated with a neuron, various neuromodulatory substances (such as biogenic amines and neuropeptides) that are involved with a range of signaling activities, can significantly influence the function of a neuron and the state of a neural circuit. And the state of a neural network influences the way in which the neuromodulators work.

Many insights about the complexities of chemical modulation/reorganization of neural circuits have derived from studies of central pattern-generating circuits in worms, insects, molluscs, and crustaceans. However, the complexities have been generalized to other invertebrate and vertebrate neural systems (Weimann and Marder 1994) and are now understood as a set of "general principles of neuromodulation at the neuron and circuit levels" (Bucher and Marder 2013). A point emphasized in this work is that a neural circuit's stable architecture may be an illusory measure of circuit functioning. The elaboration given below is based on Bargmann (2012), Bargmann and Marder (2013), Getting (1989), Marder and Bucher (2007), Marder et al. (2014), and Weimann and Marder (1994).

Multiple nonlinear processes, collectively described as "neuromodulatory," can influence a neural circuit at the cellular, synaptic, or network level. Extrasynaptic neuromodulators in the form of local hormones or co-transmitters released by neurons distal to a target neuron can significantly change the firing and synaptic properties of neurons. They can adjust the voltage parameters on which a neuron's firing threshold and conductance is based and the synaptic strength of a circuit. The plasticity of a neural circuit in terms of electrical and synaptic variability can be explained by neuromodulation. Such chemical modulation, affecting membrane excitability and synaptic function is not merely supplemental to the physical and electrical properties of a circuit. Rather, it has the strength to alter and reconfigure the functioning of a circuit, making for intrinsic circuit dynamics. And "dynamics" in this scheme also includes the dynamic selection of circuit composition: Connectivity diagrams may give parameter information—overall forms of circuit wiring. But any configuration is under the powerful shaping influence of neuromodulators. Hence, a wiring diagram can be a limited if not an illusory reference point. Chemical neuromodulation adds a whole dimension to circuit dynamics, one that cannot be captured easily by anatomic, microscopic, or electrophysiological descriptions of circuitry. What may present as a relatively dormant synapse at one time, can, under the powerful organizing influence of chemical neuromodulators, present quite differently at a different point in time. That is, molecular neuromodulators can reconfigure the circuit without changing the given synaptic layout. They do this by selectively recruiting neurons in a circuit, and in response to internal states and the behavioral context in which the circuit is being used. The same circuit, the same neuronal subset, can, within very brief temporal windows, switch through different functional roles based on the intrinsic activity of the larger circuit (Weimann and Marder 1994).

As cautioned by Marder (2012), circuit definition that hinges on neural architecture has been influenced heavily by early electrophysiologists, who in turn were influenced by electronic engineering—where circuit diagrams provide a strong organizing reference. And this has shaped a dominant notion in neuroscience that views neural circuits largely in terms of synaptic connectivity layouts which can be broken down into neuron-unit building blocks that sum synaptic inputs.[2] In huge contrast to this model, when the effects of neuromodulators, the many intrinsic properties of neurons, and the dynamics of parallel pathways and antagonistic (excitatory vs. inhibitory) relations are factored, what emerges is a picture of complex nonlinearity (Getting 1989; Bargmann and Marder 2013): In a neural network with numerous neurons, each with a different function, a neuron synapsing with multiple neurons makes for a complicated possibilities. An individual neuron in this system can be influenced through multiple parallel pathways. The network can have more than one state of oscillation that amounts to its functional output.

[2]It is also commonly said in neuroscience that the Cajalian "neuron hypothesis" and the Golgi-labeled sparse neuron diagrams that Cajal elegantly illustrated are deep historical roots of this notion.

Neuronal hubs in the network can shift dynamically, changing the network oscillatory state from one to another. Circuits can self-organize. "[N]euromodulators can activate or silence an entire circuit, change its frequency and/or phase relationships …" (Bargmann and Marder 2013, p. 486), and this is in addition to temporal variations in membrane channel activity and electrical activity across neurons. A common set of neurons may be used flexibly across different subfunctions by adjusting their firing patterns, and thereby resetting the circuit rhythm or oscillation. In some instances, determining a central oscillator is improbable, for example, when the oscillation derives from the activity of many neurons where each neuron has a different phase-response profile.[3] The complex functional significance of neuromodulation and what it spells with regard to a neuron-synapse-centered view of circuits is well encapsulated by the following excerpt from Getting (1989):

> One level of network organization is the anatomical organization, which is defined by the monosynaptic or anatomical connectivity between neurons … specified by the distribution of afferent fibers … synaptic connectivity … projection of efferents … In essence, anatomical organization defines the limits of the network and who talks to whom within the network, but does not give rise to function. The ability of a network to perform a task depends on what building block mechanisms (network, synaptic and cellular) are being expressed at a given moment. [The] anatomical network may be configured into any one of several modes, depending on the particular combination of currently active mechanisms. (p. 194)

3.3.3 Circuit Morphology as Function of Bioelectric Dynamics

In neuroscience, the term synapse references almost exclusively the gap between two neurons where neural signal transmission is mediated by chemical secretion and receptor binding. Within developmental biology, cell development, tissue organization, morphogenesis, etc., are typically framed under regulatory gene networks and biochemical gradients (again, the G–P frame). However, there is now abundant evidence showing that membrane "gap junctions" mediating the intercellular passage of ions and small molecules, constitute electric synapses. And these gap junctions, present in all cell types, including neurons, give rise to bioelectric signaling dynamics that constitute a significant epigenetic regulatory mechanism in cell behavior (see Levin 2012, 2014; Levin and Stevenson 2012; Mustard and Levin 2014; Pereda et al. 2013). A good deal of the evidence on the role of bioelectric signaling in phenotype determination comes from a vertebrate (frog) model and an invertebrate (flatworm) model contextualized within the field of regenerative biology (see Adams et al. 2016; Beane et al. 2013; Levin 2013).

[3]Certain mathematical models may offer ways around this problem—see Chap. 14 in this volume—though in this instance, the number of neural variables that would be needed for the equations are currently improbable in terms of recording and discovery.

Here, it has been demonstrated that voltage gradients and changes in membrane potential patterns across multi-cellular structures are especially important in tissue differentiation and anatomic patterning during embryogenesis and morphogenesis. However, gap junction communication is also evidenced to be important in complex patterning of the phenotype and in normal tissue physiology in both invertebrates and vertebrates (Levin 2007). The formation of entire body regions such as species-specific head anatomy is defined by gap junction-mediated bioelectric signaling and voltage gradients (see Emmons-Bell et al. 2015). The summary point is that endogenous voltage gradients play a significant role in driving the development of the face and brain, and defects in brain formation induced by genetic mutations can be over-ridden by electroceuticals—a case of the physiological state trumping the genetic state (Levin, personal communication, May 25, 2016).

These studies highlight that transmembrane ion channels and pumps determine transmembrane potential gradients across tissues—the resting potential of the cell (V_{mem})—as well as the resting potential of surrounding cells. This results in a bioelectric network topology—regions of isopotential V_{mem}—constituting a bioelectric state memory and facilitating long-range coordination of patterning activity. The studies demonstrate that this bioelectric profile, either in a stable, long-term form, or within a specific temporal window, instructively influences tissue patterning (organ identity, size, and morphology). And when the bioelectric signaling is perturbed, phenotype patterning takes on a different form. Intercellular gap junction activity is not merely a passive player in the collective function of a cell assembly. The electrical synapses they produce have dynamic, plastic effects. This has been dramatically illustrated in a recent study on planarian flatworms—known for their regenerative properties (Emmons-Bell et al. 2015): The experiment involving head regeneration demonstrated a powerful gap junction-mediated influence on phenotype. To stimulate head regrowth in a flatworm species (with normal genome), they differentially perturbed specific bioelectric networks. Gap junctions were briefly blocked with pharmacologic agents, hence altering bioelectric network topology. The resulting head and brain morphological patterns that the flatworms acquired were of forms specific to other species of flatworms despite a normal genomic sequence. This demonstrated that under the guidance of bioelectric physiological networks, different morphologies can arise in an organism. It is thought that the factor of bioelectric signaling comprises a novel form of physiological (epigenetic) regulatory network that is involved in the instruction of phenotype patterning:

> The activity of these remodeling processes after regeneration has been completed is fascinating, and implies that morphology is consistently reassessed and edited, even after large-scale re-organization events have ended. The nature of the processes that drive cell behavior toward a specific, stable end-state (when remodeling ceases) is almost entirely obscure. At this time, it is not possible in any model system to derive specific shape information (to know precisely which anatomical pattern will be a sufficient end-goal state to cause remodeling to stop) from genomic data. (Emmons-Bell et al. 2015, p. 278–282)

Neuromodulation and gap junction-mediated bioelectric networks illustrate control/modulatory systems that can override gene-regulated mechanisms to

produce cell assemblies and cell dynamics. And these are just two well-studied systems among numerous possible physiological systems that instruct a circuit phenotype over the influence of the genome. The instructive power of such processes cautions that in addition to neural circuit dynamics defined by circuit architecture and synaptic function, other complex, nonlinear physiological, and biochemical networks converge to produce a circuit phenotype. The epigenetic influence of intrinsic neural dynamics adds a stochastic element to the control of neural circuits. This obviously begs the question of what information is considered useful when framing cognitive phenomics and RDoC around circuit-level descriptors of brain function: Even if the emphasis is placed on neural-functional aspects of the circuit, it still leaves the problem that this function can be greatly modulated by distal, extra-synaptic neuromodulatory input that will not be captured by circumscribing "the circuit," be it local or long range.

3.4 Technical and Methodological Considerations in Circuit Delineation

Of the many technological and methodological issues that arise in connectomics, there are some that are especially relevant to the initiative of mapping neural circuits for the purpose of establishing circuit-based NPs. This can be said both of macroscale and microscale connectomics though microscale (high-resolution) connectomics profoundly illustrates the challenges of seeing and identifying the neural phenome in a most literal sense. It reveals a host of questions about the nature of neural circuits and the best ways to represent them.

Many of the technical issues facing MR connectomics apply to fMRI and imaging-based biomarker identification in general (and fall outside the limited focus of this discussion; see Castellanos et al. 2013). Issues concerning the presumption or inference of networks are more pertinent. The inference of large-scale neural networks in MR connectomics rests on computational methods. In particular, it rests on the application of graph theory and network science (see Behrens and Sporns 2012; and see earlier subsection on macroscale connectomics). But how nodes and edges of the network are initially defined in the applied template influences the network topology that is inferred (see Zalesky et al. 2010). And, currently, no gold standard exists for the optimal node-edge configuration as may be applicable to various brain networks (cortico-cortico, cortical-cerebellar, etc.). Surveys of the graph metrics used in MR connectome studies have shown them to vary from stable and reproducible to highly sensitive and variable (see Xia and He 2011). And, in current fMRI models of brain parcellations (the regions or nodes in a network), the parcellations can range from the order of tens to many hundreds (Craddock et al. 2013). The dependence on graph theory makes in part for an added paradoxical scenario. The graph theory approach can be interpreted as viable only "if a substantial fraction" of the connections are known Seung (2009).

Graph theory templates can vary in terms of the derivation and application of this fraction. It is also unclear that graph theory as currently applied to MR connectomics will fare well if faced with inordinate numbers of connections.

Nonetheless, it is well acknowledged in applied network science that the computational theory to support the understanding of dynamic-temporal changes in brain networks is lacking and needs urgent development (Sporns 2013). The strength of MR connectomic data and the strength of computational models are co-dependent, and the enrichment of computational models is a factor that is important in the development of NPs derived via MR connectomics (Deco and Kringelbach 2014).

3.4.1 Microscale Connectomics

In addition to novel views of brain tissue at the microscale, cellular-resolution connectomics has presented an intriguing set of issues that challenge assumptions about the notion of neural circuits at the microscale and the task of mapping them. Again, the focus below is limited to considerations that relate more directly to the prospect of delineating neural circuitry, which is a part of the phenomics agenda and which would seem to be a necessary step in describing NPs at the circuit level.

Even with automatic 3D segmentation of electron microscopy tissue sections, there are extremely tough (computational vision) challenges in merging and labeling nanometer-scale slices of brain tissue (Helmstaedter 2013; Vazquez-Reina et al. 2011): The non-regularities in neuronal branching, complex merging and termination patterns, the fact that dendritic spines can be lost in the between-section cuts, and variations in tissue preparation, are challenges yet to be overcome. The current error rate in automatic segmentation is about one mistake (in confusing neuronal splits or mergers) per mm^3 of tissue (Lichtman et al. 2014). And, complete labeling of cell types in segmented images is more easily presumed than done—Lichtman (2015)[4] has cautioned that certain structures or formations in a 3D tissue matrix confound even seasoned neurobiologists in terms of identity—connectomics at the microscale currently encounters a certain amount of tissue that escapes easy classification.

Dense reconstruction of cortical tissue (Kasthuri et al. 2015) reveals that the multiple synapses that an axon may make varies from weak to strong, a finding that is significant in terms of "the circuit" expressing as a NP. Such a key aspect of circuit profiles can only be observed through such dense connectomic analysis or through in vivo cell-level imaging (e.g., optogenetics). Obviously, neither of these methods is currently feasible in the living human.

Kasthuri and Lichtman (2007), Lichtman et al. (2014), and Burns et al. (2013) have issued some sobering details around data requirements in microscale

[4]Lecture given at Boston University, titled "The Promises and Perils of Connectomics," December 4th, 2015.

imaging: A section of brain tissue measuring 1 mm^2 and 30 nm thin, imaged at a resolution where each pixel measures 4 nm × 4 nm, generates an image size that exceeds 60 gigabytes, and 3D volumetric imaging of a single cubic millimeter of brain tissue can generate 2 million gigabytes (2 petabytes) of data. With the best of current technology, imaging a 1 cubic millimeter of brain tissue has a completion time of 1 month, generating 1 terabyte (1000+ gigabytes) per day.

All of these issues as well as a host of other technical issues not described here have prompted a central question in cellular connectomics: What is an appropriate or optimal density scale at which the mapping and reconstruction should take place? And this is a most pertinent question for neural phenomics in general: In view of the technical and logistical demands and challenges of cellular connectomics, what should be the scope of the connectomic delineation of the brain? Is comprehensive and precise entity mapping down to each and every neuron and synapse, realistic? Or does phenomic delineation of the brain mean a detailed representational map involving some probabilistic reconstruction and extrapolation where regularities in tissue are seen? But if probabilistic extrapolation is to be assumed and if used as a model for a major connectomics initiative (as is the case with the Human Brain Project, see Markram 2012), there is the problem (as demonstrated by Kasthuri et al. 2015) that a certain connectomic pattern expected of a region may not actually be manifested. The best case scenario is likely one where a multiscaled connectomic representation of the brain is achieved—it is imaged from subcellular resolution to gross anatomical resolution, and with integrated multiscaled imaging and visualization tools, one can drill up or down the levels of resolution. However, without maps of functional circuity that will bring meaning to this representation, it is likely to have little utility—structural maps are not equivalent to functional maps. The brain remains the only organ where the relationship between structure and function is not well resolved. This is in large part due to its immense cellular and molecular diversity, variability across small regions, projections over long ranges, and the fact that electrical and chemical activity constitute (hard to interpret) functional activity of the brain (Lichtman and Denk 2011; Morgan and Lichtman 2013). Describing the challenges involved with imaging brain circuits at the cellular level, Lichtman and Denk (2011) raised the following possibilities on the interpretation of the data:

> As circuit analysis finally moves forward, serious questions concerning its utility will be raised. One obvious question concerns the variability in the structure of the brain at the synaptic level … [s]ome will take such variability to mean that nothing can be learned from doing this kind of tedious, data intensive and highly expensive work. Alternately, it is likely that one could learn a great deal about the game of chess by watching one game, despite the fact that it is highly unlikely that any two games are identical. It is certainly possible that certain circuit motifs will be recognizable and will be interpretable in a number of contexts (p. 622).

The compelling questions that arise in cellular connectomics inevitably challenge the notion of circuit-based NPs. It does not discount the notion but adds a set of tough operational filters. What is the scale of circuity in question and how

might these differ across different brain systems? At what scale or scales are cognitive and neural processes to be interpreted—in the case of NPs, at what levels in the circuit are they distilled and constrained? How is the association of NPs to circuits to be made if the circuits themselves pose such challenge in terms of fine profiling? What are the risks that come with making certain assumptions about neural circuits in terms of NPs?

3.4.2 Other Considerations

Simple notions about neural connections can emerge with the rise of connectomics (both microscale and macroscale), with the popular application of graph theory to neural-functional architecture, and with source-target/input-output perspectives of circuitry. That all these factors can lead to terribly simplified views of neural connections, and neural circuitry has been cogently summarized by Rockland (2015)—in describing the complexities of connectional strength, connectional heterogeneity and subtypes, connectional reciprocity, topography, and hierarchical patterns. Her caution echoes that of Marder (2012) described in Sect. 3.3.2.

And even further caution can be drawn from work in the area of evolved electronic circuits. It has illustrated in a fascinating way the challenge of identifying circuit function from structure: When designing electronic circuits, engineers often use devices called field programmable gate arrays (FPGA) that consist of a hierarchy of reconfigurable interconnects that allow the circuit elements to be rewired in different configurations. In the late 1990s, engineers at the University of Sussex, UK, had the idea of using evolutionary algorithms to design new circuits (Thompson and Layzell 1999). Evolutionary algorithms are inspired by natural evolution, where competition among different genetic designs causes the most functionally adaptive traits to be preserved. Designing with evolutionary algorithms requires that the circuit function or output be defined, while the circuit structure itself evolves. The process is in many ways similar to the way neural circuits are developed through interaction with environmental inputs (Raman and Wagner 2011; Izquierdo and Beer 2013). What is particularly interesting about circuits produced by evolutionary algorithms, and possibly of interest to connectomics, is that evolved circuits in the experiment were very different from what the engineers would have designed or envisaged through a top-down approach. Often network structures with no apparent purpose would appear. Yet, when removed, circuit function failed (Thompson 1998; Thompson and Layzell 2000). The simple lesson is that determining circuit function from structure is remarkably difficult, even in electronic circuits that can be precisely described by mathematical equations and which are much simpler than neural circuits. Some of the principles by which evolved electronic circuit function is studied may be of value to the structure-function challenge of neural circuits (Thompson and Layzell 1999).

3.5 Finding Utility in Circuit-Level Descriptors for Neurophenotype Exploration

Cognitive and neuropsychiatric phenomics, and RDoC, place great emphasis on neural circuits as an ideal index to describe brain-behavioral relationships and anomalies. The emphasis on neural circuits as NPs implies that neurocognitive and neuropsychiatric conditions, as well as variations in normal NPs, are significantly mediated at the circuit level. It suggests that disease NPs are filtered at the circuit level—that in the multiple systems from genome-to-phenome, the circuit NP provides a window that optimally constrains the view of a disorder. As conveyed, two influences of circuit-based NPs as put forth by RDoC are (a) synaptic/neurotransmitter-centered models deriving form a psychopharmacological root in psychiatry and from psychiatric genetics, and (b) low-resolution, nodal-distributed circuit models, deriving from functional neuroimaging. Both these influential roots continue to be the significant forces in behavioral neuroscience.

A broader appraisal of neural circuits, as given by this chapter (albeit still a limited appraisal), only formalizes what is fairly obvious: That in addition to understanding genomic instruction behind neural circuit phenotypes, in addition to a synaptic and connectivity perspectives, other biochemical and physiological networks as they impact circuit phenotype will need to be understood. Information about neural dynamics, neuromodulation, and coordinated activity of neurons need to be understood for a circuit connectivity map to have significance (Bargmann and Marder 2013). Similarly, these processes cannot be ignored if valid circuit-centered NPs are to be formulated.

The process of discovering circuit-based NPs will require comprehensive data at all scales. Dynamic circuit properties gained from various recording and assessment modalities; functional information to map the weighting (importance) of connections in a network; dynamic properties of cells and circuits under various conditions; and molecular-level understanding of all circuit regulatory systems, will all be required. Further, long-term circuit states and changes will need to be understood, for example, connectivity changes such as synapse elimination and addition as governed via ontogenetic programs or induced by external activity. How neural circuits shift their states dynamically as they process information under varying response and behavioral conditions, and how this change in expressed NP in turn influences the response/behavior, is one of the more complex kinds of circuit dynamics that must play into circuit NP definition. Computational models will be required for all forms of circuit definition so that circuit function can be simulated and functional breakdown can be predicted based on altered network patterns (a tool that is essential to whole-brain modeling in MR connectomics).

As Bullmore and Sporns (2009) have cautioned, while neural networks may constitute useful NPs, the metrics by which we define networks relevant to this purpose are far from defined. They emphasize the need to understand the interaction between actual neural substrate and functional networks. This may range

from isomorphic at some scales to complex, non-isomorphic at other scales, for example, when circuit function remodels and reconfigures the circuits. And this illustrates one of the major complications for circuit-based NPs: They are easier to describe in isomorphic form (e.g., synaptic arrangement and neurotransmitter corresponding to event *X* at time *X*). And the representation of this kind of circuit NP may resemble canonical synaptic and wiring diagrams. The dynamics of functional feedback and how this may model the circuit form over the long term are not as easily accessible. This form of circuit NPs may be better represented by topological diagrams of the kind used to render dynamically changing social networks. This gives even more reason that potential circuit NPs be accompanied by models that represent the circuit in situ—the larger circuity of which it is a part (also emphasized by the themes of extrasynaptic modulation). If the pinning of NPs requires a consolidated understanding of physiological systems at all levels, a daunting feat and seemingly indefinite in scope, what makes for the utility of circuit-based or other types of NPs?

G–P frameworks may involve some reductionism, but they do offer a solid scaffold on which complex synthesis can be woven. Similarly, in the context of NPs, neural circuits of any scale and their emergent functional properties can be seen as accessible reference points. They can be seen as standard scaffolds needed to address smaller (subcellular) and larger (neural network) systems associated with the circuit. Building on the known or hypothesized functional properties of circuits is the obvious starting point in trying to solidify circuit-centered NPs: If *X* number of neurons describe a circuit shown to have a well-defined emergent property, one obvious question becomes, what is the stable functional state that the circuit maintains in order to produce the property? Even considering the differential contributions of firing rates, intrinsic and extrinsic modulatory influences, etc., the function as a whole defined, for example, as an oscillatory pattern, a sequential firing pattern, or some combination of excitation and inhibition, make for a circumscribed organizational unit. Attempting to demarcate such a system for which normal and abnormal patterns can be established is to attempt to mark a circuit-based NP. The approach does not preclude modulatory variables nor does it preclude the possibility of more than one stable state of the circuit, each in response to a different permutation of contextual or modulatory variables. *Circuit-centered NPs need not presume a causative or associated mechanism beyond the immediate neural or biochemical processes that can be firmly tied to the manifesting NP*. The causative associations given to the NP may be adjusted as more knowledge is gained. A circuit NP is a convenient locus around which all kinds of functional or physiological data can be associated.

Neural circuits define an optimal intermediate platform from which "drilling down" and "drilling up" can proceed: Specific neuronal and subcellular components that are tied to specific circuit functions can be identified (downstream); and neuronal connectivity patterns driving collective neuronal activity can be mapped (upstream). It makes a good starting point from which to join the dots and fill the blanks. Though, as described in earlier parts of this chapters, there are wide-ranging scales of circuitry, and potentially many classes of circuit NPs. Ironically, one

of the best reasons for pitching cognitive and neural phenotypes at the circuit level may have little to do with a rationale from neuroscience but a lot to do with a cognitive pitch, "cognitive" here referring to cognitive appeal on the part of researchers and clinicians. The model of a circuit-level NP as a functional system/unit that is positioned approximately midway between subcellular/cellular systems on one side and circuits systems, neuroanatomic nodes, and structures on the other side, may be conveniently simplistic but strategic. This strategic point also happens to correspond with an approximate center point in the breadth of the landscape of neuroscience—with molecular neuroscientists on one end of the spectrum and cognitive/behavioral neuroscientists at the other end. Circuit-level NPs is the zone of overlap—a common language to which neuroscientists on all strata of the spectrum can relate.

References

Adams DS, Uzel SG, Akagi J et al (2016) Bioelectric signalling via potassium channels: a mechanism for craniofacial dysmorphogenesis in KCNJ2-associated Andersen-Tawil syndrome. J Physiol 594(12):3245–3270

Bargmann CI (2012) Beyond the connectome: how neuromodulators shape neural circuits. BioEssays 34(6):458–465

Bargmann CI, Marder E (2013) From the connectome to brain function. Nat Methods 10(6):483–490

Beane WS, Morokuma J, Lemire JM et al (2013) Bioelectric signaling regulates head and organ size during planarian regeneration. Development 140(2):313–322

Behrens TE, Sporns O (2012) Human connectomics. Curr Opin Neurobiol 22(1):144–153

Berardi NA, Maffei L (2015) Brain structural and functional development: genetics and experience. Dev Med Child Neurol 57(Suppl 2):4–9

Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232(2):331–356

Bullmore E, Sporns O (2009) Complex brain networks: graph theoretical analysis of structural and functional systems. Nat Rev Neurosci 10(3):186–198

Burns R, Roncal WG, Kleissas D et al (2013) The open connectome project data cluster: scalable analysis and vision for high-throughput neuroscience. Sci Stat Database Manag

Castellanos FX, Di Martino A, Craddock RC et al (2013) Clinical applications of the functional connectome. Neuroimage 80:527–540

Craddock N, Jones L, Jones IR et al (2010) Strong genetic evidence for a selective influence of GABAA receptors on a component of the bipolar disorder phenotype. Mol Psychiatry 15(2):146–153

Craddock RC, Jbabdi S, Yan CG et al (2013) Imaging human connectomes at the macroscale. Nat Methods 10(6):524–539

Deco G, Kringelbach ML (2014) Great expectations: using whole-brain computational connectomics for understanding neuropsychiatric disorders. Neuron 84(5):892–905

Emmons-Bell M, Durant F, Hammelman J et al (2015) Gap junctional blockade stochastically induces different species-specific head anatomies in genetically wild-type *Girardia dorotocephala* flatworms. Int J Mol Sci 16(11):27865–27896

Fornito A, Bullmore ET (2012) Connectomic intermediate phenotypes for psychiatric disorders. Front Psychiatry 3(32):1–15

Fornito A, Zalesky A, Breakspear M (2015) The connectomics of brain disorders. Nat Rev Neurosci 16(3):159–172
Fries P (2005) A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. Trends Cogn Sci 9(10):474–480
Getting PA (1989) Emerging principles governing the operation of neural networks. Annu Rev Neurosci 12:185–204
Hassan BA, Hiesinger PR (2015) Beyond molecular codes: simple rules to wire complex brains. Cell 163(2):285–291
Helmstaedter M (2013) Cellular-resolution connectomics: challenges of dense neural circuit reconstruction. Nat Methods 10(6):501–507
Hyman SE (2002) Neuroscience, genetics, and the future of psychiatric diagnosis. Psychopathology 35(2–3):139–144
Izquierdo EJ, Beer RD (2013) Connecting a connectome to behavior: an ensemble of neuroanatomical models of C. elegans klinotaxis. PLoS Comput Biol 9(2):e1002890
Johnson MB, Kawasawa YI, Mason CE et al (2009) Functional and evolutionary insights into human brain development through global transcriptome analysis. Neuron 62(4):494–509
Kandel ER (2007) In search of memory: the emergence of a new science of mind, 1st edn. W. W. Norton and Company, New York
Kasthuri N, Lichtman JW (2007) The rise of the 'projectome'. Nat Methods 4(4):307–308
Kasthuri N, Hayworth KJ, Berger DR et al (2015) Saturated reconstruction of a volume of neocortex. Cell 162(3):648–661
Kim IJ, Zhang Y, Yamagata M et al (2008) Molecular identification of a retinal cell type that responds to upward motion. Nature 452(7186):478–482
Kirov G, Pocklington AJ, Holmans P et al (2012) De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. Mol Psychiatry 17(2):142–153
Levin M (2007) Gap junctional communication in morphogenesis. Prog Biophys Mol Biol 94(1–2):186–206
Levin M (2012) Molecular bioelectricity in developmental biology: new tools and recent discoveries: control of cell behavior and pattern formation by transmembrane potential gradients. BioEssays 34(3):205–217
Levin M (2013) Reprogramming cells and tissue patterning via bioelectrical pathways: molecular mechanisms and biomedical opportunities. Wiley Interdiscip Rev Syst Biol Med 5(6):657–676
Levin M (2014) Molecular bioelectricity: How endogenous voltage potentials control cell behavior and instruct pattern regulation *in vitro*. Mol Biol Cell 25:3835–3850
Levin M, Stevenson CG (2012) Regulation of cell behavior and tissue patterning by bioelectrical signals: challenges and opportunities for biomedical engineering. Annu Rev Biomed Eng 14:295–323
Lichtman (2015) "The Promises and Perils of Connectomics." Lecture given at Boston University, December 4th, 2015
Lichtman JW, Denk W (2011) The big and the small: challenges of imaging the brain's circuits. Science 334(6056):618–623
Lichtman JW, Pfister H, Shavit N (2014) The big data challenges of connectomics. Nat Neurosci 17(11):1448–1454
Linden DE (2012) The challenges and promise of neuroimaging in psychiatry. Neuron 73(1):8–22
Marder E (2012) Neuromodulation of neuronal circuits: back to the future. Neuron 76(1):1–11
Marder E, Bucher D (2007) Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. Annu Rev Physiol 69:291–316
Marder E, Thirumalai V (2002) Cellular, synaptic and network effects of neuromodulation. Neural Netw 15(4–6):479–493

Marder E, O'Leary T, Shruti S (2014) Neuromodulation of circuits with variable parameters: single neurons and small circuits reveal principles of state-dependent and robust neuromodulation. Annu Rev Neurosci 37:329–346

Markram H (2012) The human brain project. Sci Am 306(6):50–55

Mathalon DH, Sohal VS (2015) Neural oscillations and synchrony in brain dysfunction and neuropsychiatric disorders: it's about time. JAMA Psychiatry 72(8):840–844

Miniaci MC, Kim JH, Puthanveettil SV et al (2008) Sustained CPEB-dependent local protein synthesis is required to stabilize synaptic growth for persistence of long-term facilitation in Aplysia. Neuron 59(6):1024–1036

Monakow CV (1969) Die lokalisation im grosshirn und derabbau derfunktion durchkortikale herde. In: Harris G, Pribram KH (eds) Mood, states and mind. Penguin Books, London, England, pp 27–37

Morgan JL, Lichtman JW (2013) Why not connectomics? Nat Methods 10(6):494–500

Morris SE, Cuthbert BN (2012) Research domain criteria: cognitive systems, neural circuits, and dimensions of behavior. Dialogues Clin Neurosci 14(1):29–37

Mustard JL, Levin M (2014) Bioelectrical mechanisms for programming growth and form: taming physiological networks for soft body robotics. Soft Robot 1(3):169–191

Nesse RM, Stein DJ (2012) Towards a genuinely medical model for psychiatric nosology. BMC Med 10:5

Oldham MC, Konopka G, Iwamoto K et al (2008) Functional organization of the transcriptome in human brain. Nat Neurosci 11(11):1271–1282

Parikshak NN, Gandal MJ, Geschwind DH (2015) Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders. Nat Rev Genet 16(8):441–458

Paulsen O, Sejnowski TJ (2000) Natural patterns of activity and long-term synaptic plasticity. Curr Opin Neurobiol 10(2):172–179

Pereda AE, Curti S, Hoge G et al (2013) Gap junction-mediated electrical transmission: regulatory mechanisms and plasticity. Biochim Biophys Acta 1828(1):134–146

Raman K, Wagner A (2011) The evolvability of programmable hardware. J R Soc Interface 8(55):269–281

Rockland KS (2015) About connections. Front Neuroanat 9(61):1–7

Seung HS (2009) Reading the book of memory: sparse sampling versus dense mapping of connectomes. Neuron 62(1):17–29

Singer W (1999) Neuronal synchrony: a versatile code for the definition of relations? Neuron 24(1):49–65, 111–125

Sirevaag AM, Greenough WT (1988) A multivariate statistical summary of synaptic plasticity measures in rats exposed to complex, social and individual environments. Brain Res 441(1–2):386–392

Siri B, Quoy M, Delord B et al (2007) Effects of Hebbian learning on the dynamics and structure of random networks with inhibitory and excitatory neurons. J Physiol Paris 101(1–3):136–148

Sporns O (2013) Making sense of brain network data. Nat Methods 10(6):491–493

Thompson A (1998) On the automatic design of robust electronics through artificial evolution. In: Sipper M, Mange D, Pérez-Uribe A (eds) Proceedings of Evolvable systems: from biology to hardware: second international conference, ICES 98, Lausanne, Switzerland, 23–25 September 1998. Springer, Berlin, Heidelberg, pp 13–24

Thompson A, Layzell P (1999) Analysis of unconventional evolved electronics. Commun ACM 42(4):71–79

Thompson A, Layzell P (2000) Evolution of robustness in an electronics design. In: Miller J, Thompson A, Thomson P et al (eds) Proceedings of Evolvable systems: from biology to hardware: third international conference, ICES 2000, Edinburgh, Scotland, UK, 17–19 April 2000. Springer, Berlin, Heidelberg, pp 218–228

Vazquez-Reina A, Michael G, Huang D, Lichtman J, Miller E (2011) Segmentation fusion for connectomics. In: IEEE international conference on computer vision, Barcelona, Spain, 6–13 November 2011, pp 177–184

Weimann JM, Marder E (1994) Switching neurons are integral members of multiple oscillatory networks. Curr Biol 4(10):896–902

Xia M, He Y (2011) Magnetic resonance imaging and graph theoretical analysis of complex brain networks in neuropsychiatric disorders. Brain Connect 1(5):349–365

Zalesky A, Fornito A, Harding IH et al (2010) Whole-brain anatomical networks: does the choice of nodes matter? Neuroimage 50(3):970–983

Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Annu Rev Physiol 64:355–405

Part II
The Endophenotype Concept

Chapter 4
Cognitive Phenotypes and Endophenotypes: Concepts and Criteria

Carrie E. Bearden, Anderson Winkler, Katherine H. Karlsgodt and Robert Bilder

4.1 The Value of Quantitative Traits for Unraveling Complex Disease

Determining individual genes that are associated with a given disorder in order to improve diagnosis, predict future prognosis, or develop individualized treatments is a primary goal of genetics research. In particular, narrowing down a wide field of treatment options based on genotype has the potential to significantly decrease the time needed to identify an appropriate medication, especially in the context of complex disorders such as neuropsychiatric disease. Thus far, however, genetic mapping techniques have been more successful in identifying replicable genetic variants associated with common diseases that are typically characterized by objective diagnostic assessments of disease status, as compared to psychiatry,

C.E. Bearden (✉) · K.H. Karlsgodt · R. Bilder
Semel Institute for Neuroscience and Human Behavior,
Department of Psychiatry and Biobehavioral Sciences,
University of California Los Angeles, Los Angeles, USA
e-mail: cbearden@mednet.ucla.edu

K.H. Karlsgodt
e-mail: karlsgodt@psych.ucla.edu

R. Bilder
e-mail: rbilder@mednet.ucla.edu

A. Winkler
Oxford Centre for Functional MRI of the Brain, Nuffield Department
of Clinical Neurosciences, University of Oxford, Oxford, UK
e-mail: winkler@fmrib.ox.ac.uk

C.E. Bearden · K.H. Karlsgodt
Department of Psychology, University of California, Los Angeles, CA, USA

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_4

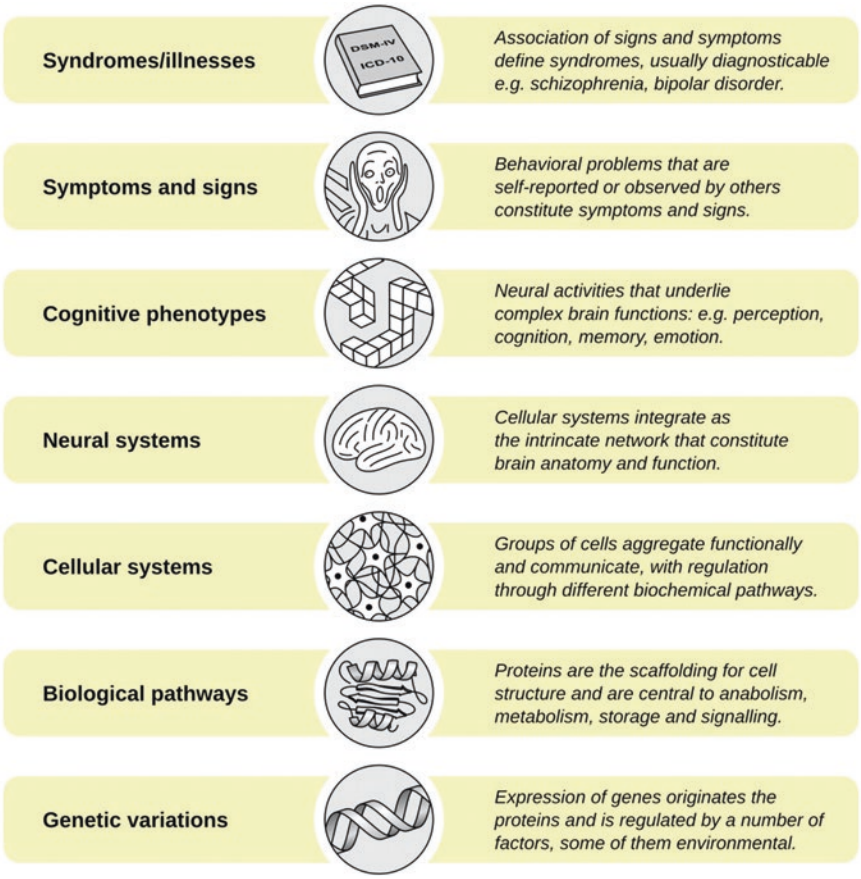

Fig. 4.1 Multilevel model of intermediate traits that bridge the genome–phenome gap

where diagnosis may be more complex. These mappable diseases have an identified biological basis and measure phenotypic features shared relatively uniformly among affected individuals. For example, type II diabetes is diagnosed based on fasting glucose 7.0 mmol/L or higher, as measured by a simple blood assay. This phenotypic feature is at the core of the diagnosis, although other disease components may vary between affected individuals.

For mental illness, it is currently not possible to make diagnoses based on simple biological assays. Thus, the phenotypic features are generally assessed by subjective ratings, and individuals are assigned a diagnosis based on the report of symptoms, which are not consistently present in all or most individuals assigned that diagnosis. There is a growing interest in the development of quantitative assays, which may provide a more objective means of rating psychopathology. Further validation for the use of these objective ratings in psychiatry could be achieved by identifying the genetic basis of such assessments. Therefore, there

has been an increase in efforts to attempt to genetically map endophenotypes—quantifiable characteristics such as brain structure or neurocognitive performance that are hypothesized to be closer to the biology represented by the actions of risk genes than the observable manifestations of psychopathology, i.e., psychiatric symptoms (Fig. 4.1) (Bilder 2008; Gottesman and Gould 2003). To advance our understanding of biological mechanisms involved in the etiology of complex neuropsychiatric disorders, it is critical to translate findings not just from higher level symptoms, but from multiple physiological levels, including the neural systems and cellular and molecular levels, into a new classification system for psychiatric disorders that is based on pathophysiologic and etiologic processes, rather than relying entirely on overt symptom clusters (Hyman 2007).

4.2 Phenotypes, Endophenotypes, and Biomarkers

As pointed out in Chap. 1, terms and constructs such as 'biomarker,' 'phenotype,' and 'endophenotype' or 'intermediate trait' point are used throughout this volume; the terms are also sometimes used interchangeably. It is important to define the terms and constructs—there are subtle distinctions that can make a key difference in interpretation. The term phenotype (from Greek *phainein*, 'to show' + *typos*, 'type') simply refers to an organism's observable characteristics or traits, such as its morphology. Phenotypes result from the expression of an organism's genes, as well as the influence of environmental factors and the interactions between these factors. The term 'endophenotype' refers to a hypothetical construct or a 'latent trait' that in theory cannot be directly observed. This term comes from genetic epidemiology and is used to parse behavioral symptoms into more stable and objectively measurable traits with a (hopefully) simpler genetic architecture than the directly observable phenotype (Gottesman and Gould 2003). The validity of an endophenotype can be supported by determining the consistency of association among and the predictability of manipulations on a set of *observable phenotypes*. This terminology differs from that originally proposed by Gottesman and Shields who described endophenotypes as 'internal phenotypes discoverable by a biochemical test or microscopic examination' (Gottesman and Shields 1973). According to Gottesman and Gould (2003), this term was adapted from John and Lewis, who originally applied the term to explain concepts in evolution and insect biology (John and Lewis 1966). They wrote that the geographical distribution of grasshoppers was related to some feature not apparent in their 'exophenotypes'; this feature was 'the endophenotype, not the obvious and external but the microscopic and internal.' In this volume, the usage of the term 'endophenotype' will be more consistent with that of Matthysse, who focused on modeling of latent traits, with the acknowledgement that empirical measurements are often remote from gene products (Matthysse et al. 1986; Matthysse and Holzman 1987). Criteria for a useful endophenotype are discussed more extensively below; overall, the ultimate value of identifying endophenotypes is in their convergent validation of

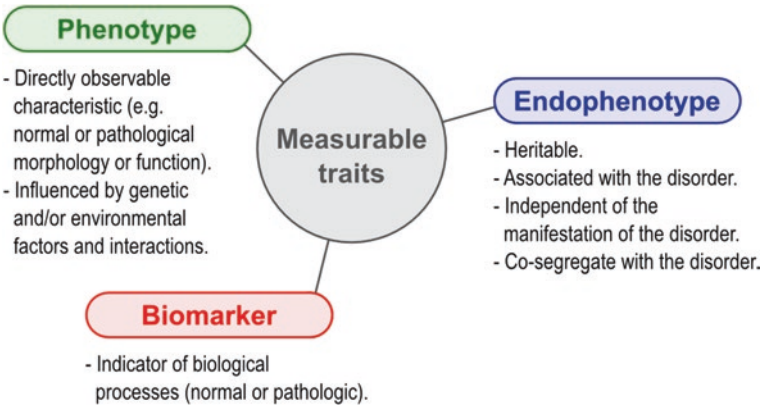

Fig. 4.2 Distinctions between characteristics of a phenotype, biomarker, and endophenotype

specific neural systems models. It should be noted that a biomarker, or biological marker, can be conceptualized as any indicator of a biological state. It is a characteristic that is objectively measurable, and may be an indicator of normal or pathological biological processes, and may be sensitive to therapeutic intervention. A common feature of all of these terms is that they are all quantitative traits, which are—at least in theory—more objectively measurable than psychiatric diagnosis. In addition, quantitative traits should have greater statistical power than would the analysis of categorical (i.e., binary) traits (Fig. 4.2).

4.3 Why Cognitive Phenotypes in Particular?

Cognitive dysfunction is a significant dimension of many psychiatric illnesses. As such, cognitive abnormalities offer quantitative phenotypes for genomic studies and clinical trials and provide strong bridging relations to informative neural systems models. Neuropsychological deficits are increasingly viewed as having substantial relevance to clinical outcomes, leading to the view that cognitive impairments may indeed be more important than the symptoms now used to diagnose the disorder, both in terms of advancing our understanding of disease pathophysiology and for using as potential treatment targets (Bilder et al. 2009a). Measurement of cognitive constructs is highly scalable, enabling phenotype assessments to be conducted with the high throughput necessary for adequately powered genomic association studies and multicenter clinical trials. Here, a *cognitive phenotype* is defined as the broad set of measurable parameters associated with cognition, including not only behavioral symptoms and specific neurocognitive probes, but also physiological and structural indices which we can use to characterize cognitive function at the neural systems and cellular and molecular levels.

Further, because certain cognitive phenotypes are present across multiple diagnostic entities (e.g., memory impairment is prominent in schizophrenia, dementia, and depression), treatments that more directly target these specific phenotypes—rather than clinical diagnosis—may yield even more dramatic public health benefits.

As such, a more complete understanding of the genetic architecture and neural underpinnings of cognitive processes such as long-term or working memory could provide clues about the biological pathways that influence these disorders. To the extent that these cognitive measures are sensitive to the function of genes that also predispose to these illnesses, quantitative indices of cognitive function (i.e., cognitive phenotypes) could be used, either independently or in conjunction with clinical diagnostic information, to identify genes that confer disease risk (Bearden and Freimer 2006; Gottesman and Gould 2003).

4.4 Are There Necessary and Sufficient Criteria for a Viable Cognitive Phenotype?

Recently, a number of publications have catalogued criteria for evaluating the validity and utility of putative *endophenotypic* markers (Table 4.1). While some criteria are universally agreed upon, others are more controversial. In particular, certain quantitative traits may be informative at a biological level and yet may not be heritable (and thus not likely to be useful for a study in which the ultimate goal is to identify genetic etiology). Although it is difficult to specify a priori which criteria are most critical, we have previously proposed tentative guidelines to assist in such endeavors. Table 4.2 offers the conditions that we believe to be both *necessary and sufficient* for a useful endophenotype, with additional criteria to guide optimally informative investigations relevant to cognitive phenotypes. Here, we can think of a cognitive phenotype as a special case of an endophenotype, and although the ultimate goal of any given cognitive phenotype under investigation may not be to identify the underlying genetic basis of the trait, we believe that the trait should nevertheless be firmly grounded in neuroscience, in order to allow translational research.

4.4.1 Reproducibility

One of the primary requirements for a cognitive phenotype is that it can be reliably assessed. The general assumption that such measures have better reliability—that is, the measures are more accurate and more 'objective' than psychiatric diagnoses—has not been adequately evaluated for many candidate cognitive phenotypes commonly cited in the literature. To accurately assess reliability, there must be multiple assessments of the same subject conducted by different

Table 4.1 Previously proposed endophenotype criteria

Gottesman and Gould (2003)
(1) Associated with illness, in the population (2) Heritable (3) State-independent (manifests in whether or not illness is active) but age-normed and might need to be elicited by a challenge (e.g., glucose tolerance test in relatives of diabetics) (4) Within families, endophenotype, and illness cosegregate (5) An endophenotype identified in probands is found in their unaffected relatives at a higher rate than in the general population
Skuse (2001)
(1) Measurable reliability, both over time and by different observers (2) Measures genetic susceptibility to illness, in all those with the susceptibility locus should manifest the endophenotype (3) Specific to the disorder in question.
Doyle et al. (2005)
(1) Should co-occur with the condition of interest (2) Can be measured reliably (3) Evidence of heritability (4) Shows familial overlap with the disorder in question (5) The same genetic factors should influence both susceptibility to disease and performance on the endophenotype measure
Waldman (2005)[a]
(1) Good psychometric properties (2) Associated with the disorder and its symptoms in the general population (3) Stable over time (i.e., expressed whether or not the disorder is currently manifested (4) Expressed at a higher rate in unaffected relatives of probands than in the general population (5) Within families, endophenotypes and illness cosegregate (6) Heritable (7) Common genetic influences underlie endophenotype and disorder (8) Shows association and/or linkage with candidate gene(s) that underlie the disorder and should show association with the gene over and above the gene's association with the diagnosis or symptoms (9) Should *mediate* association and/or linkage between candidate gene and disorder (i.e., the effects of a gene on a disorder are expressed—either fully or in part—through the endophenotype) (10) Should *moderate* association and/or linkage between the candidate gene and disorder (i.e., the effects of a gene on a disorder are stronger in disordered individuals with the endophenotype)

[a]Criteria 1–7 are important for validating the validity and utility of putative endophenotypes in molecular genetic studies; Criteria 8–10 indicate the utility of putative endophenotypes within candidate gene studies

raters (interrater reliability) and at different time points (test–retest reliability). Reliability is particularly important, as variability in methods used for assessment and measurement error contributes non-trivial sources of variability to any candidate trait studied. In general, measures of neurocognitive function are widely considered to be valuable endophenotypes, in large part due to their demonstrated reliability and stability over time (Rund 1998). However, there is considerable variability in the psychometric properties of cognitive measures. For example,

Table 4.2 Necessary and sufficient criteria for meaningful endophenotypes

• *Endophenotypes should be at least moderately heritable* and be detectable in family members of individuals with disorders associated with that phenotype
• *Endophenotypes should be associated with causes rather than effects of disorder* (i.e., should be part of the causal pathway from genes to disorders, rather than effects (sequelae) of disorders)
• Should have good reliability (internal consistency), test–retest reliability (at least within a particular state and preferably across states in illnesses with an episodic pattern), and reasonably good psychometric properties (e.g., discriminating power across a broad range of individual differences) as well as good concurrent validity (convergent and divergent validity) with respect to hypothesized endophenotypes
• Endophenotypes should vary continuously in the general population (quantitative, ideally normally distributed traits)
• Should be associated with risk for a particular disorder (although need not be disease-specific)
Additional Criteria (*for optimally informative candidate endophenotypes*)
• Latent endophenotype constructs should relate to reasonably well-characterized neural systems models
• Should involve homologies of expression across species (to enable the development of animal models)

the Wisconsin Card Sort Test is perhaps the most well-known and widely used measure of frontal executive function. Executive functions (i.e., processes which facilitate adaptation to novel situations by means of modulation and control of more fundamental cognitive skills, thought to rely on functioning of the prefrontal cortex) are one of the cognitive domains in which schizophrenic patients and their relatives most reliably demonstrate impairment (Goldberg et al. 1993). This pattern of findings is generally interpreted as evidence for a dysfunctional frontal lobe system. However, the temporal stability of this measure has rarely been examined, and some investigators have noted considerable within-subject variability over time in younger and more acutely symptomatic schizophrenia patients (Seidman et al. 1991). In addition, the Wisconsin Card Sort Test is ill suited for repeated testing (i.e., as in a clinical trial); as once one determines the 'rules,' the test is no longer measuring the same construct as it did prior to rule acquisition. These findings additionally suggest that an important element in the assessment of endophenotypes is that they can be conducted as much as possible when subjects are asymptomatic. The feasibility of testing patients who are asymptomatic may vary by disorder; for instance, this is more difficult for schizophrenia, in which patients typically have significant residual symptomatology, than for bipolar disorder, which tends to have a more episodic course.

One important caveat to point out is that for some diseases with a characteristic fluctuating course (e.g., multiple sclerosis), measurement of cognitive phenotypes during symptomatic exacerbation may actually be more informative regarding the pathophysiology of illness, than it would be in between episodes. It is possible that various neurobiological indicators—assessed indirectly via neurocognitive phenotypic assays—may be revealed selectively during periods of acute illness and

may not otherwise be readily apparent. In other words, some phenotypes may be dynamic, and the degree to which they are related to symptom onset, as well as the temporal connection to symptom onset, could also be extremely informative. This is a topic that warrants further attention.

An additional methodological issue is that a trait may be strongly mediated by confounding environmental variables. For instance, increased beta-endorphin levels in response to alcohol have been proposed as a biomarker for alcoholism risk, but beta-endorphin levels are also strongly affected by acute alcohol consumption (Vescovi et al. 1992); thus, this measure would not be considered 'state-independent.' In addition, the performance on most cognitive tasks declines with age (Tucker-Drob 2011), and there are known gender differences for measures of verbal abilities and certain visuospatial abilities like mental rotation (Suzuki et al. 2011). As such, controlling for possible covariates in statistical analyses may help to better define candidate endophenotypes that are known to be affected by environmental or demographic factors.

4.4.2 Heritability

An important requirement for endophenotypes, in particular, is that they must be sufficiently heritable to be mapped through genome-wide analysis, either by linkage or by association (Blangero et al. 2003; Gottesman and Gould 2003). Yet currently, only limited data exist regarding the heritability of many proposed candidate cognitive phenotypes (Freimer and Sabatti 2003). For example, dysfunctional temporal processing is implicated in core deficits in time estimation in individuals with attention deficit/hyperactivity disorder (ADHD). This trait has been linked to cerebellar dysfunction (Castellanos and Tannock 2002) and is thus considered to be an attractive candidate endophenotype with a plausible neurobiological basis. However, the heritability of this trait is not yet known and, as such, would not be an optimal choice for genetic mapping studies, unless its familial aggregation is first assessed in the pedigrees in which mapping is to be conducted. In general, cognitive phenotypes vary widely with regard to the evidence for their heritability, and it is important that efforts be made in the near future to obtain such evidence.

4.5 The Use of Endophenotypes for Genetic Mapping Studies

It has also been hypothesized that such intermediate phenotypes (endophenotypes) reflect a simpler genetic architecture than disease diagnoses. Although this assertion is still unproven, it is currently being tested in linkage and association studies, with the expectation that these traits will be easier to map than categorical disease

phenotypes (Bearden and Freimer 2006; Gottesman and Gould 2003). One potential benefit of the endophenotype approach is the ability to help to clarify the carrier status of family members of individuals affected with psychiatric disorders. Many of these relatives, while not meeting full criteria for the disorder, are still presumed to carry predisposing genotypes (i.e., to display incomplete penetrance). However, we currently have no means to objectively identify these individuals. In individuals at genetic risk for schizophrenia, there is clear evidence that cognitive dysfunction is inherited as part of the genetic vulnerability to the illness (Bohlken et al. 2016; Delawalla et al. 2008; Rasetti and Weinberger 2011). For example, in addition to the behavioral changes shown by patients with schizophrenia, their unaffected relatives also have working memory deficits (Cannon et al. 2000; Egan et al. 2001; Karlsgodt et al. 2011a; Tuulio-Henriksson et al. 2002) and episodic memory deficits (Karlsgodt et al. 2011a; Karnik-Henry et al. 2012; Toulopoulou et al. 2003).

An important interpretation to keep in mind when evaluating such evidence is that heritability reflects the magnitude of the overall genetic effects on a trait. Heritability does not indicate the total number of genes that may be involved, or the relative contributions of each of those genes, nor does it inform us regarding the complexity of the genetic architecture of a given trait (Almasy 2003; see also Chap. 5). Thus, overall heritability of a trait may reflect the effects of one gene, many genes, or the additive or interactive effects of any number of genes. In addition, a putative endophenotype may be no less 'genetically complex' than the categorical phenotype itself. For example, working memory is commonly considered a putative endophenotype for schizophrenia spectrum disorders. However, working memory itself is a complex process that can be influenced by a number of different genetic factors, including dopamine signaling, glutamate signaling, GABA signaling, white matter integrity, and gray matter integrity all of which have individual genetic influences (Karlsgodt et al. 2011a).

4.6 Specificity of Cognitive Phenotypes and Their Association with Categorical Disease Phenotypes

One issue that arises when considering broad cognitive traits, such as IQ, working or long term memory function, is the relative sensitivity and specificity of the measures to specific disorders. Some investigators (Skuse 2001; Hasler et al. 2004) have suggested that the utility of such measures depends on how specific they are for a particular disorder. However, when common endophenotypes are found across disorders, while it may indicate non-specific brain dysfunction (e.g., attentional impairment), there is also the possibility that the overlap indicates common underlying neurobiological substrates (Olincy et al. 2000). For example, there is increasing evidence that some genes may predispose to both schizophrenia and psychotic bipolar illness (Berrettini 2000), as well as other mental disorders (Kendler and Walsh 1998). Indeed, increasing recognition of phenotypic overlap

between bipolar disorder and schizophrenia has fueled enthusiasm for investigating bipolar disorder-associated endophenotypes. Some traits may demonstrate specificity to those at genetic risk for bipolar disorder, such as dimensional measures of cyclothymic temperament (Evans et al. 2005) or disruptions of circadian biology (Hasler et al. 2006). However, neurocognitive markers that may be *sensitive* to bipolar disorder susceptibility (e.g., measures of processing speed, working memory, and declarative memory) may not be *specific* to bipolar disorder, in that they overlap with neurocognitive endophenotypes for schizophrenia (Seidman et al. 2002; Burdick et al. 2011). Similarly, McDonald et al. examined putative neuroanatomic endophenotypes for bipolar disorder and schizophrenia and found both unique and overlapping endophenotypic traits that characterized these illnesses (McDonald et al. 2004). As such, findings indicating overlap in intermediate phenotypes may provide important clues regarding the underlying genetic bases of these disorders.

4.6.1 Relevance to Biological Mechanisms

Strategies to optimize endophenotype discovery that will be relevant to neuropsychiatric therapeutics should target neural systems-level models. Indeed, several investigators have emphasized that it is critical for endophenotypes to be solidly grounded in the neurosciences (e.g., Castellanos and Tannock 2002). It is important that cognitive phenotypes afford homologies of expression across species, enabling both basic and clinical investigations. In general, 'top-down' approaches can start with existing syndrome definitions and attempt to dissect these into more basic behavioral, cognitive, and neural systems-level constructs. Complementary 'bottom-up' approaches can start with genomic variants and attempt to determine their functional contributions at increasingly complex levels, up to the level of neural systems function. If phenotypes are to be relevant therapeutic targets, additional information can be derived from knowledge about actions of pharmacologic agents on neural systems. Each of these strategies converges on neural systems-level constructs, and thus, models at the neural systems level provide the critical link to specifying the observable phenotypes that will serve as optimal candidates for the development of rational pharmacotherapies.

Latent endophenotype constructs should relate to neural systems models that are sufficiently well studied that physiologically plausible manipulations can be conducted using currently available techniques. For example, Castellanos and Tannock (2002) propose three such cognitively based endophenotypes for attention deficit/hyperactivity disorder (ADHD), a notoriously heterogeneous diagnostic construct. In particular, they suggest abnormalities in reward-related circuitry that leads to shortened delay gradients, deficits in temporal processing that result in high intrasubject intertrial variability, and deficits in working memory are most amenable to integrative collaborative approaches that aim to uncover the causes of ADHD. Developing a strategy whereby multiple sources of information can be

investigated simultaneously, e.g., across multiple species using convergent methodology, to target specific candidate phenotypes is likely to be most informative for neuropsychiatric therapeutics.

4.6.2 Cognitive and Neuroanatomic Phenotypes in Population Samples

While traits that are both heritable and associated with disease are obviously of particular interest, traits with high reliability and heritability may be valuable for genetic analysis, even when there is uncertainty regarding their association with a particular disease phenotype. If QTL for such traits can be identified, they may inform our understanding of the genes involved in normal brain development and function. In that regard, association with disease may be considered secondary to the requirements of adequate heritability and reliability of the measure. Structural neuroanatomic measures (as assessed by high-resolution MRI) are among the best examples of such traits, providing a variety of genetically uncorrelated traits that can be candidate endophenotypes (Kochunov et al. 2010; Panizzon et al. 2009). In addition to being highly heritable in large twin and family studies (Sachdev et al. 2016; Shen et al. 2016; Whelan et al. 2016; Yang et al. 2016), neuroanatomic measures also show substantial variation in the general population. Nevertheless, very large samples may still be required to identify common genetic variants associated with quantitative variation in neuroanatomic traits; for example, the ENIGMA (Enhancing Neuro Imaging Genetics through Meta-Analysis) Consortium recently identified a common variant relevant to hippocampal and intracranial volume in a sample of over 20,000 individuals (Stein et al. 2012). Although it has been widely hypothesized that brain-imaging endophenotypes would have large effect sizes, somewhat disappointingly, this does not appear to be the case, at least for the specific volumetric traits assessed in the recent ENIGMA genome-wide meta-analysis, which had comparable effect sizes to those observed in other genome-wide association studies of complex traits (Hindorff et al. 2009). Functional MRI (fMRI) also shows great promise as a tool for endophenotype investigations. However, conducting fMRI investigations on a genome-wide scale is computationally challenging. Biological interpretability of such studies is also challenging. Such studies may prove particularly fruitful when the neurobiologic effects of a well-validated candidate gene are examined.

4.6.3 Successful Utilization of Endophenotypes in the Study of Non-psychiatric Complex Traits

The application of allied phenotypes, or endophenotypes, has been successful in the context of other complex illnesses (Cho et al. 2009; Comuzzie et al. 1997; Kathiresan et al. 2009a). However, difficulties in choosing appropriate

endophenotypes for mental disorders have limited their use in psychiatry, where relatively less is known about the biological mechanisms that predispose to illness than in other areas of medicine. As one particular example, asthma is a common, multiply determined disorder, the development of which is related to both genetic and environmental factors. Asthma is characterized by the production of high levels of immunoglobulin E (IgE) in response to common allergens. Atopy, which refers to the presence of IgE-mediated skin test responses to allergens, is detected by skin prick tests or by measurement of specific serum IgE titers against allergens or elevation of the total serum IgE concentration. The total serum IgE has a heritability of 40–50% (Palmer et al. 2000). It can be measured by standardized protocols and is lognormally distributed, with well-defined effects of age and sex. When elevated, it has a close relationship with asthma; consequently, this measure has been successfully used as a quantitative trait to map susceptibility genes for atopy and asthma. In particular, Anderson and colleagues (Anderson et al. 2002) first identified an association between total serum IgE concentration and a novel microsatellite located at 13q14, which they subsequently used in a comprehensive SNP map to localize the underlying quantitative trait locus (QTL) (Zhang et al. 2003). Similar notable examples in other areas of medicine include the identification of genomic regions associated with a quantitative trait, plasma Lp(a) lipoprotein levels, which is a risk factor for coronary artery disease (Kathiresan et al. 2009b).

4.6.4 Feasibility of Animal Models

Translational studies in animal models provide a valuable way of interrogating the underlying biology of specific cognitive phenotypes. For example, both linkage (Schwab et al. 2003; Straub et al. 1995) and association studies (Kirov et al. 2004; Straub et al. 2002; Tang et al. 2003) have implicated dystrobrevin binding protein-1 (dysbindin or DTNBP1) as a promising susceptibility gene for schizophrenia. Postmortem studies reveal that schizophrenia patients show reduced dysbindin mRNA or protein in prefrontal cortex (PFC) (Tang et al. 2009; Weickert et al. 2004, 2008) and hippocampus (Talbot et al. 2004; Weickert et al. 2004). Both dysbindin variation (Burdick et al. 2007; Zinkstok et al. 2007) and its chromosomal locus (chromosome 6p) (Hallmayer et al. 2005; Posthuma et al. 2005) have been associated with cognitive impairments characteristic of schizophrenia. Dysbindin has also been associated with spatial working memory (Donohoe et al. 2007), a key endophenotype for schizophrenia (Cannon et al. 2000; Glahn et al. 2003). Moreover, working memory impairments in schizophrenia are associated with PFC dysfunction (Goldman-Rakic 1994), while dysbindin variation associates with measures of PFC function in healthy subjects (Fallgatter et al. 2006). However, given the complexity of the genetic variation in human patients, it is difficult to isolate the effects of individual genes. Accordingly, mice with a large genomic deletion in the dysbindin gene have been used in a number of cognitive

and neurofunctional studies. For example, dysbindin mutant mice have demonstrated poor working memory performance and functional changes within the PFC (Jentsch et al. 2009; Karlsgodt et al. 2011b) that may correspond to the aspects of prefrontal dysfunction in schizophrenia patients. This example demonstrates the unique contribution that animal models can make to probe the mechanistic underpinnings of genetic changes that result in the observed endophenotypes.

4.6.5 Multivariate Phenotype Approaches

While multivariate approaches are gaining more attention as genome–phenome studies increasingly obtain multiple types of phenotypic data, there is no consensus yet about the most effective means to combine such information. One strategy for dealing with a large number of measured traits is to first focus on those that demonstrate the greatest heritability. The creation of a correlation matrix of all variables measured in a particular study permits investigators to remove from further analysis of those traits that are highly intercorrelated. In such cases, investigators can choose to analyze the trait with the best psychometric properties.

However, even after using the data reduction procedures described above, multiple traits may be retained for analysis. If traits share a common genetic etiology, analyzing them in combination may improve the genetic signal and narrow the chromosomal region of interest. There are several ways to combine variables, including creating summary measures of endophenotypic variables, using principal components analysis (PCA) and/or factor analysis. While these approaches can reduce the dimension of the problem (e.g., by focusing on just the first few factors), the results may be difficult to interpret. Multivariate linkage analysis can be a powerful means to deal with the analysis of multiple traits. Rather than combining traits into a single outcome, as in PCA, the multiple traits are analyzed simultaneously using a variance components approach. Marlow et al. (2003) achieved success with this approach, in the context of a genome-wide, multivariate analysis of six quantitative traits related to dyslexia. This multivariate approach has many advantages over analyzing multiple correlated traits in univariate analyses; in particular, it allows for correction for multiple testing, increased power by using the covariance between measures, and clarification of the pattern of QTL influence on multiple trait phenotypes.

Multivariate analysis is not without drawbacks, however. If the traits are not truly influenced by the same sets of genetic loci, power may be decreased as compared to univariate analyses, due to the increased number of parameters one must fit in a multivariate analysis, and the increased degrees of freedom (Williams et al. 1999). This problem may be overcome by performing multivariate linkage analysis only on the suite of traits that appear to show univariate linkage to the same genomic region.

4.6.6 The Endophenotype Ranking Value: Approach to High-Dimensional Phenotyping

Applying an endophenotype approach within families has numerous strategic benefits (e.g., simultaneous identification of endophenotypes, increased power to localize and identify genes, and increased power to detect the effects of rare functional variants) over the dominant paradigm in the literature currently, which focuses on the collections of unrelated individuals and relies solely upon categorical diagnoses. While the endophenotype concept has been widely advocated in psychiatric genetics (Gottesman and Gould 2003; Kathiresan et al. 2009a; Ritsner and Strous 2010), a standardized approach for the identification of viable endophenotypes is lacking. Most studies nominate candidate traits based solely on phenotypic correlations between disease risk and a quantitative risk factor to define putative endophenotypes. However, the endophenotype concept fundamentally depends upon the existence of joint genetic determination of both endophenotype and disease risk, or pleiotropy (Blangero et al. 2003; Gottesman and Gould 2003). This can be most efficiently tested using a family-based study design, in which one can assess both the heritability of the endophenotype and its genetic correlation with disease. Glahn et al. developed the endophenotype ranking value (*ERV*), a novel objective index of the genetic utility of endophenotypes for an illness (Glahn et al. 2012) (see Almasy et al. Chap. 5, this volume, for further discussion). This variable provides an unbiased and empirically derived method for choosing appropriate endophenotypes in a manner that balances the strength of the genetic signal for the endophenotype and the strength of its relation to the disorder of interest. It is defined using the square root of the heritability of the illness (h_i^2), the square root of the heritability of the endophenotype (h_e^2), and their genetic correlation (ρ_g) and is expressed in the following formula:

$$\mathrm{ERV}_{ie} = \left| \sqrt{h_i^2} \sqrt{h_e^2} \rho_g \right|$$

ERV values vary between 0 and 1; higher values indicate that the endophenotype and the illness are more strongly influenced by shared genetic factors. This method necessitates that endophenotypes be heritable and have some level of pleiotropy with the illness in question, thus reducing the heterogeneity of the disease and focusing on the proportion of shared genetic factors influencing both the endophenotype and the illness measure. An advantage of the *ERV* approach is that large numbers of potential endophenotypes can be efficiently assessed prior to conducting molecular genetic analyses, analogous to high-throughput screening methods developed for drug discovery. Furthermore, the *ERV* approach is applicable to any heritable disease and any set of potentially relevant traits.

Glahn and colleagues (Glahn et al. 2012) applied this approach to a high-dimensional set of over 11,000 traits derived from behavioral, neurocognitive, neuroanatomic, and transcriptomic phenotypic domains, within the context of large randomly ascertained pedigrees. Using this strategy, they were able to identify a set of significant endophenotypes for recurrent major depression (rMDD), which were utilized along with disease status in bivariate linkage analysis to identify a genome-wide significant quantitative trait locus exhibiting pleiotropic effects on both a transcriptome endophenotype and disease risk.

4.6.7 Data Mining Using Bioinformatics Approaches

Given the explosion of information from studies employing widely varying methodologies, resources for integrating and summarizing this information are sorely needed (i.e., a *cognitive phenotype informatics architecture*). A major obstacle has been the lack of a common semantic infrastructure for characterizing the psychological aspects of cognitive neuroscience investigations (Poldrack et al. 2011). There is considerable ambiguity in the way that terms are used in cognitive neuroscience; many similar cognitive processes are defined in the literature using distinct terms (Sabb et al. 2008), and conversely, the same cognitive process (e.g., working memory) may have several distinct definitions (Poldrack et al. 2011). The *Cognitive Atlas* (CA) is a resource that aims to develop a framework for such a semantic infrastructure, through collaborative knowledge building (accessible online at http://www.cognitiveatlas.org). This atlas provides the basic functionality for the specification of knowledge about cognitive processes and tasks, which can ultimately be linked to existing and planned data structures that represent genomic, proteomic, and other biological knowledge. This approach can open revolutionary data-mining prospects for both pathophysiological modeling and drug discovery. Finally, in the post-genomic era, one of the biggest challenges faced by interdisciplinary scientists is the lack of tools to manage the complexity of knowledge rapidly being amassed across disparate methods, models, and data types (Sabb et al. 2008). Informatics resources can advance the collation of empirical knowledge that will help to bridge the currently wide gap between genome, cognitive constructs, and disease syndromes (Bilder et al. 2009b). Such tools can advance our understanding of the genetic architecture of memory by helping researchers to identify previously unsuspected relationships across disciplinary boundaries, select specific phenotypic measures, and develop multilevel models that specify both within- and between-level associations. Figure 4.3 shows an example of this approach, using http://www.pubatlas.org/, a database visualization resource for multilevel data (see also Chap. 15).

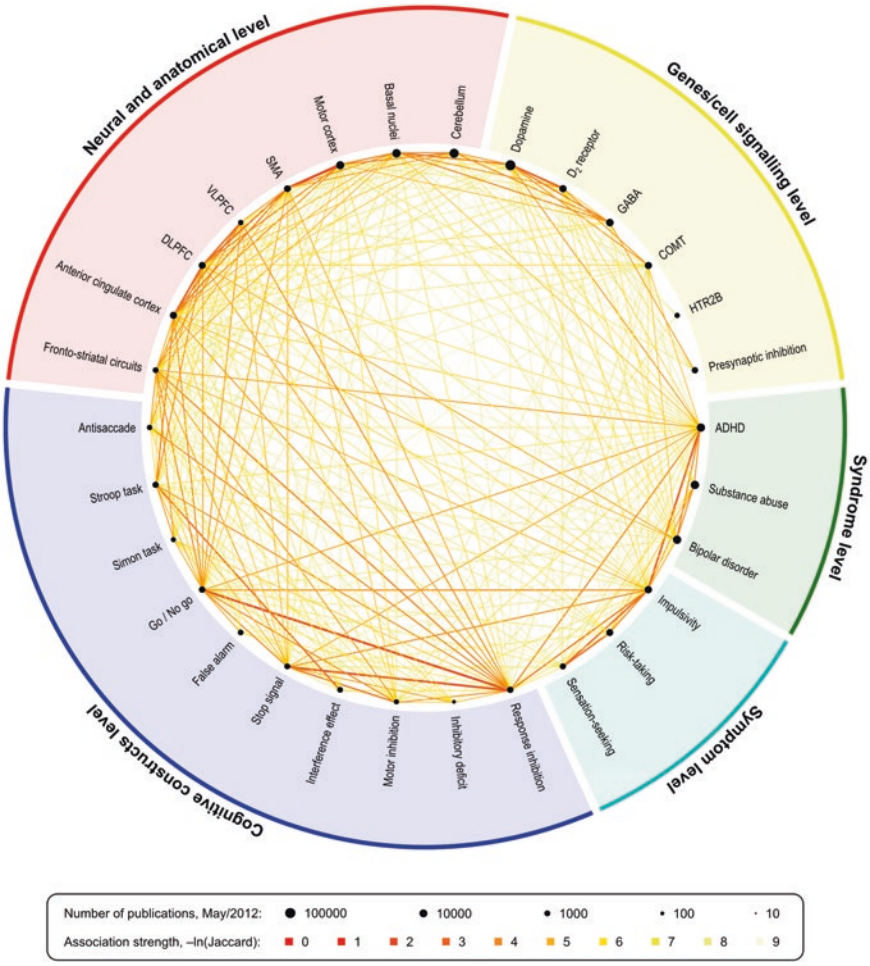

Fig. 4.3 Relationships between keywords in published literature can expose interesting links between fields and *highlight areas* where important pieces may be missing, as well as evidence emergent patterns of research. In this figure, each point around the circle represents the relative quantity of publications of a given keyword in relation to the others, in logarithmic scale, as retrieved from PubMed in May 2012. The links represent the strength of the association, scaled by the natural logarithm of the Jaccard coefficient. The smaller numbers indicate stronger associations. See also http://www.pubatlas.org/ for more literature mining and visualization tools

4.7 Future Directions

Some challenges arise when discussing cognitive, rather than strictly biologically based, endophenotypes, and coming to a consensus on these issues is an important goal of the field and of this chapter. The challenges are in part based in the

complexity of cognitive measures which may themselves contain a number of heritable subcomponents. For instance, attention or processing speed, which may themselves be heritable, can affect performance on a higher level task used as the endophenotype of interest. As a result, these complex phenotypes may not map as clearly or neatly onto genotypes as measures such as the fasting glucose levels used as an endophenotype for diabetes. Finally, the focus of endophenotypes, cognitive or otherwise, is typically on their utility for mapping genotypes related to disease and dysfunction. However, these markers may be important indicators of cognitive and behavioral variance even in healthy populations that can describe important differences between individuals. One aspect of this that has been particularly understudied is the concept of biomarkers or endophenotypes for above-average performance—for instance, that in highly gifted children. As the field of cognitive phenotyping continues to develop, it will be interesting to see how these issues are incorporated into our broader understanding of endophenotypes. It is clear that research on complex neuropsychiatric disorders needs to move beyond the purely descriptive, and the widespread use of biologically based cognitive phenotypes may be able to move us forward in that endeavor.

References

Almasy L (2003) Quantitative risk factors as indices of alcoholism susceptibility. Ann Med 35(5):337–343

Anderson GG, Leaves NI, Bhattacharyya S et al (2002) Positive association to IgE levels and a physical map of the 13q14 atopy locus. Eur J Hum Genet 10(4):266–270

Bearden CE, Freimer NB (2006) Endophenotypes for psychiatric disorders: ready for primetime? Trends Genet 22(6):306–313

Berrettini WH (2000) Are schizophrenic and bipolar disorders related? A review of family and molecular studies. Biol Psychiatry 48(6):531–538

Bilder RM (2008) Phenomics: building scaffolds for biological hypotheses in the post-genomic era. Biol Psychiatry 63(5):439–440

Bilder RM, Sabb FW, Cannon TD et al (2009a) Phenomics: the systematic study of phenotypes on a genome-wide scale. Neuroscience 164(1):30–42

Bilder RM, Sabb FW, Parker DS et al (2009b) Cognitive ontologies for neuropsychiatric phenomics research. Cogn Neuropsychiatry 14(4–5):419–450

Blangero J, Williams JT, Almasy L (2003) Novel family-based approaches to genetic risk in thrombosis. J Thromb Haemost 1(7):1391–1397

Bohlken MM, Brouwer RM, Mandl RC et al (2016) Genetic variation in schizophrenia liability is shared with intellectual ability and brain structure. Schizophr Bull sbw034

Burdick KE, Goldberg TE, Funke B et al (2007) DTNBP1 genotype influences cognitive decline in schizophrenia. Schizophr Res 89(1–3):169–172

Burdick KE, Goldberg TE, Cornblatt BA et al (2011) The MATRICS consensus cognitive battery in patients with bipolar I disorder. Neuropsychopharmacology 36(8):1587–1592

Cannon TD, Huttunen MO, Lonnqvist J et al (2000) The inheritance of neuropsychological dysfunction in twins discordant for schizophrenia. Am J Hum Genet 67(2):369–382

Castellanos FX, Tannock R (2002) Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. Nat Rev Neurosci 3(8):617–628

Cho SC, Jung SW, Kim BN et al (2009) Temperament and character among Korean children and adolescents with anxiety disorders. Eur Child Adolesc Psychiatry 18(1):60–64
Comuzzie AG, Hixson JE, Almasy L et al (1997) A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. Nat Genet 15(3):273–276
Delawalla Z, Csernansky JG, Barch DM (2008) Prefrontal cortex function in nonpsychotic siblings of individuals with schizophrenia. Biol Psychiatry 63(5):490–497
Donohoe G, Morris DW, Clarke S et al (2007) Variance in neurocognitive performance is associated with dysbindin-1 in schizophrenia: a preliminary study. Neuropsychologia 45(2):454–458
Doyle AE, Faraone SV, Seidman LJ et al (2005) Are endophenotypes based on measures of executive functions useful for molecular genetic studies of ADHD? J Child Psychol Psychiatry 46(7):774–803. PMID: 15972070
Egan MF, Hyde TM, Bonomo JB et al (2001) Relative risk of neurological signs in siblings of patients with schizophrenia. Am J Psychiatry 158(11):1827–1834
Evans L, Akiskal HS, Keck PE Jr et al (2005) Familiality of temperament in bipolar disorder: support for a genetic spectrum. J Affect Disord 85(1–2):153–168
Fallgatter AJ, Herrmann MJ, Hohoff C et al (2006) DTNBP1 (dysbindin) gene variants modulate prefrontal brain function in healthy individuals. Neuropsychopharmacology 31(9):2002–2010
Freimer N, Sabatti C (2003) The human phenome project. Nat Genet 34(1):15–21
Glahn DC, Therman S, Manninen M et al (2003) Spatial working memory as an endophenotype for schizophrenia. Biol Psychiatry 53(7):624–626
Glahn DC, Curran JE, Winkler AM et al (2012) High dimensional endophenotype ranking in the search for major depression risk genes. Biol Psychiatry 71(1):6–14
Goldberg TE, Hyde TM, Kleinman JE et al (1993) Course of schizophrenia: neuropsychological evidence for a static encephalopathy. Schizophr Bull 19(4):797–804
Goldman-Rakic PS (1994) Working memory dysfunction in schizophrenia. J Neuropsychiatry Clin Neurosci 6(4):348–357
Gottesman II, Shields J (1973) Genetic theorizing and schizophrenia. Br J Psychiatry 122(566):15–30
Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160(4):636–645
Hallmayer JF, Kalaydjieva L, Badcock J et al (2005) Genetic evidence for a distinct subtype of schizophrenia characterized by pervasive cognitive deficit. Am J Hum Genet 77(3):468–476
Hasler G, Drevets WC, Manji HK et al (2004) Discovering endophenotypes for major depression. Neuropsychopharmacology 29(10):1765–1781
Hasler G, Drevets WC, Gould TD et al (2006) Toward constructing an endophenotype strategy for bipolar disorders. Biol Psychiatry 60(2):93–105
Hindorff LA, Sethupathy P, Junkins HA et al (2009) Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci USA 106(23):9362–9367
Hyman SE (2007) Can neuroscience be integrated into the DSM-V? Nat Rev Neurosci 8(9):725–732
Jentsch JD, Trantham-Davidson H, Jairl C et al (2009) Dysbindin modulates prefrontal cortical glutamatergic circuits and working memory function in mice. Neuropsychopharmacology 34(12):2601–2608
John B, Lewis KR (1966) Chromosome variability and geographic distribution in insects. Science 152(3723):711–721
Karlsgodt KH, Bachman P, Winkler AM et al (2011a) Genetic influence on the working memory circuitry: behavior, structure, function and extensions to illness. Behav Brain Res 225(2):610–622
Karlsgodt KH, Robleto K, Trantham-Davidson H et al (2011b) Reduced dysbindin expression mediates *N*-methyl-D-aspartate receptor hypofunction and impaired working memory performance. Biol Psychiatry 69(1):28–34

Karnik-Henry MS, Wang L, Barch DM et al (2012) Medial temporal lobe structure and cognition in individuals with schizophrenia and in their non-psychotic siblings. Schizophr Res 138(2–3):128–135

Kathiresan S, Voight BF, Purcell S et al (2009a) Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 41(3):334–341

Kathiresan S, Willer CJ, Peloso GM et al (2009b) Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 41(1):56–65. doi: 10.1038/ng.291

Kendler KS, Walsh D (1998) The structure of psychosis: syndromes and dimensions. Arch Gen Psychiatry 55(6):508–509

Kirov G, Ivanov D, Williams NM et al (2004) Strong evidence for association between the dystrobrevin binding protein 1 gene (DTNBP1) and schizophrenia in 488 parent-offspring trios from Bulgaria. Biol Psychiatry 55(10):971–975

Kochunov P, Glahn DC, Lancaster JL et al (2010) Genetics of microstructure of cerebral white matter using diffusion tensor imaging. Neuroimage 53(3):1109–1116

Marlow AJ, Fisher SE, Francks C et al (2003) Use of multivariate linkage analysis for dissection of a complex cognitive trait. Am J Hum Gen 72(3):561–570

Matthysse S, Holzman PS (1987) Genetic latent structure models: implication for research on schizophrenia. Psychol Med 17(2):271–274

Matthysse S, Holzman PS, Lange K (1986) The genetic transmission of schizophrenia: application of Mendelian latent structure analysis to eye tracking dysfunctions in schizophrenia and affective disorder. J Psychiatr Res 20(1):57–67

McDonald C, Bullmore ET, Sham PC et al (2004) Association of genetic risks for schizophrenia and bipolar disorder with specific and generic brain structural endophenotypes. Arch Gen Psychiatry 61(10):974–984

Olincy A, Ross RG, Harris JG et al (2000) The P50 auditory event-evoked potential in adult attention-deficit disorder: comparison with schizophrenia. Biol Psychiatry 47(11):969–977

Palmer LJ, Burton PR, Faux JA et al (2000) Independent inheritance of serum immunoglobulin E concentrations and airway responsiveness. Am J Respir Crit Care Med 161(6):1836–1843

Panizzon MS, Fennema-Notestine C, Eyler LT et al (2009) Distinct genetic influences on cortical surface area and cortical thickness. Cereb Cortex 19(11):2728–2735

Poldrack RA, Kittur A, Kalar D et al (2011) The cognitive atlas: toward a knowledge foundation for cognitive neuroscience. Front Neuroinform 5:17

Posthuma D, Luciano M, Geus EJ et al (2005) A genomewide scan for intelligence identifies quantitative trait loci on 2q and 6p. Am J Hum Genet 77(2):318–326

Rasetti R, Weinberger DR (2011) Intermediate phenotypes in psychiatric disorders. Curr Opin Genet Dev 21(3):340–348

Ritsner MS, Strous RD (2010) Neurocognitive deficits in schizophrenia are associated with alterations in blood levels of neurosteroids: a multiple regression analysis of findings from a double-blind, randomized, placebo-controlled, crossover trial with DHEA. J Psychiatr Res 44(2):75–80

Rund BR (1998) A review of longitudinal studies of cognitive functions in schizophrenia patients. Schizophr Bull 24(3):425–435

Sabb FW, Bearden CE, Glahn DC et al (2008) A collaborative knowledge base for cognitive phenomics. Mol Psychiatry 13(4):350–360

Sachdev PS, Thalamuthu A, Mather KA et al (2016) White matter hyperintensities are under strong genetic influence. Stroke 47(6):1422–1428

Schwab SG, Knapp M, Mondabon S et al (2003) Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. Am J Hum Genet 72(1):185–190

Seidman LJ, Pepple JR, Faraone SV et al (1991) Wisconsin card sorting test performance over time in schizophrenia. Preliminary evidence from clinical follow-up and neuroleptic reduction studies. Schizophr Res 5(3):233–242

Seidman LJ, Faraone SV, Goldstein JM et al (2002) Left hippocampal volume as a vulnerability indicator for schizophrenia: a magnetic resonance imaging morphometric study of nonpsychotic first-degree relatives. Arch Gen Psychiatry 59(9):839–849

Shen KK, Dore V, Rose S et al (2016) Heritability and genetic correlation between the cerebral cortex and associated white matter connections. Hum Brain Mapp 37(6):2331–2347

Skuse DH (2001) Endophenotypes and child psychiatry. Br J Psychiatry 178(5):395–396

Stein JL, Medland SE, Vasquez AA et al (2012) Identification of common variants associated with human hippocampal and intracranial volumes. Nat Genet 44(5):552–561

Straub RE, MacLean CJ, O'Neill FA et al (1995) A potential vulnerability locus for schizophrenia on chromosome 6p24-22: evidence for genetic heterogeneity. Nat Genet 11(3):287–293

Straub RE, Jiang Y, MacLean CJ et al (2002) Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 71(2):337–348

Suzuki K, Shikishima C, Ando J (2011) Genetic and environmental sex differences in mental rotation ability: a Japanese twin study. Twin Res Hum Genet 14(5):437–443

Talbot K, Eidem WL, Tinsley CL et al (2004) Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. J Clin Invest 113(9):1353–1363

Tang JX, Zhou J, Fan JB et al (2003) Family-based association study of DTNBP1 in 6p22.3 and schizophrenia. Mol Psychiatry 8(8):717–718

Tang J, LeGros RP, Louneva N et al (2009) Dysbindin-1 in dorsolateral prefrontal cortex of schizophrenia cases is reduced in an isoform-specific manner unrelated to dysbindin-1 mRNA expression. Hum Mol Genet 18(20):3851–3863

Toulopoulou T, Rabe-Hesketh S, King H et al (2003) Episodic memory in schizophrenic patients and their relatives. Schizophr Res 63(3):261–271

Tucker-Drob EM (2011) Global and domain-specific changes in cognition throughout adulthood. Dev Psychol 47(2):331–343

Tuulio-Henriksson A, Haukka J, Partonen T et al (2002) Heritability and number of quantitative trait loci of neurocognitive functions in families with schizophrenia. Am J Med Genet 114(5):483–490

Vescovi PP, Coiro V, Volpi R et al (1992) Plasma beta-endorphin, but not met-enkephalin levels are abnormal in chronic alcoholics. Alcohol Alcohol 27(5):471–475

Waldman ID (2005) Statistical approaches to complex phenotypes: evaluating neuropsychological endophenotypes for attention-deficit/hyperactivity disorder. Biol Psychiatry 57(11):1347–1356. PMID: 15950007

Weickert CS, Straub RE, McClintock BW et al (2004) Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. Arch Gen Psychiatry 61(6):544–555

Weickert CS, Rothmond DA, Hyde TM et al (2008) Reduced DTNBP1 (dysbindin-1) mRNA in the hippocampal formation of schizophrenia patients. Schizophr Res 98(1–3):105–110

Whelan CD, Hibar DP, van Velzen LS et al (2016) Heritability and reliability of automatically segmented human hippocampal formation subregions. Neuroimage 128:125–137

Williams JM, de Leeuw M, Black MD et al (1999) Factors associated with outcomes of persistent truncus arteriosus. J Am Coll Cardiol 34(2):545–553

Yang Z, Zuo XN, McMahon KL et al (2016) Genetic and environmental contributions to functional connectivity architecture of the human brain. Cereb Cortex 26(5):2341–2352

Zhang Y, Leaves NI, Anderson GG et al (2003) Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. Nat Genet 34(2):181–186

Zinkstok JR, de Wilde O, van Amelsvoort TA et al (2007) Association between the DTNBP1 gene and intelligence: a case-control study in young patients with schizophrenia and related disorders and unaffected siblings. Behav Brain Funct 3:19

Chapter 5
The Strategy and Utility of the Endophenotype Approach to Neurobehavioral Function

Ellen Quillen, David Glahn and Laura Almasy

5.1 Introduction

The current lack of understanding as to the root causes of psychiatric illnesses hinders improvements in prevention, diagnosis, and treatment. While certainly influenced by environmental factors, there is ample evidence for a genetic component to a wide variety of mental disorders, as well as normal cognitive traits. Heritability estimates, a measure of the proportion of variation in a trait attributable to genetic variation, display a broad range for many disorders depending on the methodology and population used for calculation but may be as high as 90% for autism-spectrum disorders (Ronald and Hoekstra 2011), 32% for Tourette's syndrome (Mathews and Grados 2011), 80% for schizophrenia (Sullivan et al. 2003), 79% for bipolar disorder (Kendler et al. 1993), 79% for attention-deficit/hyperactivity disorder (Lichtenstein et al. 2010), 65% for obsessive–compulsive disorder (Van Grootheest et al. 2005), 32% for generalized anxiety disorder (Hettema et al. 2001), 43% for

E. Quillen
Department of Genetics, Texas Biomedical Research Institute, San Antonio, USA
e-mail: equillen@txbiomedgenetics.org

D. Glahn
Department of Psychiatry, Yale School of Medicine, New Haven, USA
e-mail: david.glahn@yale.edu

D. Glahn
Olin Neuropsychiatric Research Center Institute of Living, Hartford Hospital, Hartford, USA

L. Almasy (✉)
South Texas Diabetes and Obesity Institute, University of Texas Rio Grande Valley School of Medicine, Edinburg, USA
e-mail: almasy@txbiomedgenetics.org

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_5

panic disorder (Hettema et al. 2001), and 80% for general intelligence (Deary et al. 2010). Despite these large heritabilities, only a tiny fraction of the variation in these disorders has been explained.

Traits with high heritability cannot be assumed to have a simple genetic basis. While height is among the most heritable normally varying human traits, with 93% of the variation attributed to genes (Silventoinen et al. 2003), this phenotype is believed to be under the influence of at least 44 independent genetic loci (Weedon and Frayling 2008; Weedon et al. 2008). Like many complex traits, the genetic architecture of mental illness is expected to be extremely complex with a number of biological pathways involved, multiple genetic variants contributing to variation in each pathway, and interaction among genes and with the environment. Considering the incapacitating effects of many psychiatric disorders, their relatively high frequencies, and their onset before or at reproductive age, it is unlikely that a small number of genes of large effect are directly determining disease state. Such variants would have been eliminated by evolution in relatively few generations. While some have argued that mutations underlying mental illness may be beneficial in certain environments (e.g., creative thinking and moderate paranoia) and therefore maintained by balancing selection, there is little evidence to support this supposition. Rather, the prevalence of mental illnesses is most consistent with an equilibrium between constantly occurring novel mutations that contribute to the disorders and selection eliminating these harmful mutations. The result is a large number of mutations that are individually rare and are spread throughout the genome and among families and populations, with each individual mutation contributing to a small number of cases (Keller and Miller 2006).

For more than twenty years, increased genetic liability (i.e., cumulative risk factors) in combination with environmental triggers has been recognized as the best-fitting model for most psychiatric disorders. Under this model, there are many genetic loci at which individuals may have alleles that increase or decrease the likelihood of developing a particular disorder with an individual's total liability equivalent to the summed likelihood across the genome. Expression of the disorder is a product of the genetic liability and the environmental risk factors for disease pushing an individual over a disease "threshold" after which an individual is considered affected. Because of this, the dichotomization of an underlying distribution into affected and unaffected status is sometimes referred to as the liability-threshold model of disease (McGue et al. 1983, 1985).

From a diagnostic perspective, clinicians are interested predominantly in the end product of these genetic and environmental interactions and whether the manifestation is significant enough to require treatment. In contrast, the geneticist is focused on understanding only what factors increase the genetic liability, regardless of the distal disease phenotype, because the alleles influencing genetic liability, not the environmental triggers, are heritable. In practice, however, symptoms are frequently the only recorded manifestation of liability. This illustrates why a dichotomous (i.e., affected/unaffected) diagnostic approach may be appropriate for determining the need for treatment, but insufficient for describing disease liability in genetic research. The endophenotype approach provides a means for identifying quantitative

traits that are correlated with disease liability such that they can be used to improve understanding of the biological, pathological, and etiological underpinnings of disease. In addition to operationalizing studies based on a liability-threshold model of disease, the use of endophenotypes, particularly in genetic research, increases power to identify causative factors. The following discussion of the shortcomings of existing research methods and the advantages of an endophenotype approach will illustrate the utility of the latter for parsing genetic liability for neurobehavioral traits.

5.2 Shortcomings of Existing Research Methods

The use of symptom-based diagnostic criteria to determine the genes, biochemistry, or neurological pathways involved in mental illness has led to few answers about the proximate or ultimate causes of neurobehavioral function. As a result, pharmacological treatments minimize symptoms without addressing core pathology. Furthermore, most treatments have been developed as the result of trial-and-error testing leaving a number of disease domains—e.g., cognitive deficits in schizophrenics—that cannot be treated (Carter and Barch 2007; Green et al. 2004a, b; Kirkpatrick et al. 2006) or for which the only currently known treatments have debilitating side effects (Gartlehner et al. 2008; Meyer 2010) which can lead to noncompliance with treatment regimens (Breen and Thornhill 1998; Keller et al. 2002; Nose et al. 2003). While the goal of identifying novel therapeutic targets is a primary motivation for most psychiatric research, current methodologies are failing in large part due to the near-exclusive focus on diagnostic criteria in classifying research subjects without regard to variation in symptoms within affected individuals, subclinical symptoms in unaffected individuals, or the appearance of similar symptoms across disorders.

Recent research into the underlying etiology of neurobehavioral function has considered neuroanatomical structure and function through MRI and fMRI techniques, examined biological markers in blood and brain tissue, and compared the genomes, transcriptomes, and methylomes of individuals with and without psychiatric disorders. While all of these techniques have the potential to be useful, the shared flaw in these methodologies is a case–control design which assumes (1) homogenous phenotypes among cases and controls, (2) reliable differentiation between individuals with and without a particular disorder, and (3) causal genetic variants specific to those affected with a particular disorder. Unfortunately, these assumptions do not hold for the majority of neurobehavioral traits.

5.2.1 Heterogeneity of Symptomatology

The majority of psychiatric diseases are understood entirely on the basis of their symptomatology because the etiology and pathogenesis remain largely

unexplained. Considering its prominence in diagnosis and research, one would expect symptomatology to be consistent from case to case. However, both diagnostic criteria and quantitative measures of affected individuals indicate a high degree of heterogeneity of symptoms and severity for many neurobehavioral traits. Diagnosis of schizophrenia, to take one example, relied on the presence of any combination of two or more of five symptoms (in the DSM-IV-TR). The revised DSM (V) requires that at least one of the symptoms be delusions, hallucinations, or disorganized speech, but incorporate no specific quantitative measures for delineating schizophrenia. The diagnostic criteria for schizophrenia implicitly demonstrate the heterogeneous nature of the disorder, but this has also been commented on directly. A list of attempts at a single, over-arching definition of schizophrenia is more poetic than quantifiable—"a weakening of the mainsprings of volition," "loss of inner unity of mental activities," "structural loosening of associations," "intrapsychic ataxia," "neurointegrative defect," "cognitive dysmetria," or "disconnection disorder" (Jablensky 2006). The difficulty in identifying a shared etiology among the various manifestations of schizophrenia is likely due to the correlated but independent depressive, positive, and negative symptoms of schizophrenia (Stefanis et al. 2002).

While it is possible that identical underlying genetic causes could manifest in different symptoms among affected individuals, it is more likely that etiological heterogeneity underlies the variability of symptoms among individuals. Studies that rely exclusively on a case–control framework (sometimes referred to as a retrospective case study) based on clinical diagnosis implicitly assume the former. This heterogeneity of symptomatology is not limited to schizophrenia; rather, it has been documented for depression (Chen et al. 2000; Zimmermann et al. 2009), bipolar disorder (Müller-Oerlinghausen et al. 2002), autism-spectrum disorders (Bruining et al. 2010), and attention-deficit/hyperactivity disorder (Mick et al. 2005), among other disorders.

It is noteworthy that the observed heterogeneity is not simply among clinically identified subtypes of a disorder. In the case of bipolar disorder (BPD), DSM categorization is based primarily on the severity of the disorder and the frequency of cycling. While severity is an important consideration in determining course of treatment, variation among affected individuals is better described by quantitative measures of specific symptoms. Studies applying factor analysis to a range of psychiatric symptoms present among bipolar individuals identified 6 independently varying (orthogonal) factors that accounted for significant proportions of the variance in the sample. These factors were associated with psychosis, irritability and aggression, dysphoria, accelerated thought stream, hedonia, and hyperactivity (Cassidy et al. 1998; Gupta et al. 2009). Similar studies of attention-deficit/hyperactivity disorder (ADHD) have found that reaction time and IQ both vary substantially among affected individuals and their relatives. However, the correlations between ADHD symptoms and IQ and between ADHD and reaction time are independent of one another, suggesting unique etiological pathways (Kuntsi et al. 2010; Wood et al. 2010).

Due to the increase in the frequency of diagnosis over recent decades, a great deal of research has focused on autism-spectrum disorders (ASDs). Heterogeneity is implied by the varying names of the disorders, but is largely assumed to be of degree and not kind. While revisions to the diagnostic criteria have narrowed the definition of autism in the DSM-V, autism remains a disorder characterized by three classes of impairment—social impairments, communication impairments, and repetitive behaviors or interests. A number of researchers have questioned the likelihood of a single-shared etiology among these traits, as autistic individuals may vary in severity on each axis. Screening for autism-spectrum symptoms in randomly ascertained twins demonstrates extremely high heritability for each of these three impairments, but little covariation—suggesting at least partial independence of etiology (Ronald et al. 2006). For all psychiatric disorders, heterogeneity is a likely contributor to the lack of coherence of the research literature and the failure to find a set of genes and/or biological indicators necessary and sufficient to cause the disorders.

5.2.2 Appearance of Subclinical Forms of Disorders

The appearance of subclinical forms of psychiatric disorders, particularly in first-degree relatives of those diagnosed, is further evidence for the continuous nature of these traits and complicates a case–control design for all research studies. These complications include individuals who endorse some but not all symptoms, a later age of onset, or the comorbidity of the disorder with other psychiatric ailments or substance abuse. Reliance on self-reported symptoms and behavioral observation as diagnostic criteria is additionally problematic for researchers as these methods, while important in determining quality of life for patients, are difficult to quantify, are variably applied by clinicians, and can be highly subjective. As a result, research on the pathogenesis of psychiatric disorders is hindered by the difficulty in accurately differentiating those who have the disease from those who do not. The challenge of cleanly categorizing individuals as affected or unaffected suggests an underlying continuous spectrum of psychopathology.

Autism-like traits (ALTs) have been observed in unaffected parents and children of autistic individuals (Bernier et al. 2012; Constantino et al. 2010; Piven 1997) and, for some autistic traits, show a broad distribution in the general population (Hoekstra et al. 2007a, b; Skuse et al. 2005). Subclinical social impairment is particularly visible among the siblings of children with major developmental challenges (Constantino et al. 2006). These observations suggest that ASDs are not discrete disorders, but rather the extreme end of a continuous distribution of social adaptation and communication behaviors (Constantino et al. 2004). A recent study of more than 19,000 twins examined the distribution of ALTs among monozygotic and dizygotic twins and concluded that there are no clear break points between ASDs and ALTs, and the ASDs and ALTs share a common etiology rooted in

shared genetic variation (Lundström et al. 2012). These ALTs can be linked to broader behavioral problems in non-autistic individuals, although these behaviors do not entirely share a genetic etiology (Hoekstra et al. 2007a, b).

It is unsurprising that ALTs are present in the general population given the well-established continuum of severity of ASD, but continuums of many psychiatric symptoms are found in unaffected individuals. A similar distribution of subclinical symptoms has also been documented for obsessive–compulsive disorder (OCD), with obsessive and compulsive psychopathology documented in individuals with a family history of OCD (Black et al. 1992; Van Grootheest et al. 2005) as well as in the population at large (Jonnal et al. 2000; Van Grootheest et al. 2007). Less expected is the presence of subclinical tics in relatives of individuals with Tourette's disorder (Hebebrand et al. 1997; McMahon et al. 2003) or the variable distribution of affective disorder symptoms (including thought disorganization, flat affect, hypomania, depression, social dysfunction, and impulsivity) in families of patients (Glahn et al. 2007; Gur et al. 2007a, b; Hain et al. 1995; Smith et al. 2008), in the general population (Shevlin et al. 2007; Stefanis et al. 2002; Verdoux and van Os 2002), and even, controversially, in the studies of creativity (Schuldberg 2001). In adult-onset disorders such as schizophrenia, the appearance of subclinical symptoms may be prodromes of impending psychosis (Cannon et al. 2008; Ruhrmann et al. 2010), but also show substantial variation in populations where the lifetime prevalence is zero. Based on these broad distributions of symptoms, most psychiatric disorders appear to be continuous in nature with a liability-threshold model overlaid for the purposes of diagnosis (Baron and Risch 1987).

5.2.3 Similarities of Disease Domains Across Diagnoses

Researchers have yet to conclusively determine whether cognitive deficits have unique one-to-one correlations with individual psychiatric disorders, whether disruptions of independent neural processes can cause the same illness, or whether aberrant cognition can lead to several different outcomes (Corvin et al. 2012). If either the second or third concept is accurate, approaches that attempt to link a single gene, biochemical pathway, or cognitive trait with a particular disease will result in, at best, an incomplete understanding of the disorder. Shared symptomatology across disorders has led to top-down diagnostic groupings (e.g., affective disorders or mood disorders). From a research perspective, disorders sharing some but not all symptoms better support a bottom-up model of psychiatric disease based on deficits in common domains (e.g., executive function impairment or insufficient dopamine production).

A shared disease domain caused by a shared genetic defect would explain why families may exhibit multiple disorders. The family in which the "disrupted in schizophrenia 1" (*DISC1*) mutation was first identified (Blackwood et al. 2001; Jacobs et al. 1970; Millar et al. 2000) has carriers affected with schizophrenia, recurrent major depression, bipolar disorder, and various minor diagnoses (St Clair

et al. 1990). *DISC1* has also been linked to autism and Asperger's syndrome, indicating it is not a disease-specific or disease family-specific variant (Kilpinen et al. 2007). Some studies have failed to link *DISC1* to schizophrenia, and a meta-analysis of 15 candidate gene and genome-wide association (GWA) studies suggests the gene may only be linked to the disorder in a subset of schizophrenics (Mathieson et al. 2011). Variation in *DISC1* has been associated with gray matter thickness and performance on tests of working memory, which would be consistent with an association with a particular domain shared among these disorders and only severely impaired in a subset of schizophrenics (Carless et al. 2011). At a genome-wide level, linkage results localized multiple regions of the genome implicated in both schizophrenia and bipolar disorder, indicative of a shared genetic etiology (Fanous et al. 2012).

Although currently lacking an identified genetic pathway, panic disorder, generalized anxiety disorder, phobias, and OCD also show substantial familial comorbidity with statistical analyses suggesting significant shared genetic variance but independent environmental variance (Hettema et al. 2001). In addition to the familial comorbidity, individual comorbidity also suggests shared impairment of neural functioning among these disorders. Reports of increased prevalence of ADHD and OCD among individuals with Tourette's disorder (TD) led to the observation that individuals diagnosed with TD+ADHD or TD+OCD had more socially inappropriate behaviors and complex vocal tics than individuals diagnosed with TD alone. Brain imaging studies of individuals with TD, ADHD, or OCD show consistent involvement of the cortico–striato–thalamo–cortical circuits in the expression of these disorders (O'Rourke et al. 2011). The likelihood of a common impairment is bolstered by statistical evidence for a joint genetic basis to OCD and TD and to OCD and ADHD in addition to significantly shared environmental influences (Mathews and Grados 2011).

While the DSM does not technically allow for the codiagnosis of ADHD and ASDs, ALTs are significantly more common in individuals diagnosed with ADHD than among their relatives (Reiersen et al. 2007). Furthermore, approximately half of the genetic variation influencing ADHD symptoms also influences ALTs (Reiersen et al. 2008; Ronald et al. 2008). In research, the use of hierarchical diagnostic criteria where individuals with both an ADHD and an ASD are classed exclusively as autistic or an individual with manic symptoms who subsequently presents with schizophrenic symptoms will be classified exclusively as schizophrenic masks the domains shared by multiple disorders.

A polygenic model of psychiatric illness (Fig. 5.1) predicts that disturbances in one domain (caused by a particular set of genes) will result in many different illnesses depending on the other implicated domains (and other, possibly overlapping, sets of genes) in combination with environmental influences. This is likely the case for psychotic symptoms, such as delusions and hallucinations, which are common to multiple disorders. Schizoaffective disorder is a way to express comorbidity among psychotic and mood disorders and evidence from a study of monozygotic and dizygotic twins suggests this comorbidity may be due to a common genetic liability. Approximately 67–95% of the additive genetic liability of

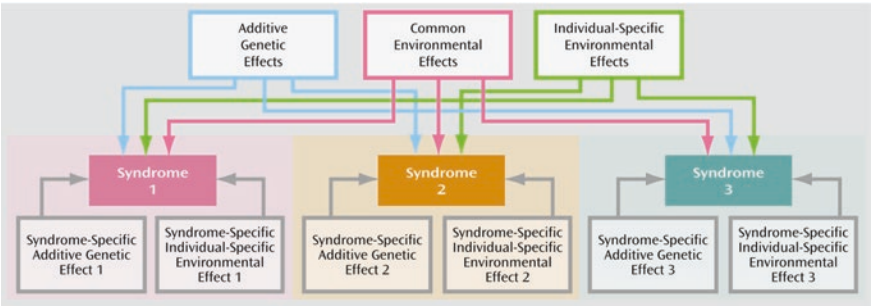

Fig. 5.1 Effects contributing to variance in liability to diseases. From (Cardno et al. 2002)

schizophrenia, schizoaffective disorder, and manic syndromes are shared with the remainder syndrome-specific symptoms (Cardno et al. 2002). Anxiety-related behaviors in children show a similar pattern of partially overlapping genetic and environmental influences on general distress, separation anxiety, fear, obsessive–compulsive behaviors, and shyness/inhibition (Eley et al. 2003). In other cases, as with major depression and generalized anxiety disorder, the underlying genetic liability appears to be identical, with the specific manifestation determined by the environment (Kendler 1992).

A final line of evidence for shared disease domains across disorders comes from pharmacological interventions. Selective serotonin reuptake inhibitors (SSRIs) were developed for treating major depression and other mood disorders. However, they are increasingly prescribed for anxiety disorders (including obsessive–compulsive disorder, panic disorder, and generalized anxiety disorder) and eating disorders (Preskorn et al. 2004).

5.3 Advantages of an Endophenotype-Based Approach

A number of issues have been raised regarding the use of clinical diagnoses in research: (1) heterogeneity of symptoms, (2) appearance of subclinical forms of the disorder in relatives, (3) reliance on self-report, (4) dichotomization of continuous traits, and (5) impairment of similar cognitive domains across multiple disorders and variation in unaffected individuals. The endophenotype approach, in combination with the associated etiological model, addresses each of these issues. An endophenotype approach is rooted in a disease model in which additive genetic effects cause errors in one or more neurological pathways which interact with environmental influences to cause impairments in particular domains and result in the disease(s) of interest (Fig. 5.1). Since psychiatric disorders are the end result of mutations in many genes that influence multiple pathways, identifying these genes is facilitated by isolation of these individual pathways.

Because endophenotypes are continuous individual traits, the issues of heterogeneity of symptoms, subclinical forms, and dichotomization do not apply. Furthermore, endophenotypes are typically measured directly by researchers, removing the subjective nature of self-reported symptoms. Generally domain-specific deficits in a particular endophenotype may be unique to a particular disorder or shared across multiple syndromes, and variation in the endophenotypes is likely to be continuous across affected and unaffected individuals. The inclusion of unaffected and subclinically affected individuals enables larger sample sizes in studies looking for the underlying genes and pathways contributing to the endophenotype and trait of interest. These specific pathways can then become novel therapeutic targets and may be used to refine diagnostic criteria.

5.3.1 Endophenotypes Are Considered More Closely Related to the Root of the Disorder

The endophenotype approach requires the acceptance that psychiatric disorders are heterogeneous and must be deconstructed into separate domains for effective study. In addition to solving the previously enumerated problems with applying subjective diagnostic criteria to research, endophenotypes are considered to be closer to root neuropathologies and genetic causes which inherently increase statistical power in detecting gene action. Because endophenotypes represent individual disease domains and/or unique biological pathways, one would expect fewer genetic and environmental factors to contribute to the endophenotype than to the observed expression of the disorder. It is difficult, however, to provide direct support for this assertion except in retrospective consideration of studies that have succeeded in identifying genes associated with endophenotypes.

The best evidence for this assertion comes from the identification of genes showing consistent association with an endophenotype but inconsistent association with diagnosis. The link between the dopamine transporter gene *DAT1* and ADHD was inconclusive following initial genome-wide studies. However, this locus is strongly related to measures of neuropsychological impairment (variation in sustained attention, response variability, and spatial-attentional asymmetries) known to vary among individuals with ADHD (Bellgrove et al. 2005). Additional analyses have also found that *DAT1*, along with the dopamine receptor *DRD4*, is better associated with endophenotypes than with ADHD as a discrete phenotype. Furthermore, the interaction effect between *DAT1* and environmental influences such as maternal smoking is more pronounced when considering hyperactivity endophenotypes. These observations support a role for *DAT1* in regulating specific symptoms of ADHD, even though it was poorly replicated when the distal phenotype was the sole outcome variable (Turic and Swanson 2010).

In what is sometimes referred to as a reverse endophenotype study, a various cognitive endophenotypes are compared in individuals with and without the

DISC1 mutation. This design elucidates the intermediate pathway between disease and genotype, rather than exploiting a previously established link between endophenotype and disease, to identify related genes. The studies linked *DISC1* mutations with variation in brain anatomy (hippocampal structure, white matter integrity, prefrontal gray matter, and cortical thickness), development (cortical maturation), and cognition (short- and long-term memory and visual memory) (Brandon and Sawa 2011). These endophenotypes, particularly those denoting cognitive impairments, are not unique to schizophrenia and additional studies have demonstrated that *DISC1* is most active during the development of the cerebral cortex and glutamate receptors, giving credence to the hypothesis that mutations in the gene may result in structural liabilities in the development of one or more disorders, rather than causing a particular disorder directly (Kamiya et al. 2012).

The corollary to the observation that the endophenotype has a closer relationship to the biological process leading to the diagnosis is that the genetic influences on the endophenotype will be easier to detect than those on the distal phenotype. However, this is not necessarily the case, and some endophenotypes may prove highly polygenic (Flint and Munafò 2007). While it is still reasonable to expect fewer genes contribute to endophenotypes than to the distal phenotype, there are a number of additional advantages which highlight the utility of the endophenotype approach.

5.3.2 Case–Control Studies Lack Statistical Power

The existing case–control framework is flawed for studying genetic, cognitive, and neurological causes of psychiatric disorders that are highly polygenic. Mendelian disorders, such as Huntington's disease, are regulated by a single gene of extremely large influence and can be detected by either linkage (family-based) or association (population-based) studies. A few genes with modest influence on psychiatric disorders have been identified, but replicating linkage and association results for most neurobehavioral traits has proved difficult. This stems from an incompatibility between the underlying assumptions of the case–control approach and what is known about the underlying genetic architecture of neurobehavioral function. Linkage-based methods have the highest power for identifying rare genetic variants of large effect relative to the phenotypic variation in the sample. Association-based methods, in contrast, are designed to identify relatively common variants of smaller effect. The genetic architecture of psychiatric phenotypes is extremely complex, with the International Schizophrenia Consortium estimating the involvement of hundreds of genes of small effect and similar numbers of genes expected for most disorders (Purcell et al. 2009).

Statistical power in genetic studies is based on the sample size, the distribution of the phenotype, and the distribution of the causative variants in the sample. Because there is no way to know a priori the distribution of the causative variants, achieving sufficient power to identify many genes of such small effect

necessitates either impossibly large sample sizes or a change in the distribution of the phenotype in the sample. Given the low prevalence of some disorders, it can be extremely difficult to obtain the thousands of affected individuals necessary for a statistically powerful genetic association study. Case–control studies require more samples in general, and more variants are likely to contribute to the disease as a whole than to a single, component endophenotype. Consortia have been formed to merge samples and facilitate meta-analyses in an attempt to increase power (Neale et al. 2010; Psychiatric GWAS Consortium 2012; Ripke et al. 2011; Sklar et al. 2011).

These consortia have addressed the sample-size problem, but case–control designs still suffer from a decrease in power as a result of heterogeneity within both the cases and controls. This heterogeneity is due to the dichotomization of what is likely a continuous phenotypic spectrum, and changing the distribution of the phenotype by considering a quantitative trait will substantially increase statistical power for any given sample size. As a result, consortia are increasingly including endophenotype collection to maximize power after few results were found in the case–control genome-wide association studies (Chan 2011; Gur et al. 2007a, b; Hasler and Northoff 2011).

5.3.3 Quantitative Traits Enhance Statistical Power

Quantitative, or continuously distributed, traits have a number of advantages in the study of the genetics of psychiatric disorders. Conceptually, quantitative traits more closely match the real distribution of disease liability as assessed by the heterogeneity within affected individuals and the appearance of subclinical forms in relatives. Statistically, a study design based on a quantitative trait will always have greater power than one based on cases and controls.

The endophenotype approach has been successfully applied to genetic research on other complex diseases (such as heart disease) which share many of the research and statistical challenges previously discussed. As with psychiatric disorders, the precise cause of heart disease may vary among patients and may onset later in life. Heart disease also exhibits comorbidity and overlapping etiology with high blood pressure, obesity, type 2 diabetes, and other disorders. The use of known risk factors (e.g., high cholesterol) as endophenotypes proved useful in identifying causative genetic loci influencing risk of developing heart disease due to a number of statistical advantages inherent in quantitative trait analysis (Almasy and Blangero 2001).

The issue of statistical power to identify variants of small effect has been a perennial challenge for researchers and quantitative traits will always be statistically more powerful than dichotomized traits in genetic analyses (Wijsman and Amos 1997). Consider a sample of individuals exhibiting a normally distributed, continuous trait which perfectly correlates with disease risk. Applying a threshold model of disease, individuals who fall in the top 10% of the distribution are considered

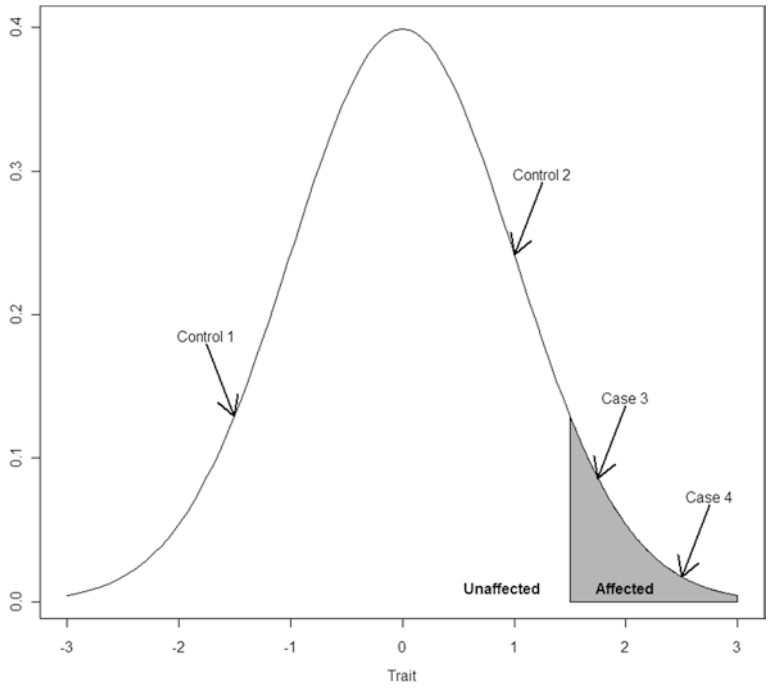

Fig. 5.2 Distribution of endophenotype across cases and controls illustrates relationship between liability and diagnosis

affected while those in the bottom 90% are unaffected. Despite the continuous distribution of disease liability, a case–control analysis will ignore the variation within the two groups making the design less efficient. This is best illustrated by overlaying case–control status onto an endophenotype as shown in Fig. 5.2. Individuals who fall in the shaded portion of the endophenotype distribution are affected with the distal phenotype or disease. Under a case–control model, control 1 and control 2 are considered to share a (lack of) liability for disease (0), and case 3 and case 4 are also considered to share a liability (1). Therefore, genetic markers will only be considered as associated with the disorder if they are present in cases 3 and 4 but absent in controls 1 and 2. In reality, markers absent in 1, but found in the other three individuals, are more likely to be contributing to the disorder.

The magnitude of this loss of statistical power is most extreme for traits, including most psychiatric disorders, that are at low prevalence in the general population, even if heritability of the disorder is high (Blangero et al. 2003). For a low prevalence disorder, knowing that someone is unaffected (i.e., a control) tells you very little about their potential underlying liability. Use of endophenotypes also increases the proportion of the population suitable for study since endophenotypes can be assessed in unaffected relatives or samples not ascertained for disease status because the endophenotype is an index of disease risk, not disease status.

The endophenotype approach is also advantageous in dissecting disease domains. Schizophrenia is a disease marked by both positive and negative symptoms. The positive symptoms are generally more responsive to antipsychotic medication, but the effectiveness of pharmacological intervention varies among patients. The type and severity of negative symptoms differs among patients, as does the response to pharmacological intervention (which may produce adverse effects which mimic negative symptoms). Considering symptoms individually, and comparing symptoms across disorders, has led to the association of individual negative symptoms with specific structural abnormalities and defects in neurological receptors. Because these negative symptoms are not directly correlated with positive symptoms or present in all schizophrenics, considering them independently has enabled these breakthroughs is a way that classing study participants as either affected or unaffected would not. Furthermore, targeting intervention based on the specific neurological pathway impacted should improve therapeutic outcomes and minimize unnecessary side effects (Erhart et al. 2006; Laughren and Levin 2006). Cognitive deficits are an additional impairment found in many schizophrenics for which the dominant treatment is psychotherapy focused on life-skills development, and increases in environmental structure which compensates for the deficits in executive function and working memory. Such treatment is rarely able to return the patient to their premorbid level of cognition and independence, but no procognitive agents have been identified because specific cognitive endophenotypes are poorly understood (Carter and Barch 2007; Green et al. 2004a, b). Both neurocognitive deficits and negative symptoms have been found to significantly influence functional outcomes and patient's quality of life (Milev 2005). Specific consideration of these factors may have the most direct impact on improving treatment for schizophrenics.

5.4 Endophenotype Research Design

The major challenge in identifying endophenotypes for psychiatric disorders is the relative lack of consistently identified biological pathways and risk factors when compared to other complex disorders. Whereas high blood pressure and increased cholesterol are obvious choices for endophenotypes when considering heart disease, pinpointing suitable endophenotypes for psychiatric disorders continues to be a substantial hurdle to the initiation of large-scale studies.

5.4.1 Adjudicating Neurocognitive Endophenotypes

Continuous traits can be ascertained from clinical measures of neuropsychological impairments, neuroanatomical features assessed with MRI, functional studies using fMRI or EEG, gene expression levels, blood chemistry, and a wide variety of other means discussed in the following chapters. Whatever the source of the

endophenotype is, it is essential that the correlations with the disease of interest are due to underlying genetic liability, not a by-product of treatment or degeneration due to disease progression (Almasy and Blangero 2001). In other words, the most fundamental determinant of a successful endophenotype is a shared genetic basis with the distal disease. More specifically, the utility of a neurocognitive endophenotype is judged based on (1) the cosegregation with illness, (2) heritability, (3) state independence, (4) accuracy and reliability of measurement, and (5) manifestation in unaffected relatives of the proband (Gottesman and Gould 2003).

There are a number of practical considerations for endophenotypes which are obligatory for any phenotype under study. Within an individual, measures should be repeatable and stable over time and objective enough that results are consistent across clinicians. In contrast to many diagnostic symptoms, endophenotypes must be present and consistent across active and inactive periods of illness. This requirement ensures the endophenotype indexes disease risk and not disease status, as does the ability of endophenotypes to be measured quantitatively in both affected and unaffected individuals. The distribution of the endophenotype should be continuous across the affected and unaffected individuals, as is the genetic risk for the disorder.

In addition to these considerations, endophenotypes must have two extremely important genetic characteristics—heritability, a measure of the strength of the genetic signal, and pleiotropy, a genetic correlation with disease risk. Because of these considerations, endophenotype discovery is best done in extended pedigrees where heritability and genetic liability can be assessed. Concentrating a sample into as few pedigrees as possible maximizes power to assess heritability and genetic correlation. Defined as the proportion of phenotypic variance attributable to genetic variance, heritability can be calculated by comparing the observed phenotypic similarity among relatives to their genetic similarity due to their relationship (kinship). While traits that are not heritable may be useful for identifying individuals at risk of developing the disorder, in the same way that smoking is a risk for lung cancer, heritability is an indicator of underlying genetic causation and so is essential for both endophenotypes and disorders used in any genetic analysis. Heritability can be estimated by a number of computer programs (including SOLAR) which has the advantage of explicitly controlling for shared environmental influences (Almasy and Blangero 1998).

From the heritability of the disorder and the genetic distance from an affected individual, genetic liability can be assessed for each individual in a pedigree in the absence of a priori knowledge of the genes involved. This is essentially a way of quantifying the information recorded when a family history is requested from patients. Endophenotypes must be correlated with genetic liability (i.e., familial genetic risk) such that the endophenotype reliably differentiates between affected individuals, unaffected individuals with a family history, and unaffected individuals with no family history. A χ^2 test may be used to assess the ability of the endophenotype to differentiate between classes of individuals (affected individuals, unaffected relatives with moderate liability, and unrelated controls with no known liability for the disorder). However, this test is not sensitive to shared environmental influences which may mimic familial relationships (e.g., prenatal smoking and environmental toxin exposure). To decompose the phenotypic correlations (ρ_p)

into environmental (ρ_e) and genetic correlations (ρ_g), bivariate genetic analyses should be performed (Glahn et al. 2007, 2010).

The endophenotype ranking value (ERV) is an empirical, unbiased method for comparing the utility of endophenotypes for genetic research. The ERV varies from 0 to 1 and balances the strength of genetic signal for the endophenotype (heritability of the endophenotype, h_e^2) and for the disorder (heritability of the disorder, h_i^2) with the genetic correlation between the traits (ρ_g). The ERV is applicable to any type of trait and, as gene expression and other data-intensive analyses become more prevalent, it can rapidly screen thousands of putative endophenotypes, including transcript levels to identify the ones with the best potential to localize genes influencing liability to the disorder of interest (Glahn et al. 2011). It should be noted that the endophenotype will rarely perfectly map with the disease of interest. Because endophenotypes generally track only a single disease domain (e.g., verbal memory), imperfect correlation is due to the heterogeneity of symptoms within a disorder as well as the potential overlap of domains with normal variation and/or other disorders which may be present, but not queried in the sample. Endophenotypes for the same disorder may demonstrate pleiotropic effects with the disease of interest but not one another. This means that the same genes can contribute to both the endophenotype and the disease, but the same set of genes is unlikely to influence all endophenotypes associated with a particular illness. This was the case in an analysis of endophenotypes for recurrent major depression where a locus associated with both ventral diencephalon volume and depression liability was not associated with endophenotypes for behavioral or neurocognitive measures or transcriptional activation (Glahn et al. 2011).

A final concern may be the specificity of the endophenotype. However, this is not a necessary requirement for a useful endophenotype, and removal of nonspecific endophenotypes may be detrimental. Specificity can be assessed either for a particular disorder or for a particular disease domain by comparing trait values in individuals with the focal trait (e.g., schizophrenia or verbal memory impairment) to relatives with a distinct disorder (e.g., major depression or locational impairment) (Glahn et al. 2007). However, given the rampant comorbidity of disorders and clear evidence for shared disease domains implying a common etiology, insisting on specificity may result in the removal of valuable endophenotypes that index risk factors shared between disorders. Specificity should only be a concern if an investigator is exclusively interested in genetic variation that distinguishes between disorders.

5.4.2 Studies of Endophenotypes in Randomly Ascertained Samples

While initial identification of endophenotypes requires studies of affected individuals, one of the advantages of an endophenotype approach to gene localization and identification is the ability to conduct research in a normal population where the endophenotype varies (Almasy and Blangero 2001). This relies on the assumption

that the underlying genetic factors determining the most extreme phenotypes also influence variation within the mid-ranges of the distribution. This assumption is a reasonable extension of the liability-threshold model for psychiatric disease, so one would expect a randomly ascertained sample to exhibit variation in the relevant genes. Considering the difficulty in identifying large numbers of affected individuals, particularly multiplex families for some diseases, using randomly ascertained samples has a profound impact on the speed with which genetic studies can be completed. Additionally, a study may be done more efficiently by adding endophenotype assessments to an already-genotyped sample.

Endophenotypes can be analyzed using any study design developed for quantitative traits, including association studies of unrelated individuals. However, large, extended pedigrees have greater power to identify quantitative trait loci (QTLs) than association studies of unrelated individuals and so may be preferable for psychiatric diseases where the individual effect size of each locus is expected to be small. Large pedigrees have the additional advantage of being more likely to contain multiple copies of rare variants which, in light of the failures of genome-wide association studies to identify common alleles of large effect, likely account for much of the heritability of complex traits.

While many assume that studies of unaffected samples would have less power to detect linkage, large pedigrees ascertained by disease status of one or two probands have only a slight increase in power compared to families randomly ascertained based on large pedigree size (Williams et al. 1999). Furthermore, ascertaining on disease status in smaller families can introduce bias into results which is avoided in families ascertained independently of phenotype (Comuzzie and Williams 1999). More importantly, randomly ascertained samples can be studied for many traits of interest.

Several studies have already localized genes or loci contributing to endophenotype variation in healthy populations. A recent gene-based study of endophenotypes for cognitive ability identified a relationship between *RORB*, a candidate gene for bipolar disorder, and verbal intelligence in a sample of healthy adults (Ersland et al. 2012). Combined samples including both affected and unaffected individuals have also been analyzed, including in a study identifying several novel genetic loci associated with brain activation in regions linked to working memory (Potkin et al. 2009). Finally, it is also possible to consider the distribution of endophenotypes exclusively within affected individuals. Among individuals with schizophrenia, but not in healthy controls, *ZNF804A* is associated with hippocampal volume and memory processing, suggesting spared cognitive performance but increased social deficits in the subset of patients homozygous for this mutation (Donohoe et al. 2011).

5.4.3 Endophenotypes, Genes, and Nosology

A final advantage of the endophenotype approach is the influence it can have on nosology. Psychiatric disorders are commonly divided into subtypes based

on symptom severity or specific clinical attributes. Because endophenotypes are defined by a genetic underpinning, their use in classifying patients for treatment would result in biologically more homogeneous groups more likely to respond similarly to a particular treatment. In addition to better differentiation of disorders, endophenotypes highlight potential biological and genetic relationships among disorders which may otherwise be considered independent and therefore not relevant in determining family history.

Finally, because endophenotypes are related to disease liability, they could be used to identify individuals with an increased risk prior to the onset of symptoms. For example, cognitive deficits are a potentially useful diagnostic endophenotype for risk of developing schizophrenia and affective disorders. Such deficits are frequently apparent prior to the onset of any psychoses (Erlenmeyer-Kimling et al. 2000) and closely related to eventual disease status in individuals with a family history of the disorder (Green et al. 2004a, b). With increasing reliability, brain neuroanatomical patterns may also be used to predict transition to disease (Koutsouleris et al. 2009). Identifying individuals at increased risk of developing a mental disorder or detecting the disorder at an early stage could lead to preemptive environmental or pharmacological interventions which would dramatically increase the patient's quality of life.

5.5 Conclusions

As the end goal of all psychiatric research is to improve diagnosis and outcome for individuals with mental illness, it is important to recognize that the first step in psychiatric genetic research is the development of a basic understanding of the genes and pathways involved. This may best be achieved by divorcing trait identification from diagnostic criteria. The basis for judging the utility of diagnostic criteria for treatment and for research is not the same, because genetic research seeks to identify genetic factors which increase the risk of the disorder and only indirectly predict the likelihood of developing the disorder.

Unlike environmental triggers which may contribute to the onset of the disease, genetic liability is stable throughout life and heritable in the absence of clinical expression of the disorder, or it would not be possible for diseases to "skip a generation." Despite differences between the research and diagnostic perspective of disease, the endophenotype approach is consistent with clinical observations of symptom heterogeneity, subclinical manifestations in relatives, and shared disease domains across disorders—all of which present challenges for researchers and clinicians alike. A deeper understanding of the many component biological pathways involved in each neurobehavioral trait will create an environment where applied research can flourish. If the endophenotype approach succeeds where a case–control framework has failed, the new risk variants identified have the potential to refine our understanding and treatment of mental illness through new biological insights which may lead to novel therapeutic targets, increased sensitivity of biomarkers, earlier detection or prevention of disease, determine individual etiology, and direct individualized care for each patient.

Acknowledgments This work was supported in part by NIH grants R01 GM031575, R01 MH061622, U01 AA08403, and R01 MH059490. The authors would like to thank Marc A. Quillen for his insightful comments on this chapter.

References

Almasy L, Blangero J (1998) Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Gen 62(5):1198–1211

Almasy L, Blangero J (2001) Endophenotypes as quantitative risk factors for psychiatric disease: rationale and study design. Am J Med Genet B Neuropsychiatr Genet 105(1):42–44

Baron M, Risch N (1987) The spectrum concept of schizophrenia: evidence for a genetic-environmental continuum. J Psychiatric Res 21(3):257–267

Bellgrove MA, Hawi Z, Kirley A et al (2005) Dissecting the attention deficit hyperactivity disorder (ADHD) phenotype: sustained attention, response variability and spatial attentional asymmetries in relation to dopamine transporter (DAT1) genotype. Neuropsychologia 43(13):1847–1857

Bernier R, Gerdts J, Munson J et al (2012) Evidence for broader autism phenotype characteristics in parents from multiple-incidence autism families. Autism Res 5(1):13–20

Black DW, Noyes JR, Goldstein RB, Blum N (1992) A family study of obsessive-compulsive disorder. Arch Gen Psychiatry 49(5):362–368

Blackwood DH, Fordyce A, Walker MT et al (2001) Schizophrenia and affective disorders–cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. Am J Hum Gen 69(2):428–433

Blangero J, Williams JT, Almasy L (2003) Novel family-based approaches to genetic risk in thrombosis. J Thromb Haemost 1(7):1391–1397

Brandon NJ, Sawa A (2011) Linking neurodevelopmental and synaptic theories of mental illness through DISC1. Nat Rev Neurosci 12(12):707–722

Breen R, Thornhill JT (1998) Noncompliance with medication for psychiatric disorders: reasons and remedies. CNS Drugs 9(6):457–471

Bruining H, de Sonneville L, Swaab H et al (2010) Dissecting the clinical heterogeneity of autism spectrum disorders through defined genotypes. PLoS ONE 5(5):e10887

Cannon TD, Cadenhead K, Cornblatt B, Woods SW (2008) Prediction of psychosis in youth at high clinical risk. Arch Gen Psychiatry 65(1):28–37

Cardno A, Rijsdijk F, Sham P et al (2002) A twin study of genetic relationships between psychotic symptoms. Am J Psychiatry 4:539–545

Carless MA, Glahn DC, Johnson MP et al (2011) Impact of DISC1 variation on neuroanatomical and neurocognitive phenotypes. Mol Psychiatry 16(11):1096–1104, 1063

Carter CS, Barch DM (2007) Cognitive neuroscience-based approaches to measuring and improving treatment effects on cognition in schizophrenia: the CNTRICS initiative. Schizophr Bull 33(5):1131–1137. doi:10.1093/schbul/sbm081

Cassidy F, Forest K, Murry E, Carroll BJ (1998) A factor analysis of the signs and symptoms of mania. Arch Gen Psychiatry 55(1):27–32

Chan RCK (2011) Consortium for the human information and neurocognitive endophenotype (CHINE) in mainland China: an example from neurological soft signs for neuropsychiatric disorders. Chin Sci Bull 56(32):3409–3415. doi:10.1007/s11434-011-4715-5

Chen LS, Eaton WW, Gallo JJ, Nestadt G (2000) Understanding the heterogeneity of depression through the triad of symptoms, course and risk factors: a longitudinal, population-based study. J Affect Disord 59(1):1–11

Comuzzie AG, Williams JT (1999) Correcting for ascertainment bias in the COGA data set. Genet Epidemiol 17(Suppl 1):S109–S114

Constantino JN, Gruber CP, Davis S et al (2004) The factor structure of autistic traits. J Child Psychol Psychiatry 45(4):719–726
Constantino J, Lajonchere C, Lutz M et al (2006) Autistic social impairment in the siblings of children with pervasive developmental disorders. Am J Psychiatry 163:294–296
Constantino JN, Zhang Y, Frazier T et al (2010) Sibling recurrence and the genetic epidemiology of autism. Psychiatry 167(11):1349–1356
Corvin A, Donohoe G, Hargreaves A et al (2012) The cognitive genetics of neuropsychiatric disorders. Curr Top Behav Neurosci 12:579–613
Deary IJ, Penke L, Johnson W (2010) The neuroscience of human intelligence differences. Nat Rev Neurosci 11(3):201–211
Donohoe G, Rose E, Frodl T et al (2011) ZNF804A risk allele is associated with relatively intact gray matter volume in patients with schizophrenia. NeuroImage 54(3):2132–2137
Eley TC, Bolton D, O'Conner TG et al (2003) A twin study of anxiety-related behaviours in preschool children. J Child Psychol Psychiatry 44(7):945–960
Erhart SM, Marder SR, Carpenter WT (2006) Treatment of schizophrenia negative symptoms: future prospects. Schizophr Bull 32(2):234–237
Erlenmeyer-Kimling L, Rock D, Roberts S et al (2000) Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: the New York High-Risk Project. Am J Psychiatry 157(9):1416–1422
Ersland KM, Christoforou A, Stansberg C et al (2012) Gene-based analysis of regionally enriched cortical genes in GWAS data sets of cognitive traits and psychiatric disorders. PLoS ONE 7(2):e31687
Fanous AH, Middleton FA, Gentile K et al (2012) Genetic overlap of schizophrenia and bipolar disorder in a high-density linkage survey in the Portuguese Island population. Am J Med Genet B Neuropsychiatr Genet 159b(4):383–391
Flint J, Munafò MR (2007) The endophenotype concept in psychiatric genetics. Psychol Med 37(2):163–180
Gartlehner G, Thieda P, Hansen RA (2008) Comparative risk for harms of second-generation antidepressants: a systematic review and meta-analysis. Drug Saf 31(10):851–865
Glahn DC, Almasy L, Blangero J (2007) Adjudicating neurocognitive endophenotypes for schizophrenia. Am J Med Gen Part B: Neuropsychiatr Genet 144(2):242–249
Glahn DC, Almasy L, Barguil M et al (2010) Neurocognitive endophenotypes for bipolar disorder identified in multiplex multigenerational families. Arch Gen Psychiatry 67(2):168–177
Glahn DC, Curran JE, Winkler A et al (2011) High dimensional endophenotype ranking in the search for major depression risk genes. Biol Psychiatry 71(1):6–14
Gottesman II, Gould TD (2003) Reviews and overviews the endophenotype concept in psychiatry: etymology and strategic intentions. Psychiatry, 636–645
Green MF, Kern RS, Heaton RK (2004a) Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. Schizophr Res 72(1):41–51
Green MF, Nuechterlein KH, Gold JM et al (2004b) Approaching a consensus cognitive battery for clinical trials in schizophrenia: the NIMH-MATRICS conference to select cognitive domains and test criteria. Biol Psychiatry 56(5):301–307
Gupta SC, Sinha VK, Praharaj SK, Gandotra S (2009) Factor structure of manic symptoms. Aust N Z J Psychiatry 43(12):1141–1146
Gur RE, Calkins ME, Gur RC et al (2007a) the consortium on the genetics of schizophrenia: neurocognitive endophenotypes. Schizophr Bull 33(1):49–68
Gur RE, Nimgaonkar VL, Almasy L et al (2007b) Neurocognitive endophenotypes in a multiplex multigenerational family study of schizophrenia. Am J Psychiatry 164(5):813–819
Hain C, Maier W, Hoechst-Janneck S, Franke P (1995) Subclinical thought disorder in first-degree relatives of schizophrenic patients. Results from a matched-pairs study with the thought disorder index. Acta Psychiatr Scand 92(4):305–309
Hasler G, Northoff G (2011) Discovering imaging endophenotypes for major depression. Mol Psychiatry 16(6):604–619

Hebebrand J, Klug B, Fimmers R et al (1997) Rates for tic disorders and obsessive compulsive symptomatology in families of children and adolescents with Gilles de la Tourette syndrome. J Psychiatric Res 31(5):519–530
Hettema J, Neale M, Kendler KS (2001) A review and meta-analysis of the genetic epidemiology of anxiety disorders. Am J Psychiatry 158:1568–1578
Hoekstra RA, Bartels M, Hudziak JJ et al (2007a) Genetic and environmental covariation between autistic traits and behavioral problems. Twin Res Hum Genet 10(6):853–860
Hoekstra RA, Bartels M, Verweij CJH, Boomsma DI (2007b) Heritability of autistic traits in the general population. Arch Pediatr Adolesc Med 161(4):372–377
Jablensky A (2006) Subtyping schizophrenia: implications for genetic research. Mol Psychiatry 11(9):815–836
Jacobs PA, Brunton M, Frackiewicz A et al (1970) Studies on a family with three cytogenetic markers. Ann Hum Genet 33(4):325–336
Jonnal A, Gardner C, Prescott C, Kendler K (2000) Obsessive and compulsive symptoms in a general population sample of female twins. Am J Med Genet B Neuropsychiatr Genet 96(6):791–796
Kamiya A, Sedlak TW, Pletnikov MV (2012) DISC1 pathway in brain development: exploring therapeutic targets for major psychiatric disorders. Front Psychiatry 3:25
Keller MC, Miller G (2006) Resolving the paradox of common, harmful, heritable mental disorders: which evolutionary genetic models work best? Behav Brain Sci 29(4):385–404; discussion 405–52
Keller MB, Hirschfeld RMA, Demyttenaere K, Baldwin DS (2002) Optimizing outcomes in depression: focus on antidepressant compliance. Int Clin Psychopharmacol 17(6):265
Kendler KS, Neale MC, Kessler RC et al (1992) Major depression and generalized anxiety disorder: same genes, (Partly) different environments? Arch Gen Psychiatry 49(9):716–722
Kendler KS, Pedersen N, Johnson L et al (1993) A pilot Swedish twin study of affective illness, including hospital-and population-ascertained subsamples. Arch Gen Psychiatry 50(9):699
Kilpinen H, Ylisaukko-Oja T, Hennah W et al (2007) Association of DISC1 with autism and Asperger syndrome. Mol Psychiatry 13(2):187–196
Kirkpatrick B, Fenton WS, Carpenter WT, Marder SR (2006) The NIMH-MATRICS consensus statement on negative symptoms. Schizophr Bull 32(2):214–219. doi:10.1093/schbul/sbj053
Koutsouleris N, Meisenzahl EM, Davatzikos C et al (2009) Use of neuroanatomical pattern classification to identify subjects in at-risk mental states of psychosis and predict disease transition. Arch Gen Psychiatry 66(7):700–712
Kuntsi J, Wood AC, Rijsdijk F et al (2010) Separation of cognitive impairments in attention-deficit/hyperactivity disorder into 2 familial factors. Arch Gen Psychiatry 67(11):1159
Laughren T, Levin R (2006) Food and drug administration perspective on negative symptoms in schizophrenia as a target for a drug treatment claim. Schizophr Bull 32(2):220–222
Lichtenstein P, Carlström E, Råstam M et al (2010) The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. Am J Psychiatry 167(11):1357–1363
Lundström S, Chang Z, Rästam M et al (2012) Autism spectrum disorders and Autisticlike traits. Arch Gen Psychiatry 69(1):46–52
Mathews C, Grados M (2011) Familiality of tourette syndrome, obsessive-compulsive disorder, and attention-deficit/hyperactivity disorder: heritability analysis in a large sib-pair sample. J Am Acad Child Adolesc Psychiatry 50(1):46–54
Mathieson I, Munafò MR, Flint J (2011) Meta-analysis indicates that common variants at the DISC1 locus are not associated with schizophrenia. Mol Psychiatry 1–8
McGue M, Gottesman II, Rao DC (1983) The transmission of schizophrenia under a multifactorial threshold model. Am J Hum Gen 35(6):1161–1178
McGue M, Gottesman II, Rao DC (1985) Resolving genetic models for the transmission of schizophrenia. Genet Epidemiol 2(1):99–110
McMahon WM, Carter AS, Fredine N, Pauls DL (2003) Children at familial risk for Tourette's disorder: child and parent diagnoses. Am J Med Gen Part B: Neuropsychiatr Genet 121B(1):105–111

Meyer JM (2010) Antipsychotics and metabolics in the post-CATIE era. Curr Top Behav Neurosci 4:23–42
Mick E, Spencer T, Wozniak J, Biederman J (2005) Heterogeneity of irritability in attention-deficit/hyperactivity disorder subjects with and without mood disorders. Biol Psychiatry 1;58(7):576–582
Milev P, Ho BC, Arndt S, Andreasen NC (2005) Predictive values of neurocognition and negative symptoms on functional outcome in schizophrenia: a longitudinal first-episode study with 7-year follow-up. Am J Psychiatry 162(3):495–506
Millar JK, Wilson-Annan JC, Anderson S et al (2000) Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum Mol Genet 9(9):1415–1423
Müller-Oerlinghausen B, Berghöfer A, Bauer M (2002) Bipolar disorder. Lancet 359:241–247
Neale BM, Medland SE, Ripke S et al (2010) Meta-analysis of genome-wide association studies of attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 49(9):884–897
Nose M, Barbui C, Tansella M (2003) How often do patients with psychosis fail to adhere to treatment programmes? A systematic review. Psychol Med 33(07):1149–1160
O'Rourke JA, Scharf JM, Platko J et al (2011) The familial association of tourette's disorder and ADHD: the impact of OCD symptoms. Am J Med Genet B Neuropsychiatr Genet 156B(5):553–560
Piven J, Palmer P, Jacobi D et al (1997) Broader autism phenotype: evidence from a family history study of multiple-incidence autism families. Am J Psychiatry 154:185–190
Potkin SG, Turner JA, Guffanti G et al (2009) A genome-wide association study of schizophrenia using brain activation as a quantitative phenotype. Schizophr Bull 35(1):96–108
Preskorn S, Ross R, Stanga C (2004) Selective seratonin reuptake inhibitors. In: Alarcon RD (ed) Antidepressants: past, present, and future. Springer Science+Business Media, Massachussetts, pp 241–262
Psychiatric GWAS Consortium, Ripke S, Wray NR et al (2012) A mega-analysis of genome-wide association studies for major depressive disorder. Mol Psychiatry 18(4):497–511
Purcell SM, Wray NR, Stone JL et al (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460(7256):748–752
Reiersen AM, Constantino JN, Volk HE, Todd RD (2007) Autistic traits in a population-based ADHD twin sample. J Child Psychol Psychiatry 48(5):464–472
Reiersen A, Constantino J, Grimmer M et al (2008) Evidence for shared genetic influences on self-reported ADHD and autistic symptoms in young adult Australian twins. Twin Res Human Genet 11(6):579–585
Ripke S, Sanders A, Kendler K et al (2011) Genome-wide association study identifies five new schizophrenia loci. Nat Genet 43(10):969–U77
Ronald A, Hoekstra RA (2011) Autism spectrum disorders and autistic traits: a decade of new twin studies. Am J Med Genet B Neuropsychiatr Genet 156B(3):255–274
Ronald A, Happé F, Bolton P et al (2006) Genetic heterogeneity between the three components of the autism spectrum: a twin study. J Am Acad Child Adolesc Psychiatry 45(6):691–699
Ronald A, Simonoff E, Kuntsi J et al (2008) Evidence for overlapping genetic influences on autistic and ADHD behaviours in a community twin sample. J Child Psychol Psychiatry 49(5):535–542
Ruhrmann S, Schultze-Lutter F, Salokangas RKR et al (2010) Prediction of psychosis in adolescents and young adults at high risk: results from the prospective european prediction of psychosis study. Arch Gen Psychiatry 67(3):241–251
Schuldberg D (2001) Six subclinical spectrum traits in normal creativity. Creativity Res J 13(1):5–16
Shevlin M, Murphy J, Dorahy MJ, Adamson G (2007) The distribution of positive psychosis-like symptoms in the population: a latent class analysis of the national comorbidity survey. Schizophrenia Res 89(1–3):101–109
Silventoinen K, Sammalisto S, Perola M et al (2003) Heritability of adult body height: a comparative study of twin cohorts in eight countries. Twin Res 6(5):399–408

Sklar P, Ripke S, Scott LJ et al (2011) Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat Genet 43(10):977–983
Skuse DH, Mandy WPL, Scourfield J (2005) Measuring autistic traits: heritability, reliability and validity of the social and communication disorders checklist. Br J Psychiatry 187:568–572
Smith MJ, Barch DM, Thompson PA, Scernansky JG (2008) Subclinical expression of schizphrenia-like symptoms in non-psychotic siblings of individuals with schizophrenia. Schizophr Res 103:324–325
St. Clair D, Muir W, Carothers A et al (1990) Association within a family of a balanced autosomal translocation with major mental illness. Lancet 336(8706):13–16
Stefanis NC, Hanssen M, Smirnis NK et al (2002) Evidence that three dimensions of psychosis have a distribution in the general population. Psychol Med 32(2):347–358
Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psych 60(12):1187–1192
Turic D, Swanson J (2010) DRD4 and DAT1 in ADHD: functional neurobiology to pharmacogenetics. J Pharmgenomics Pers Med 61–78
Van Grootheest DS, Cath DC, Beekman AT, Boomsma DI (2005) Twin studies on obsessive-compulsive disorder: a review. Twin Res Hum Genet 8(5):450–458
Van Grootheest DS, Cath DC, Beekman AT, Boomsma DI (2007) Genetic and environmental influences on obsessive-compulsive symptoms in adults: a population-based twin-family study. Psychol Med 37(11):1635–1644
Verdoux H, van Os J (2002) Psychotic symptoms in non-clinical populations and the continuum of psychosis. Schizophrenia Res 54(1–2):59–65
Weedon MN, Frayling TM (2008) Reaching new heights: insights into the genetics of human stature. Trends Genet 24(12):595–603
Weedon MN, Lango H, Lindgren CM et al (2008) Genome-wide association analysis identifies 20 loci that influence adult height. Nat Genet 40(5):575–583
Wijsman EM, Amos CI (1997) Genetic analysis of simulated oligogenic traits in nuclear and extended pedigrees: summary of GAW10 contributions. Genet Epidemiol 14(6):719–735
Williams JT, Begleiter H, Porjesz B et al (1999) Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. II. Alcoholism and event-related potentials. Am J Hum Gen 65(4):1148–1160
Wood AC, Asherson P, Van Der Meere JJ, Kuntsi J (2010) Separation of genetic influences on attention deficit hyperactivity disorder symptoms and reaction time performance from those on IQ. Psychol Med 40(06):1027–1037
Zimmermann P, Bruckl T, Nocon A (2009) Heterogeneity of DSM-IV major depressive disorder as a consequence of subthreshold bipolarity. Arch Gen Psychiatry 66(12):1341–1352

Chapter 6
Limitations of the EP Concept in an Idealized Gene–Phene Framework

Amy Vashlishan-Murray

Acronyms and Abbreviations

GWAs	Genome-wide association studies
SNP	Small nucleotide polymorphism
CNV	Copy number variant
fMRI	Functional magnetic resonance imaging
COMT	Catechol O-methyltransferase enzyme
5-HTT	5-hydroxytryptamine transporter
MAOA	Monoamine oxidase-A inhibitor
DRD4	Dopamine D4 receptor

This critique is launched against an idealized gene–phene framework where EPs occupy a neat intermediary position between the genome and the phenome. As a contribution to this objective, the assessment of several underlying issues that are tied to EPs may render the idealized framework unsteady to the point that genetic instructions and neatly tractable signal transmission are no longer imagined to simply drive the formation of CPs via EPs in a direct and easily dissectible manner.

A. Vashlishan-Murray (✉)
Department of Communication Sciences and Disorders, Emerson College, Boston, USA
e-mail: a_vashlishan_murray@emerson.edu

A. Vashlishan-Murray
Department of Molecular Biology, Massachusetts General Hospital, Boston, USA

A. Vashlishan-Murray
Department of Neurobiology, Harvard Medical School, Boston, USA

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_6

Here, we will consider the EP concept in relationship to the criteria that define it and to the rhetoric of its promise for isolating genetic contributors of psychiatric disease. In doing so, this chapter will first address assumptions that underlie the EP concept and the genetic studies that it generally proposes to support. Further, it will evaluate the communication of genetic associations with EPs and the implications of this communication on clinical interpretation and utility.

Many of the genotype–phenotype associations that have been published thus far may represent overestimations or may be overemphasized. There is a need to establish validity criteria and standards for all genome-wide association study (GWAS) findings and to communicate valid findings responsibly within the scientific community and beyond. As the EP concept was born out of a desire to bring a more scientific and objective dimension to classification and diagnosis of psychiatric disease, it is particularly important to not misrepresent the utility of genetic information from association studies using EPs.

6.1 Heritability: Missing and Misused

6.1.1 Missing Heritability in GWAS

Over just the past decade, genome-wide association studies have become a popular strategy for identifying potential genetic underpinnings of disease. This approach takes the advantage of the sequenced human genome and more recent knowledge of the variation that exists in this sequence among individuals. Single DNA letter variations or single nucleotide polymorphisms (SNPs) serve as representative markers for other sequence variants in nearby regions of the genome. A large collection of SNPs positioned throughout the genome is genotyped at once in a large population of individuals with and without a particular disease or trait of interest. SNPs that are found to occur more frequently in groups with the disease or trait are said to be associated with the disease. Where candidate genes were once selected to support hypotheses about genetic etiology of a disease or trait, the whole genome becomes the candidate.

While this approach has successfully led to the identification of genes involved with disease, the research community has recently witnessed "diminishing returns" (Goldstein 2009; Hirschhorn 2009; Hunter and Kraft 2007; Baker 2010). Even where statistically strong associations are detected, it is often the case that the presence of the disease-associated genetic variant accounts for a very small percentage of the variation in the trait in the population. For example, the first height-associated genetic variant, HMGA2, is reliably more prevalent among people who are labeled as tall, but it explains less than 0.3% of the genetic influence on height in these study participants (Weedon et al. 2007). Additionally, as strong as the association may be, the presence or absence of the height-associated HMGA2 variant accounts for only 0.5–1.0 cm of the differences in height in the population. This is not a SNP that is useful for predicting one's height.

The number of variants that will be needed to explain the genetic underpinnings of a complex trait or disease is unknown (Maher 2008). A subsequent study identified more than 40 genetic variants associated with height, for example, but even these many variants only collectively explained roughly 5% of height's heritability (Gudbjartsson et al. 2008; Maher 2008). This gap between the small bit of heritability genetic associations have accounted for and the total proportion of the trait's variance that is estimated to be attributable to genetic variation has been dubbed the "missing heritability" (Maher 2008; Manolio et al. 2009). Further, this gap is not an issue relevant only to select traits, but will invariably be concern for all complex phenotypes.

In response to this heritability gap between projected and actual genetic explanations for variation, the objective for GWAS has frequently been reimagined. When progress from candidate gene studies slowed, GWASs were originally conceived to identify key common variants with large effects on complex disease (Freimer and Sabatti 2007; Hardy and Singleton 2009; Hunter and Kraft 2007; Todd and Farrall 1996). When few such common, large-effect variants were identified, the hunt turned toward seeking numerous common variants of smaller effect (Maher 2008). When such small-effect common variants still did not account for a lion's share of the heritability estimate, sights were directed toward rare variants of large effect (Baker 2010; Ng et al. 2010; Nielsen 2010; The 1000 Genomes Project Consortium 2010).

Might it take thousands of weak-impact variants to explain all of the genetic contribution to variation in a trait? Might we need to employ different statistical analyses to better consider how genetic variants may impact the phenotypes synergistically (Yang et al. 2010)? Could important variants with larger impact on the phenotype of interest be missed by the "net" of GWAS because they are found so infrequently in the population? And importantly for the context of this discussion, does the EP concept help make more attainable the goal of identifying some combination of these different types of underlying genetic variants? Or, does it simply seek to shift focus to a different part of the equation in light of the diminishing returns from GWAS to date?

Implicit in the promise of the EP concept, as it is most commonly presented, is that redefining the phenotype might impact the "missing heritability" problem and make the search for genetic contributions more fruitful. If complex phenotypes are poorly defined, it will inevitably make it more difficult to find meaningful associations between these phenotypes and the genetic variants of their bearers. A strategy that employs EPs proposes to examine quantifiable features of a complex trait in lieu of the more unwieldy parent phenotype.

Distilling a relatively heterogeneous disease such as schizophrenia into more reliably quantifiable subparts initially suggests a potential inroad into facilitating genetic dissection. However, "missing heritability" is even a problem for complex traits like height that are already relatively quantifiable. Thus, even if repackaging psychiatric phenotypes with the EP concept to put them on equal footing with seemingly straightforward traits such as height may not ultimately be more productive for identifying genetic etiology. It may be that the heritability estimates

that provide the motivation for seeking genetic explanations are actually a root of the problem.

6.1.2 Assumptions About Heritability

Heritability is used to describe how much of the phenotypic variation in a population is due to genetic variation as opposed to environmental variation or variation in gene–environment interactions (Sober 2001). Heritability is given a value between 0.0 (genetic variation does not impact phenotypic variation) and 1.0 (genetic variation accounts for all of the phenotypic variation) for a particular phenotype or EP. By design, the interest in heritability studies is on describing the heritable component, while environment is typically regarded as potential confounder to be controlled, rather than as a variable to be examined. As a result, the impact of the environment on heritability estimates is often overlooked.

Environment, in fact, plays a major role in heritability estimates. Heritability is measured for a specific population, at a specific time, interacting with a specific environment (Beckwith 2008; Sober 2001). Thus, if environment changes, the heritability estimate may also change (Beckwith 2008; Schaffner 2006). Turkheimer et al., for example, have shown that IQ is highly heritable in families of high socioeconomic status, but that the same trait measured the same way exhibits near-zero heritability in families of low socioeconomic status (Turkheimer et al. 2003). In one population and environment, variation in IQ is largely attributable to genetic variation, but in a different population and low-resource environment, differences in IQ are almost entirely attributable to environment. Heritability is not a fixed entity, nor can it be extrapolated from the population, time, and environment where it was calculated and explained in another population, time, and environment.

The fact that heritability estimates are not stable over time has particular implications for its application to the EP framework. Work by Flynn et al. demonstrates that the average IQs of many populations across the globe have steadily increased over the past 50 years (Flynn 2007). This makes one question the value of aiming to identify concrete genetic influences on CPs, which may, like IQ, be highly fluid. It may be that EPs can be measured precisely, but that accuracy may not be meaningful in its application to psychiatric disease if the measurement is not temporally stable.

There is also an assumption inherent in the EP strategy that the *genetic risk* of an EP that one may uncover will be temporally stable. In addition to studies that indicate that heritability estimates are in flux, there is little support for such an assumption given what is understood about the variability of gene expression over time. There is a need to follow up association studies with rigorous molecular studies that investigate how variation in a gene or nearby regulatory region affects the function of a gene product or its expression (Green et al. 2008). Although lifetime gene expression patterns are not mapped out for the majority of genes, it is

well-established that gene expression varies with development and in response to the environment making it likely that particular genetic variants will have greater or reduced impact on phenotypic variance at different times and during different circumstances.

An additional set of concerns surrounding the heritability concept is linked the fact that populations of twins are used to generate heritability estimates. First, as discussed above, there is a reason to question the application of findings from heritability studies performed in a particular population to the population at large. When that population is a special case population like twins, even more caveats can be extracted. For example, identical twins likely experience a number of common developmental circumstances (Ainslie and O'Loughlin 1987). Thus, similarities in twins' environment may contribute to measured phenotypes in a way that would not be the case with two genetically non-identical individuals.

In the typical twin study design, heritability is calculated by comparing the phenotypic variance between pairs of identical twins (who theoretically lack genetic variance) and fraternal twins (who vary genetically). It is assumed that each pair of twins shares their environment to the same extent. Known as the "equal environment assumption," this idea permits environment to be ignored so that any differences in behavior among fraternal twin pairs that are not observed among identical twin pairs can be attributed to genetic differences.

However, behavior does not happen in a vacuum, and the interactions between environment and behavioral phenotype are not a one-way street. Behaviors occur partly as a response to the environment, but the environment itself is also shaped in response to phenotypes like appearance or behavior (Sober 2001). It follows that identical twins that share more phenotypic features might be treated more similarly than fraternal twins, potentially shaping their own behavioral responses and leading them to act more similarly. A seminal study by Ainslie et al. determined that the environments of identical twins during their first year of life were disproportionately more similar than the environments of non-identical twin pairs (Ainslie and O'Loughlin 1987). For heritability estimates to be valid, members of identical and fraternal twin pairs would need to each experience equal trait-relevant environments, but it is not clear that that is possible.

Further, it is also unclear what constitutes the "trait-relevant environment." In studies used to measure heritability, environment is considered as a sort of catchall category for everything that is not genetic. The hope is that any aspects of environment that are relevant to the trait are included, but there is no effort to define what these aspects are. Heritability studies "in principle allow one to make claims regarding the effects of both genes and environment without ever having actually measured anything about genes or the environment," notes one critique (Beckwith 2008).

There is also reason to be concerned that some relevant aspects of environment fail to be accounted for in heritability studies. *Intrauterine* environments, for example, are not factored into heritability studies but could certainly have a lifelong phenotypic impact (Maher 2008; Prescott et al. 1999). There is also evidence that environmental exposures can directly impact gene expression without

altering genetic sequence. Patterns of environmentally induced genetic modifications (termed epigenetic for "near" or "on" genetic) that are created in one generation can be passed on to influence the next (Lam et al. 2007; Waterland and Jirtle 2003). Thus, to incorporate all relevant aspects of the environment, it seems one would need to consider the environments in which parents and grandparents developed, making the issue far more complicated.

With the current approach, these epigenetic influences would be reflected in the genetic/heritable component of influence on a trait. Thus, not only is it difficult to determine the trait-relevant environment for a trait, but also it is not simple to tease apart the genetic and environmental influences. And even when one is not considering environmental influences that can be inherited in the form of epigenetic changes, the environment itself may be passed on socially, economically, and behaviorally. This is supported by studies that demonstrate social sources of shared twin behavior that were previously attributed to genetic influences (Horwitz et al. 2003a, b). As Teri Maniolo of the National Human Genome Research Institute summarizes it: "Heritability estimates are basically what clusters in families, and environment clusters in families" (Maher 2008).

6.1.3 Heritability Applied to the Endophenotype Concept

The arguments above suggest that heritability estimates that are relied upon to identify useful EPs may be incomplete or inaccurate. However, even beyond concerns over how environment is accounted for in studies to determine heritability, there are additional concerns over how heritability is understood and used in relationship to the EP concept. In summarizing the criteria for meaningful EPs, Bearden and Freimer say that EPs should be "at least moderately heritable" (Bearden and Freimer 2006). It is unclear what the requirement for "moderate" heritability means in practical terms.

The candidate EP "working memory," for example, has an estimated heritability of 43–49% (Ando et al. 2001). A heritability estimate of approximately 50% means that variation in the environment will impact variation in working memory *at least as much* as genetic contributors will, yet the authors refer to these heritability estimates as "moderately high" (Ando et al. 2001). Based on this study and these estimates, working memory later becomes interpreted as "highly heritable" elsewhere in the literature (e.g., Flint and Munafo 2007).

If a key objective of the EP strategy involves linking genotypes with their complex phenotypic manifestations, then it would only make sense to pursue EPs for which there is convincing evidence that there is a strong underlying genetic basis to the EP. Yet, there is no agreed-upon heritability threshold for making a potentially meaningful EP. Nor is there an agreed-upon standard for labeling the magnitude of heritability estimates in practical terms. Further, there is no correlation between heritability estimates and the number of genes involved in influencing differences in a trait.

Given the issues with finding genetic variation to account for heritability predictions as well as issues with generating heritability predictions in the first place, it may be wise to reconsider the emphasis on heritability as criteria central to the EP concept. As previously mentioned, this volume questions the requirement of heritability for useful intermediate phenotypes in crafting a definition of CPs that diverges from its ordinarily gene-focused origin in the EP concept. Interestingly, in other commentary, Kendler and Neale emphasize that EPs may also reflect the impact of the environment. They suggest that "researchers might be interested in finding EPs that provide more useful indexes of environmental factors" (Kendler and Neale 2010).

Despite how important the environment may be for etiological research in psychiatric disorders, the EP concept is often emphasized as a path for discovery of root causes with an eye only to the genome. In fact, as the EP concept appears to be generally defined outside of this volume, what Kendler and Neale describe above would not be considered an EP but a "biological marker," as EP is intended to specifically signify variation that has genetic underpinnings (Gottesman and Gould 2003). It is true that to support motivation for identifying genetic underpinnings, heritability is a necessary criterion for an EP. But, given the potential confounding elements of study design for arriving at heritability estimates, and the limited success GWAS has had in accounting for them, EPs may be more valuable to the field when the vision of deconstructing psychiatric diagnoses is revised to place greater value on environmental contributors. Focus on CPs or biological markers, which could be environmental or genetic in origin, would better embrace the likely contributors to psychiatric disease and leave more important windows open for exploration of causal factors.

6.2 Consideration of Genetic Complexity

6.2.1 Many Interacting Genes

The Psychiatric GWAS Consortium Steering Committee recognizes the complexity of psychiatric disease. "The genetic architecture of mental disorders is very complex and may be difficult to solve using standard GWA approaches" (2009). A key objective of the EP strategy is to simplify complex behavioral phenotypes, defining the features of psychiatric disease more precisely in order to make the phenotype more genetically tractable (Flint and Munafo 2007; Gottesman and Gould 2003; Kendler and Neale 2010). One critique of the EP concept, however, is that many EPs such as response inhibition, contingency detection, or perceptual tasks are, themselves, complex behavioral traits.

First, it is necessary to recognize that EPs are influenced by many genes (polygenicity) and that the genes that influence EPs are likely to influence many phenotypes (pleiotropy) (Green et al. 2008). Both polygenicity and pleiotropy are logical correlates of the complexity and connectivity of neural circuits and

neurotransmitter systems. Serotonergic signaling, for example, relies upon the functioning of many gene products, including those that process, package, and transport serotonin, not to mention the many gene products involved in release and reception of the neurotransmitter. Further, any single gene that affects serotonergic signaling will necessarily affect any neural circuit, brain region, or behavioral process that utilizes serotonin.

Genes that function broadly as part of neurotransmitter systems are frequently associated with psychological phenotypes. For example, genes for the 5-hydroxytryptamine transporter (5-HTT), the monoamine oxidase-A inhibitor (MAOA), and the dopamine D4 receptor (DRD4) have become favorites for asserting association with psychological phenotypes as diverse as depression, anxiety, antisocial behavior, attention-deficit hyperactivity disorder, schizophrenia, and addiction (Nordquist and Oreland 2010; Ptacek et al. 2011). However, the same reasons that make these genes interesting to cognitive neuroscientists should make us skeptical of the value and informativeness of these associations. Hans Brunner, a geneticist on the team who described an association between abnormal aggressive behavior and MAOA, later realized that "a direct causal relationship between a single gene and a specific behavior is highly unlikely" due in part to the "highly complex effects of MAOA deficiency on neurotransmitter function" (Brunner 1996). This idea is also nicely summarized in a review by Green et al. who note that "as many of the genes that are of interest to cognitive neuroscience code for elements of diffuse neuromodulatory systems, one should not expect them to be particularly limited in their functional relevance" (2008). In other words, how important is a variant in a gene that is responsible for regulating levels of all monoamine neurotransmitters throughout the brain, for example, to a specific single behavior?

An implication of the polygenicity of neuronal phenotypes is that many contributing genes may interact to affect the phenotype in non-additive ways. The phenomenon of epistasis describes such interactions, which result when gene products function in a molecular pathway. Any two genes may have a synergistic affect or may interact to have a dampening effect on a phenotype.

The barrier to dissecting the genetic contributions to complex CPs exists not just in identifying involved genes, but also in teasing apart the action of one gene upon another. However, non-additive interactions are difficult to account for as well as difficult to predict. GWAS are not well equipped for taking epistasis into account, and efforts to do so typically require hypotheses about interacting partners based on molecular data that are not ordinarily generated by genome-wide approaches.

Polygenicity itself does not preclude identification of genetic contributors to variation in psychiatric disease, but it does make the picture more complex. "To fill in all the heritability gaps" summarizes Brendan Maher in a *Nature* News Feature, "researchers may need better and more varied models of the entire network of genes and regulatory sequences, and of how they act together to produce a phenotype." EPs have the opportunity to make this complexity more tractable when they aim to contribute discrete, measurable, causally related phenotypes for psychiatric disease to aid in such modeling.

6.2.2 More Simple Genetic Architecture?

The genetic architecture that will be required to explain the heritability of the complex phenotypes (intermediate or otherwise) of cognitive neuroscience requires consideration of significantly more than SNPs. Copy number variations (CNVs), for example, are a non-SNP variant of interest thought to contribute to complex phenotypes (Maher 2008). CNVs are stretches of DNA sequence that are repeated a varying number of times in different individuals. CNVs have been associated with psychiatric disease such as schizophrenia, for which few SNP-based genetic associations have been identified (Stefansson et al. 2008; The International Schizophrenia Consortium 2008).

CNVs often occur de novo in an individual rather than clustering with family history, and they are not as well-characterized throughout the genome as SNPs. In the case of schizophrenia, studies have identified a 1.15-fold increase in genomic CNVs compared to controls, but the impact of this subtle change is unknown (The International Schizophrenia Consortium 2008). While there is no evidence that CNVs will help account for significant proportions of the heritability of mental disorders, and no reason to believe that EPs will focus in on this particular type of complexity in any specific way, they represent an aspect of complex genetic architecture that the previous GWAS would have been unable to detect.

Given the assortment of genetic complexity from which EPs are not likely immune, it may be naive to think that it will be any easier to account for the genetic contributions of EPs that represent complex neurological processes and behaviors than it would be to do so for psychiatric disease itself. This concern is addressed in an analysis by Flint and Munafo where the authors calculate "genetic effect sizes" of variants associated with psychiatric disorders and with candidate EPs (Flint and Munafo 2007). Where genetic loci are found to be associated with psychiatric disorders or EPs, they seek to establish how big a contribution the genetic variant has on variance in the phenotype. Their meta-study approach directly tests the assumption that EPs will identify more simple underlying genetic architecture with larger effects.

Meta-study analysis of an association between a variant in the catechol O-methyltransferase enzyme (COMT) with schizophrenia estimated that the variant accounted for less than 0.2% of the phenotypic variation (and did not, in fact, support the association in a statistically significant way) (Flint and Munafo 2007). COMT variant association with working-memory EP measures such as the Wisconsin Card Sorting Task and the N-back task each accounted for less than 0.5% of the phenotypic variance. Even utilizing the more physiological P300 event-related potential EP did not generate a larger effect size, the COMT variant, in this case, accounting for less than 0.1% of the phenotypic variance. The authors conclude that EPs are not likely to be "any easier to dissect at a genetic level than the disorders to which they are related."

6.3 Reliability, Validity, and Utility in Concept and Communication

6.3.1 Reliable Measurements

The potential utility of the EP concept in psychiatry is highly dependent upon the selection of EPs that are reliably quantifiable and that predictably act as precursors to or components of psychiatric disease. Cognitive-neurogenetic studies are only as good as their ability to validly and specifically measure mental phenotypes (Green et al. 2008). However, the reliability of measurement for some EPs as well as the validity in using them as a proxy for particular psychiatric disease is not given.

Schizophrenia has been a poster-child of sorts for promoting the need for an EP approach. It is a psychiatric disease that varies in severity among individuals and is defined as a collection of symptoms subject to clinical interpretation. The spectrum nature of schizophrenia can lead it to be defined differently between or even within studies, which can underlie some of the difficulty in replicating prospective genetic associations (Gunter 2008). This makes the idea of a quantifiable intermediate EP attractive.

However, not all potential intermediate traits that can be measured can be measured reliably. For example, there are several critiques of imaging-based definition of phenotypes. Functional magnetic resonance imaging (fMRI), which reveals local blood oxygenation in the brain in real time, has been used to define potential intermediates between genes and psychiatric disease. For over a decade, researchers have used fMRI to attempt to link candidate gene findings to objective measures of psychiatric diseases such as schizophrenia, depression, and autism (Chi 2009).

A major critique of fMRI is that it is not consistently interpreted. One three-dimensional fRMI image, reports David Goldstein of the Duke Institute for Genome Sciences and Policy, can contain more than 50,000 picture elements of data (Chi 2009). The implication is that these data can be interpreted in multiple ways, leading to inconsistencies in how phenotypes are defined which may be no different from the inconsistencies in how the parent disease phenotype is defined. Additionally, analyses that examine the stability of such imaging phenotypes over time have found high variability (Kendler and Neale 2010).

There is also concern over interpreting the validity of EPs that rely upon imaging such as fMRI in relationship to psychiatric disease. Activity in particular regions of the brain, such as the amygdala, is involved in a host of neural processes and mental states. Thus, is it not possible to infer particular mental states or complex disease states from altered activity in various regions of the brain (Miller 2008). Despite making a biological link between a genetic variant and psychiatric disease, fMRI-based phenotypes may actually reveal little about the neural mechanisms of human cognition (Miller 2008).

In one study, a variant of ZNF804A, a gene of unknown function found to be associated with schizophrenia via GWAS, was correlated with alterations in correlated activity between the dorsolateral prefrontal cortexes and with the hippocampus (Esslinger et al. 2009). While this demonstrates a functional correlate of a genetic variation and is viewed as a validation of the EP strategy in psychiatry (Esslinger et al. 2009), others have questioned the ability of fMRI to accurately measure functional connectivity with data that average neural activity over windows almost as long as a second (Cela-Conde et al. 2009). While other neuroimaging techniques such as EEG, discussed in a chapter of this volume, improve this temporal resolution, there is still a risk of explanative "storytelling" to tie genes or psychiatric diseases to data that may be subjectively interpreted.

6.3.2 Defining and Communicating Endophenotypes

As emphasized by the examination of fMRI above, it is a key that measurement of EPs be more reliable than measurement of psychiatric disease itself (Kendler and Neale 2010). However, many traits that are considered by some to be EPs seem to be as difficult to define and interpret as many psychiatric diseases. In a review of the endophenotype concept, Flint and Munafo (Flint and Munafo 2007) list "personality" as an EP measure for anxiety, depression, and schizophrenia.

Reliably describing personality does not seem more promising or less unwieldy than doing so for these types of spectrum psychiatric diseases. Even where personality is parsed out into dimensional traits such as neuroticism which can be measured with tools such as Eysenck's scale (Smoller and Tsuang 1998; Willis-Owen et al. 2005), the test–retest correlations for such metrics are not remarkable ($r = 0.70$) (Kendler and Neale 2010). While measurement reliability in this case is greater than that for interview-based assessment of the psychiatric disease major depression ($r = 0.43$) (Foley et al. 1998; Kendler and Neale 2010), it is unclear whether this is a big enough step in the right direction to support the notion that personality, as an EP, is closer to the biological basis of psychiatric disease.

Further, there seems to be little evidence thus far that "EPs," like the genetics of personality, will help unravel the genetic basis of psychiatric disease. A 2010 GWAS of Cloninger's temperament scales revealed no genetic variants that significantly contributed to personality variation (Verweij et al. 2010). The power and size of this study suggest that variants that explain 1% of the variation in personality or greater do not contribute to personality trait variation. Other GWAS for neuroticism (Shifman et al. 2008) and the Big Five traits (Terracciano et al. 2010) identified only associations with small effect sizes, many of which failed subsequent replication (Verweij et al. 2010).

This brings to light a more general need to consider, and standardize, communication around the EP concept (Gottesman and Gould 2003). This is, in fact, a need which the publication of this volume on CPs appears to identify and address.

Without consensus around the definition of and expectations for an EP, the line between symptom and functional EP can be blurred. EPs seek to be causally linked to genotypes with the expectation that, through the EP, genotypes will be causally linked to a heterogeneous psychiatric disorder of interest. However, there seem to be no clear guiding standards to help one distinguish whether a particular intermediate phenotype of interest is involved in the cause of psychiatric disease or is evidence/effect of that disease.

For example, a table of EP measures in a review of the EP concept (Flint and Munafo 2007) lists several putative EPs, particularly many of those it categorizes as "psychological," which seem to simply be descriptive of psychiatric disease itself. "Perception of affect" and "subthreshold mood lability" seem to be more accurately labeled diagnostics or symptoms of anxiety disorder and bipolar disorder, respectively, than trait intermediates between genes and disease. Similarly, the developmental EP "age at first word" seems to one of the diagnostic criteria for autism rather than a phenotype associated with causes of the condition.

It is necessary to make a careful distinction between trait and state markers (Fridhandler 1986) in discussion of putative EPs. EPs would presumably be trait markers in that they would help to indicate disease risk and play roles in eventual development of psychiatric disease. By contrast, state markers would be diagnostic indicators of existing disease. Although the distinction between trait and state seems subtle, only the former supports the aim of the EP concept to help unravel the path of disease etiology.

Kendler and Neale argue that the field has paid insufficient attention to potential causal claims surrounding EPs. An EP's association with causes rather than effects of a disorder may be requisite for delivery on the promise of providing insight into disease etiology (Bearden and Freimer 2006). Most risk indicators utilized for psychiatric disease operate under a "liability index model" where genetic variance may separately influence the risk indicator/EP and the psychiatric disease (Kendler and Neale 2010). Such pathways are minimally informative as they do not contain falsifiable causal claims (Kendler and Neale 2010). Only when a risk indicator is related to genetic contributors and a psychiatric disease as an intermediate in a linear path (mediational model), can it be tested for causal connections with psychiatric disease phenotypes and truly be designated an EP (Kendler and Neale 2010; Walters and Owen 2007).

The distinction made between the liability index and mediational model for an endophenotype's relationship to genetic influences and psychiatric disease is an important one, as many EP models have incorporated unrealistic assumptions (Kendler and Neale 2010). It is important to recognize that all genetic influences on EPs will not necessarily affect psychiatric disease and that all genetic influences on psychiatric disease will not necessarily be via an EP. Importantly, environmental risk factors for psychiatric disease will likely impact EPs and will need to be considered (Kendler and Neale 2010).

Consideration of the relationship of EPs to psychiatric disease and to genetic variation raises a critical question about the practical impact that EPs could have on deconstructing complex psychiatric phenotypes. There are two associations that

must exist for an EP to be informative. The EP must be associated with particular elements of genetic variation. The EP must also be associated with a psychiatric disease. Given that an association indicates only an increased frequency of a variation in a population compared to another, can a causal link made through two moderate associations be (clinically) meaningful?

A SNP may be associated with an EP in a statically significant way but may be relatively uncommon in a population. Even if this association had a large effect size (accounting for a large percentage of the variation in EP), the genetic variation may only be present in, say, 10% of those with the EP. If that endophenotype was also state independent (as the proposed criteria for the EP concept (Gottesman and Gould 2003) prescribe), it would be associated with a risk for a particular psychiatric disorder but not be disease-specific. The endophenotype might only be found in 10% of people with the psychiatric disorder. In this hypothetical example, for every 100 individuals with the genetic variation of interest, only 10 would have the EP and only 1 of those would complete that causal pathway and present with disease. This would not be helpful predicatively or diagnostically. A checklist of at least several EPs would be required for effective diagnosis of heterogeneous disease. This would seem to be a little-improved replacement for the DSM, which currently operates largely in the same fashion using clusters of diagnostic criteria.

6.3.3 Validity Standards and Communicating Associations

In addition to discussion of the need for standards for defining EPs, there is an equally urgent need to develop standards for genetic studies for which these EPs may be used (Bearden and Freimer 2006). Indeed, there has been a notable problem with replicating findings from GWAS in general (Chanock et al. 2007; Trikalinos et al. 2004; Wacholder et al. 2004), and several of the arguments raised in this chapter suggest that these problems may persist even when EPs are employed.

EPs are not immune from a need for consensus regarding validity of genetic associations. In the face of replication issues, it is evident that a high level of stringency is necessary to weed out spurious candidate genes (Green et al. 2008). However, there is no field-wide standard for study size, statistical significance, effect size, or number of required independent replications for a genetic association with a phenotype (EP or otherwise) to be considered genuine.

In fact, gene–phenotype associations tend to be overestimated in the literature. Green et al. attribute this, in part, to publication biases and pos hoc studies of subgroups (Green et al. 2008). Publication bias results when negative results or failed replication attempts remain unpublished either as a result of hesitance to submit or the unwillingness of journals to publish such results (Ioannidis 2006). Similar considerations regarding publication foster the trend of post hoc data analysis where subgroups of the original sample are evaluated in order to achieve statistical significance (Ioannidis 1998).

Additionally, overestimations of genotype–phenotype associations are fueled by the overall philosophy of the EP strategy, which embraces the assumption that the genetic effects for brain-based EPs are greater than for other phenotypes. In conjunction with the requirement of some EPs for specialized experimental equipment, such as fMRI, this may lead the field to inappropriately tolerate smaller sample sizes and fewer replication studies, which increases the risk of false positives (Green et al. 2008).

In the broader field of genetics, there has been a trend toward marketing the information from genetic associations, however prematurely, to consumers in the form of genetic tests. The marketers of these genetic either openly or implicitly misrepresent the utility of genetic associations by offering tests for genetic associations that have been identified in small studies and for which replication has often either not been attempted or has failed (Vashlishan Murray et al. 2010). In some cases, companies even rate the tests "for" various phenotypes with a star-rating system that suggests value and promotes the tests in a way that distorts the process by which scientific conclusions become believable (Vashlishan Murray et al. 2010).

Cognitive and behavioral phenotypes are among those marketed in the direct-to-consumer genetic testing industry. Tests are offered for variants that allegedly impact intelligence, memory, "avoidance of errors," and eating behavior. Each of these four genetic tests is based on genetic associations identified in studies with less than 1000 people, which have not been replicated. The test for "avoidance of errors" screens for a genetic variant of the dopamine receptor DRD2. This variant was associated with outcomes in a feedback-based learning task that was measured in a neuroimaging study of only 26 individuals (Klein et al. 2007). While there are no immediate plans to bring findings from CP studies to market in a targeted fashion, some proponents of pharmacogenomics favor the marketing of initial positive findings over the wisdom of caution and adoption of rigorous validity and utility standards (Colburn 2003).

The variants screened for in each of these genetic tests are also not expected to have a large effect size or impact on phenotype outcome. Yet in each case, they are the only variants tested in association with a trait, which seems to suggest that they are the only variants that matter. Failure to recognize the complex genetic architecture of CP is a problematic trend when genetic associations are communicated in the public arena. These variants contribute (sometimes very minimally) to variation in a phenotype or EP in the population. They are not the "genes for" particular psychological functions. It is important that as genetic data from studies based on CPs is gathered, genetic associations be communicated without suggesting genetic determinism.

6.4 Conclusion

The central idea behind the EP concept is that "with recent advances in molecular genetics, the rate-limiting step in identifying susceptibility genes for psychiatric disorders has become phenotype definition" (Smoller and Tsuang 1998). This

chapter argues, however, that phenotype definition is just one of several rate-limiting steps. The rationale behind the EP concept is a logical one. The problem is that in the context of the GWAS landscape, it shifts the blame for missing heritability once again.

As this chapter has posited, heritability itself is a problematic construct in how it is measured, communicated, and applied to the EP concept. Chief among these problems is the way environment is considered, or fails to be considered. Along with relative disinterest in the important role of the environment in shaping complex psychiatric disease, there are problematic assumptions about temporal phenotypic stability and simplified underlying genetic architecture that shape the EP concept. Lastly, this chapter points to a need for monitoring the reliability, validity, utility, and communication of the EP concept and describes the issues with these same components in the genetic association studies that the EP concept promotes.

It is notable that even the relevant genetic variation contributing to a highly heritable and readily quantifiable phenotype such as height has thus far eluded researchers using the current GWAS paradigm. Given the limitations and complexities of untangling the genetic architecture of complex disease, the most common vision for the EP concept exists as part of an idealized, and possibly untenable, gene–phene framework. Recent commentary has suggested that having one's sights trained toward extracting genetic causation—or in fact specific causation of any kind—may be misguided.

> …individual differences in complex human characteristics do not, in general, have causes, neither genetic nor environmental. Complex human behavior emerges out of a hyper-complex developmental network into which individual genes and individual environmental events are inputs. The systematic causal effects of any of those inputs are lost in the developmental complexity of the network. Causal explanations of complex differences among humans are therefore not going to be found in individual genes or environments any more than explanations of plate tectonics can be found in the chemical composition of individual rocks (Turkheimer 2011).

Seeking a genetic basis of complex psychiatric diseases may try to put something in a box that cannot be boxed. This suggests a problem with the objective of the EP concept more than with the technical embodiment of the strategy. The use of EPs has great potential to make the diagnosis of complex psychiatric disease more consistent and the biological study of complex psychiatric disease more approachable. However, it may not be reasonable to expect that this deconstruction will yield *genetic* insight. The true value of the EP concept lies in deconstructing a complex disease phenotype into measurable and manageable subfeatures that can be useful for diagnosis, rather than for identifying genetic correlates of disease.

References

Ainslie RCOK, O'Loughlin DD (1987) The early developmental context of twinship: some limitations of the equal environments hypothesis. Am J Orthopsychiatry 57(1):120–124

Ando J, Ono Y, Wright MJ (2001) Genetic structure of spatial and verbal working memory. Behav Gen 31(6):615–624
Baker M (2010) Genomics: the search for association. Nature 467(28):1135–1148
Bearden CE, Freimer NB (2006) EPs for psychiatric disorders: ready for primetime? Trends Gen 22(6):306–313
Beckwith JMC (2008) Twin studies of political behavior: untenable assumptions? Perspect Politics 6(4):785–791
Brunner H (1996) MAOA deficiency and abnormal behaviour: perspectives on an association. Ciba Found Symp 194:155–164, 164–157
Cela-Conde CJ, Maestu F, Mirasso C et al (2009) Can fMRI alone identify functional connectivity in the brain? Science 324:eLetter
Chanock SJ, Manolio T, Boehnke M et al (2007) Replicating genotype-phenotype associations. Nature 447(7145):655–660
Chi R (2009) Human genetics: hit or miss? Nature 461:712–714
Colburn WA (2003) Biomarkers in drug discovery and development: from target identification through drug marketing. J Clin Pharmacol 43(4):329–341
Esslinger C, Henrik W, Kirsch P et al (2009) Neural mechanisms of a genome-wide supported psychosis variant. Science 324(5927):605
Flint J, Munafo MR (2007) The EP concept in psychiatric genetics. Psychol Med 37(2):163–180
Flynn JR (2007) What is intelligence?. Cambridge University Press, Cambridge
Foley DL, Neale MC, Kendler KS (1998) Reliability of a lifetime history of major depression: implications for heritability and co-morbidity. Psychol Med 28(4):857–870
Freimer NB, Sabatti C (2007) Human genetics: variants in common diseases. Nature 445:828–830
Fridhandler BM (1986) Conceptual note on state, trait, and the state-trait distinction. J Pers Soc Psychol 50:169–174
Goldstein DB (2009) Common genetic variation and human traits. N Eng J Med 360(17):1696–1698
Gottesman II, Gould TD (2003) The EP concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160(4):636–645
Green AE, Munafo MR, DeYoung CG (2008) Using genetic data in cognitive neuroscience; from growing pains to genuine insights. Nat Rev Neurosci 9:710–720
Gudbjartsson DF, Walters GB, Thorleifsson G et al (2008) Many sequence variants affecting diversity of adult human height. Nat Genet 40(5):609–615
Gunter C (2008) Human disease: chipping away at psychiatric disorders. Nat Rev Gen 9(9):654
Hardy J, Singleton A (2009) Genomewide association studies and human disease. N Eng J Med 360(17):1759–1768
Hirschhorn J (2009) Genomewide association studies-illuminating biologic pathways. N Eng J Med 360(17):1699–1701
Horwitz AV, Videon TM, Schmitz MF et al (2003a) Double vision: reply to Freese and Powell. J Health Soc Behav 44(2):136–141
Horwitz AV, Videon TM, Schmitz MF et al (2003b) Rethinking twins and environments: possible social sources for assumed genetic influences in twin research. J Health Soc Behav 44(2):111–129
Hunter DJ, Kraft P (2007) Drinking from the fire hose-statistical issues in genomewide association studies. N Eng J Med 357(5):436–439
Ioannidis J (1998) Effect of the statistical significance of results on the time to completion and publication of randomized efficacy trials. JAMA 279:281–286
Ioannidis J (2006) Journals should publish all “null” results and should sparingly publish “positive” results. Cancer Epidemiol Biomarkers Prev 15:186
Kendler KS, Neale MC (2010) EP: a conceptual analysis. Mol Psychiatry 15(8):789–797
Klein TA, Neumann J, Reuter M et al (2007) Genetically determined differences in learning from errors. Science 318(5856):1642–1645

Lam MY, Heaney JD, Youngren KK et al (2007) Trans-generational epistasis between Dnd1Ter and other modifier genes controls susceptibility to testicular germ cell tumors. Hum Mol Gen 16(18):2233–2240
Maher B (2008) Personal genomes: the case of the missing heritability. Nature 456(7218):18–21
Manolio TA, Collins FS, Cox NJ et al (2009) Finding the missing heritability of complex diseases. Nature 461(7265):747–753
Miller G (2008) Neuroimaging. Growing pains for fMRI. Science 320(5882):1412–1414
Ng SB, Buckingham KJ, Lee C et al (2010) Exome sequencing identifies the cause of a mendelian disorder. Nat Genet 42(1):30–35
Nielsen R (2010) Genomics: in search of rare human variants. Nature 467(7319):1050–1051
Nordquist N, Oreland L (2010) Serotonin, genetic variability, behaviour, and psychiatric disorders—a review. Ups J Med Sci 115(1):2–10
Prescott CA, Johnson RC, McArdle JJ (1999) Chorion type as a possible influence on the results and interpretation of twin study data. Twin Res 2(4):244–249
Ptacek R, Kuzelova H, Stefano GB (2011) Dopamine D4 receptor gene DRD4 and its association with psychiatric disorders. Med Sci Monit 17 (9):RA215–220
Schaffner KF (2006) Behavior: Its nature and nurture. In: Parens E, Chapman A, Press N (eds) Wrestling with behavioral genetics: science, ethics, and public conversation. Johns Hopkins University Press, Baltimore
Shifman S, Bhomra A, Smiley S et al (2008) A whole genome association study of neuroticism using DNA pooling Mol Psychiatry 13(3):302–312
Smoller JW, Tsuang MT (1998) Panic and phobic anxiety: defining phenotypes for genetic studies. Am J Psychiatry 155(9):1152–1162
Sober E (2001) Separating nature and nurture. In: Wachbroit DWAR (ed) Genetics and criminal behavior. Cambridge University Press, Cambridge
Stefansson H, Rujescu D, Cichon S et al (2008) Large recurrent microdeletions associated with schizophrenia. Nature 455(7210):232–236
Terracciano A, Sanna S, Uda M et al (2010) Genome-wide association scan for five major dimensions of personality. Mol Psychiatry 15(6):647–656
The 1000 Genomes Project Consortium (2010) A map of human genome variation from population-scale sequencing. Nature 467(7319):1061–1073
The International Schizophrenia Consortium (2008) Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature 455:237–241
Todd JA, Farrall M (1996) Panning for gold: genome-wide scanning for linkage in type 1 diabetes. Hum Mol Genet 5(1):1443–1448
Trikalinos T, Ntzani EE, Contopoulos-Ioannidis DG, Ioannidis JP (2004) Establishment of genetic associations for complex diseases is independent of early study findings. Eur J Hum Gen 12(9):762–769
Turkheimer E (2011) Commentary: variation and causation in the environment and genome. Int J Epidemiol 40(3):598–601
Turkheimer E, Haley A, Waldron M et al (2003) Socioeconomic status modified heritability of IQ in young children. Psychol Sci 14(6):623–628
Vashlishan Murray AB, Carson MJ, Morris CA et al (2010) Illusions of scientific legitimacy: misrepresented science in the direct-to-consumer genetic-testing marketplace. Trends genet: TIG 26(11):459–461
Verweij KJ, Zietsch BP, Medland SE et al (2010) A genome-wide association study of Cloninger's temperament scales: implications for the evolutionary genetics of personality. Biol Psychol 85(2):306–317
Wacholder S, Chanock S, Garcia-Closas M et al (2004) Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 96(6):434–442
Walters JT, Owen MJ (2007) EPs in psychiatric genetics. Mol Psychiatry 12(10):886–890

Waterland RA, Jirtle RL (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23(15):5293–5300

Weedon MN, Lettre G, Freathy RM et al (2007) A common variant of HMGA2 is associated with adult and childhood height in the general population. Nat Genet 39(10):1245–1250

Willis-Owen SA, Turri MG, Munafo MR et al (2005) The serotonin transporter length polymorphism, neuroticism, and depression: a comprehensive assessment of association. Biol Psychiatry 58(6):451–456

Yang J, Benyamin B, McEvoy BP et al (2010) Common SNPs explain a large proportion of the heritability for human height. Nat Genet 42(7):565–569

Part III
Cognitive and Neural Systems

Chapter 7
Response Inhibition

Kei Mochizuki and Shintaro Funahashi

7.1 Response Inhibition: Definition and Overview

7.1.1 Response Inhibition and the Prefrontal Cortex

Response inhibition is formulated as a process to suppress the latent motor options to correctly perform the selected action. Without this controlling process, it would be impossible to perform the intended action because of the interference by other unintended behaviors. Thus, response inhibition can be considered to be one of the most important parts of executive control, and it has been thought to be related to the function of the prefrontal cortex (PFC).

A deficit in response inhibition can result in a variety of behavioral disorders. Abnormal activation of the response inhibition process may cause difficulty in motor initiation such as motor apraxia and motor neglect after prefrontal damage (Knight 1984). Hyperactive response inhibition may also be related to motor impersistence (Kertesz et al. 1985). On the other hand, insufficient inhibitory control could cause the environmental dependency syndrome (Hoffmann and Bill 1992) such as activation of the grasp reflex (Renzi and Barbieri 1992) and the compulsive manipulation of tools and imitation behavior (Lhermitte et al. 1986; Feinberg et al. 1992; Renzi et al. 1996), which often result from prefrontal

K. Mochizuki
Department of Physiology, Faculty of Medicine, Kindai University, Osaka, Japan
e-mail: keimochizuki@med.kindai.ac.jp

S. Funahashi (✉)
Kokoro Research Center, Kyoto University, Kyoto, Japan
e-mail: funahashi.shintaro.35e@st.kyoto-u.ac.jp

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_7

and anterior callosal lesion. Motor perseveration in prefrontal patients (Sandson and Albert 1984; Goldberg 1986) may also be related to the deficit in inhibitory control.

These examples clearly demonstrate that the PFC plays an essential role in response inhibition. The importance of the PFC is also apparent in the inhibitory control of higher cognitive functions such as attention. For example, patients with damage in the prefrontal and other frontal areas show deficits in the Stroop test (Stroop 1935). In the Stroop test, participants have to inhibit the natural tendency to read written words and instead state the colors of the ink with which the words are written. However, patients with frontal damage often fail to overcome the interference. Severe Stroop interference in frontal patients has been repeatedly reported in several studies (Perret 1974; Vendrell et al. 1995). The Wisconsin card sorting test (Berg 1948; Milner 1963) is another example of neuropsychological assessment that is used to test the ability of inhibitory control on attention and response set. While prefrontal patients often acquire the initial categorization rule, they fail to learn a new rule by inhibiting the old one (Milner 1963). Response inhibition is important even in tasks with quite different structures, such as the Iowa gambling task (Bechara et al. 1994; Damasio 1996), which primarily focuses on the subject's ability to learn reward contingency following emotional events.

Table 7.1 summarizes examples of neuropsychological tests of prefrontal function with response inhibition components. The general presence of response inhibition components in multiple prefrontal tests suggests the importance of the PFC in this cognitive function.

7.1.2 Go/No-Go Task

The neuropsychological tasks shown in Table 7.1 are designed to test various aspects of subject's cognitive ability. Therefore, more simple tasks are suitable for assessing the subject's capacity for response inhibition per se, especially in animal studies.

The go/no-go task is one of the most widely used tasks for assessing the ability of response inhibition (Fig. 7.1a). In this task, the subject is first instructed to associate stimuli with a go or no-go response. Either go or no-go stimulus is randomly presented in each trial. The subject's task is to respond to the go stimulus (e.g., press a key) as fast as possible while ignoring the no-go stimulus, to which they must not respond. The ability of response inhibitory control can be assessed by checking the proportion of correct nonaction in no-go trials.

This go/no-go paradigm is actually a direct experimental implementation of a bedside assessment for prefrontal patients (e.g., tapping a desk following the tester's tap while ignoring the tester's double-tap) (Drewe 1975a, b; Stuss and Benson 1984). Due to its simple task structure, the go/no-go task has been widely used in numbers of experimental studies in both humans (Simmonds et al. 2008; Chikazoe 2010) and nonhuman animals (Watanabe 1986a, b; Eagle et al. 2008).

Table 7.1 Examples of prefrontal tests and their inhibitory task component

Name	Outline of the test	Required inhibitory ability	Representative studies
Fist-palm-edge test	Repeat tapping of the fist, palm or edge of the hand onto the desk, alternately	Inhibit the tendency to keep tapping with the same hand shape	Luria (1966)
"to lose" rock-paper-scissors test	Display the appropriate rock, paper, or scissors gesture following the tester to lose, instead of to win	Suppress the natural trait to display the gesture to win	Kashima and Kato (1993), Matsubara et al. (2004)
Wisconsin card sorting test	Sort cards with different colors, shapes, and numbers of marks into four decks to identify unknown sorting rules	Inhibit sorting by a previously appropriate rule	Berg (1948), Milner (1963)
Stroop test	State the color of the ink of written words, which are the names of different colors	Ignore the meaning of the word to correctly state the color of the ink	Stroop (1935)
Trail making test (part B)	Connect numbers and letters alternately in numerical and alphabetical order	Inhibit the tendency to keep connecting symbols of the same type (numbers or letters)	Reitan (1955)
Tower of Hanoi test	Move the tower to the other end piece by piece	Suppress moving pieces directory to the other end to attain the final goal	Gagné and Smith (1962), Stuss and Benson (1984)
Iowa gambling task	Repeatedly draw cards from four decks with different overall payoffs, searching for the advantageous selection strategy	Inhibit the desire to keep choosing seemingly profitable decks that also provide sudden large losses, ultimately causing total failure	Bechara et al. (1994), Damasio (1996)

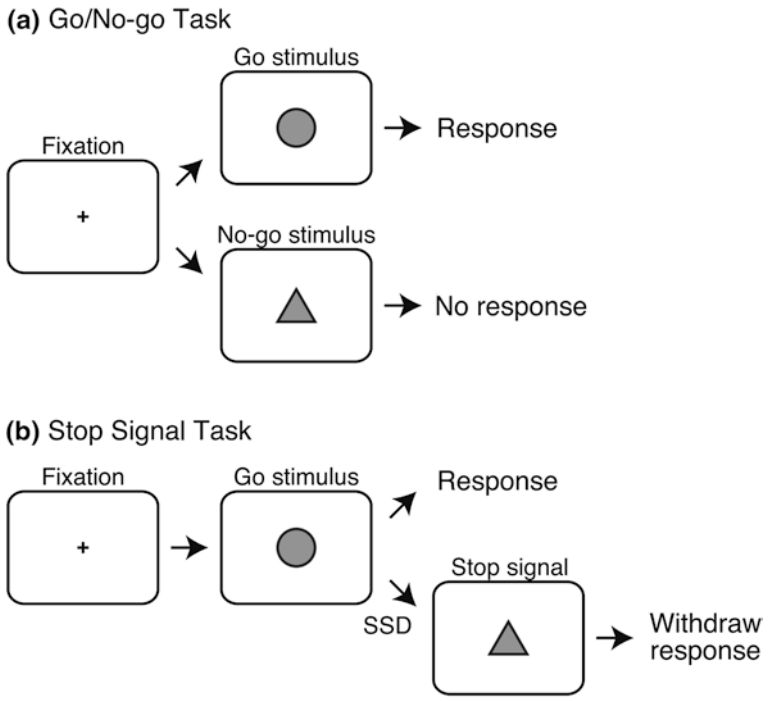

Fig. 7.1 Go/no-go task and stop signal task **a** Go/no-go task. After the fixation cue, either a "go" or "no-go" stimulus is presented. The subject has to make a response (e.g., press a key) to the go stimulus as quickly as possible, while suppressing the response for the no-go stimulus. **b** Stop signal task. In every trial, the "go" stimulus is presented, requiring the subject to respond to it. However, in some trials, a "stop" signal is presented after a short interval (SSD). In these trials, the subject must withdraw the ongoing motor execution

However, in the go/no-go task, the only observable measure of behavior related to response inhibition is the percentage of correct nonactions in no-go trials, and subtle changes in behavior caused by psychiatric disorders or experimental manipulation (e.g., modulation of neurotransmitter release by pharmacological treatment) are sometimes not correctly captured. Therefore, some other methods that can be used to capture the subject's ability of inhibiting response is needed.

7.1.3 Stop Signal Task

The stop signal task (Logan and Cowan 1984) is another popular task that is used in many studies (Fig. 7.1b). In this task, the subject is first presented with a go stimulus after an unpredictable delay. The subject has to respond to this stimulus

as quickly as possible. However, in some trials, a stop signal is presented briefly after the go stimulus (typically around a hundred milliseconds). In these trials, the subject has to withdraw the response they were preparing.

The interval between the go stimulus and the stop signal is called the stop signal delay (SSD), and trials with different SSD lengths are randomly intermingled in the task. It becomes more difficult to withdraw the ongoing response as the SSD gets longer (i.e., when the instruction to stop is given just before the subject is about to respond). From the proportion of correct withdrawal in trials with different SSDs, the length of the time needed to stop a response can be estimated. This imaginary "response time" for the stopping process to take place is called the stop signal reaction time (SSRT).

Based on the elaborate task procedure and mathematical formulation (Logan and Cowan 1984), the stop signal task and the SSRT enable the experimenter to assess the subject's ability to inhibit the response. The SSRT has been widely used in many studies (Band and van Boxtel 1999; Verbruggen and Logan 2008) and has been shown to be useful for assessing the subject's ability of response inhibition.

7.2 Cognitive and Neural Mechanisms of Response Inhibition

7.2.1 Human Neuroimaging and Neuropsychology

Early neuroimaging studies used tasks with inhibitory control components, such as go/no-go task, and implicated the involvement of the right PFC in response inhibition (Konishi et al. 1998, 1999; Garavan et al. 1999, 2002; Duncan and Owen 2000; Liddle et al. 2001; Menon et al. 2001; Durston et al. 2002; Bunge et al. 2002). Recent studies using the stop signal task have also confirmed the importance of the right PFC, especially the right inferior frontal gyrus (rIFG), in response inhibition (Rubia et al. 2003; Aron and Poldrack 2006; Sharp et al. 2010; Tabu et al. 2011). For example, Aron and Poldrack (2006) reported the activation of the rIFG in stop trials. Furthermore, stop-related activation of the rIFG was greater in subjects with shorter SSRT (i.e., those who were more proficient at inhibiting response). These results clearly indicate the important role of rIFG in response inhibition.

The importance of the rIFG during the stop signal task has also been demonstrated in neuropsychological studies (Aron et al. 2003; Hodgson et al. 2007). Patients with rIFG damage exhibited longer SSRT compared to the control, and the length of the SSRT was correlated with the volume of the damage in this brain area. Similar results were obtained with the virtual-lesion procedure by transcranial magnetic stimulation applied at the right PFC (Chambers et al. 2006).

However, some recent studies have reported that the pre-supplementary motor area (pre-SMA) and adjacent higher motor cortices also play important roles in

response inhibition (Li et al. 2006; Sharp et al. 2010; Huster et al. 2011; Tabu et al. 2011). These studies used a variation of the stop signal task to examine the neural activity related to response inhibition separately from other cognitive processes such as attention and set shifting. Another candidate structure is the basal ganglia, based on the concept of motor execution by direct and indirect pathways. These two pathways are thought to compete with each other to generate appropriate response while suppressing other unnecessary motor commands. Several studies have reported the involvement of basal ganglial nuclei during the stop signal task (Vink et al. 2005; Aron and Poldrack 2006; Chevrier et al. 2007; Li et al. 2008; Huster et al. 2011). Nevertheless, the relationship between the rIFG and other cortical and subcortical structures in the performance of the stop signal task is under debate and still requires detailed examination in future studies.

7.2.2 Animal Neurophysiology

The cognitive function required in the stop signal task has been described with the use of the race model (Logan and Cowan 1984; Band et al. 2003). In this model, the mental processes of "going" and "stopping" are postulated to be two different accumulators. These accumulators independently store the signals (or "urges") for going and stopping over time, and race to reach the threshold of activation. The accumulator that reaches the threshold first determines the animal's behavior (i.e., going or stopping). This psychological accumulation process is likely to be maintained by building-up neural activities, which are often observed in neurons in many cortical areas and resemble the signal-accumulation process predicted in the race model. Therefore, by searching for building-up neural activity during the stop signal task, we can explore the candidate of neural underpinning of response inhibition.

Indeed, this race model has successfully illustrated the neuronal mechanism of cognitive functions that require conflict resolution, such as perceptual judgment (Gold and Shadlen 2007). However, the movement preparation and control required in the stop signal task are generally fast (typically less than 500 ms). Therefore, neuroimaging studies are unsuitable for this investigation, due to the limitation in temporal resolution. Electrophysiological studies using animal subjects are necessary to understand the neuronal mechanism of response inhibition.

Based on the long history of research on oculomotor control by the prefrontal and related motor cortices, electrophysiological studies of response inhibition in monkeys have been performed using the stop signal task with eye movement. This saccadic version of the stop signal task is often called the countermanding task (Hanes and Schall 1995; Hanes et al. 1998), although the task structure is identical to that in the manual version of the stop signal task. With the use of the countermanding task, the activity of neurons has been recorded in several cortical and subcortical regions related to oculomotor control.

Neurons in the frontal eye field (FEF) have been shown to increase their firing rate before the initiation of the eye movement in visually guided saccade tasks (Bruce and Goldberg 1985; Schall et al. 1995; Hanes and Schall 1996). These neurons are called "movement cell" and thought to trigger eye movement. Hanes et al. (1998) reported that, although building-up activity was usually observed in movement cells before the initiation of a saccade, the magnitude of the building-up activity of movement cells drastically decreased when the monkey successfully inhibited the saccadic response in stop signal trials (Fig. 7.2a). Importantly, the magnitude of building-up activity differentiated slightly, but significantly, prior to the passage of the SSRT from the presentation of the stop signal. Since the SSRT is the psychologically estimated reaction time that is required to inhibit a response, neuronal activity causal to the inhibition process must be evident before the passage of the SSRT. Differential activity observed after the SSRT has elapsed should only be the reflection of the result of the competition between the "going" and "stopping" processes, which is downstream of response inhibition per se. In this sense, the activity of movement cells in the FEF could be appreciated as a neuronal candidate to inhibit a saccade in the countermanding task.

The FEF has also been known to contain another type of neurons called "fixation cell". These neurons exhibit sustained firing during fixation and cease shortly before and during saccade execution. In the stop signal task, these neurons exhibit strong transient excitation in successful stop trials (Fig. 7.2b). Difference in the

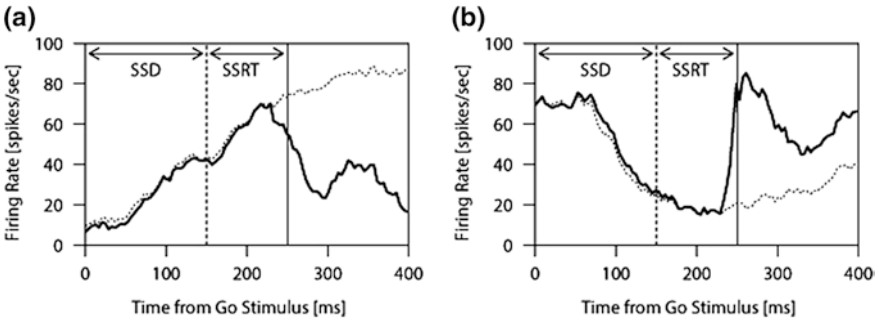

Fig. 7.2 Schematic illustrations of example activities of movement and fixation cells in the FEF. **a** Activity of the movement cell. Movement cells exhibit building-up activity after the presentation of the go stimulus in go trials (*dotted*), while the activity drastically decreases in stop trials (*solid*) in which the response was correctly withdrawn according to the stop signal. Activity is aligned to the time of the presentation of the go stimulus. *Dashed* and *solid vertical lines* indicate the time of SSD (time at which the stop signal was presented in stop trials) and SSD + SSRT (time after the passage of SSRT from the presentation of the stop signal), respectively. Note that only the change in activity before the *solid vertical line* can influence the response inhibition process. **b** Activity of the fixation cell. Fixation cells exhibit a transient decrease in spontaneous activity before and during a saccade, but quickly restart to fire in stop trials

firing rate between stop trials and go trials was again observed before the passage of the SSRT from the presentation of the stop signal (Hanes et al. 1998).

In summary, movement cells in the FEF stopped firing in successful stop trials, while fixation cells were transiently activated. The difference in neuronal activity between go and stop trials was evident before the passage of the SSRT, which was the psychologically estimated time required to complete inhibition. Therefore, the generation of eye movement could be controlled by the competition between the activity of movement cells and fixation cells in the FEF, and this competition process itself could be the neuronal underpinning of response inhibition.

Neuronal activity in other motor areas such as the supplementary eye field (SEF) and the anterior cingulate cortex (ACC) has also been examined. However, unlike the FEF, only a few neurons in the SEF exhibited differential activity between go and stop trials before the SSRT had passed (Stuphorn et al. 2000). Instead, many neurons in the SEF and ACC showed differential activation related to the errors (e.g., increased activity in stop trials with an erroneous response compared to correct go trials) and the difficulty of the task (i.e., length of the SSD) (Ito et al. 2003). Based on these activity patterns, the SEF and ACC were likely to be involved in error monitoring and conflict resolution (Stuphorn and Schall 2006). Although error monitoring and conflict resolution are also important components of motor control, these results indicate that the FEF plays a unique role in response inhibition.

7.3 Response Inhibition as a Behavioral Phenotype

7.3.1 Response Inhibition in People with ADHD

One example of the psychiatric disorders that can be assessed in terms of response inhibition is attention deficit/hyperactivity disorder (ADHD). The primary symptoms of ADHD are characterized by the difficulty in sustaining attention and hyperactive-impulsive behavior (American Psychiatric Association 2000; Pelham et al. 2005). These behavioral characteristics are likely to be related to the malfunction of the response inhibition process. In addition, this malfunction is thought to be caused by the impairment of the PFC (Madras et al. 2005; Makris et al. 2009; Shaw and Rabin 2009).

Numbers of studies have investigated the ability of response inhibition in individuals with ADHD using the stop signal task. For example, Schachar and Logan (1990) examined the SSRT of children with and without ADHD and found that the ADHD group exhibited longer (i.e., worse) SSRT compared to the age-matched control group. This result was replicated in later studies in both children (Oosterlaan et al. 1998) and adults (Bekker et al. 2005) with ADHD. Electroencephalographic recording revealed abnormal event-related potentials on frontal areas in subjects with ADHD during the performance of the stop signal task (Liotti et al. 2007). The impaired ability of response inhibition in children

with ADHD was improved by the treatment with methylphenidate (De Vito et al. 2009). These studies suggest the impaired response inhibition ability in individuals with ADHD, and this is presumably linked to the abnormal prefrontal functioning.

Furthermore, converging evidence from recent studies has suggested that the SSRT is related to genetic factors, which is essential as a behavioral phenotype. For example, studies have shown a correlation of the SSRT in twins (Schachar et al. 2011) and siblings (Rommelse et al. 2008), which suggests the influence of familial factors in the ability of response inhibition. Bidwell et al. (2007) also reported longer SSRTs in the unaffected co-twins of ADHD children, although the subclinical ADHD symptoms were controlled. While the SSRT usually improves in adolescent development in normal children, the effect of age on the improvement of SSRT was not significant in children with ADHD (Gupta and Kar 2009).

These studies indicate that the behavioral measures from the stop signal task can be used to quantitatively evaluate the subject's trend for ADHD.

7.3.2 Problems with Response Inhibition as a Behavioral Phenotype

Even though it can be used to reveal cognitive impairment in people with ADHD, the validity of response inhibition as a behavioral phenotype of ADHD is still under debate.

One of the problems regarding its validity is related to recent advances in our understanding of subtypes of ADHD. In the current diagnostic criteria, ADHD is divided into three major subtypes: predominantly hyperactive-impulsive, predominantly inattentive, and combined (American Psychiatric Association 2000). Studies of genetic contributions to ADHD have revealed a wide variety of candidate genes for ADHD (Faraone et al. 2005; Khan and Faraone 2006), which implies that, from a genetic perspective, there are multiple possible causes of ADHD. Although the number of studies which examined each ADHD subtype separately has been increasing (Nigg 2001; Nigg et al. 2002; Weiss et al. 2003; Geurts et al. 2005), differences among ADHD subtypes in the ability of the response inhibition still need to be carefully examined.

Another problem arises from the stop signal task itself. The stop signal task has a very simple task structure, and the SSRT is based on a firm mathematical framework. However, recent studies in cognitive neuroscience have reported the possible confounding effects of other cognitive processes that are necessary in the stop signal task (Tabu et al. 2011). For example, some of the recent studies have suggested that the prolonged SSRT in subjects with ADHD is caused by a decrease in the speed of information processing (Lijffijt et al. 2005; Alderson et al. 2007, 2008) and is not the result of the impairment of inhibitory control. Thus, the ability of response inhibition in people with ADHD may need to be re-examined using more sophisticated methods than the original stop signal paradigm.

Furthermore, some researchers have pointed out the diversity of the inhibition process (Duque et al. 2012; Majid et al. 2012). For example, an essential cognitive process in the stop signal task is to overcome ongoing motor execution, which was initiated by the previously presented go stimulus (Fig. 7.1b). On the other hand, in the go/no-go task, although the subject needs to exhibit response inhibition, only the no-go stimulus is presented in no-go trials. Since the go stimulus is not presented in no-go trials, motor execution is not initiated in these trials (Fig. 7.1a). Therefore, the response inhibition needed in the go/no-go task is simply not to initiate the prepared motor action. These inhibitory processes necessary in the two tasks might be supported by completely different neural circuitries. Some discrepancies in the ability of the response inhibition in people with ADHD, as well as their underlying neural mechanisms, might be the result of this difference of the task structures. Since recent studies have reported deficit in multiple types of inhibitory function in people with ADHD (Schachar et al. 2007), further examinations on this issue are necessary.

Also, despite the well-established mathematical formulation, the race model may still need to be re-examined. Psychological and neuronal models for the stop signal task hypothesize direct competition between accumulators for the going response and stopping response. However, recent studies have proposed a distinction between inaction (simply doing nothing) and intentional nonaction (Karch et al. 2009; Kühn et al. 2009a, b, 2010). These studies suggested that nonaction is represented as one of the possible "actions" in the brain. Still, whether nonaction can be classified as one kind of actions or not, and whether nonaction is equivalent to other motor actions in terms of the motor hierarchy, have to be carefully tested in future studies.

Finally, the difference of the effector used in human and animal studies could be a matter of concern. The neuronal mechanism of response inhibition has been studied mainly using saccadic eye movement in animals, while hand or arm movements are used with human subjects. Thus, it is still unclear how neuronal activity observed in animal electrophysiology corresponds to the prefrontal activation observed in human neuroimaging studies. Notably, the FEF and the SEF, the cortical areas that have been extensively examined in electrophysiological studies on response inhibition, are known to be the motor centers specialized for the control of eye movements. Therefore, the importance of these regions in the inhibitory control of manual responses is not clear. Careful examination regarding how the PFC participates in response inhibition and what neuronal mechanisms are responsible for response inhibition are needed.

7.4 Conclusion

Several lines of evidence suggest that the PFC plays an important role in response inhibition. These include results obtained from studies on human brain imaging and neuropsychology using the stop signal paradigm as well as studies on animal neurophysiology using the saccadic countermanding task. Impairment of this

ability has been thought to underlie certain types of psychiatric disorders such as ADHD. However, the effectiveness of the measures of response inhibition as a behavioral phenotype of ADHD is being reconsidered. Future studies must investigate the ability of response inhibition in both normal subjects and people with ADHD, using more elaborate tasks to reveal the genuine contribution of this cognitive function to psychiatric symptoms. At the same time, more detailed examinations of ADHD subtypes as well as their genetic and neuronal bases are also necessary. Close interactions between cognitive neuroscience and clinical psychiatry is necessary for obtaining a better understanding of the neural basis of ADHD and for the development of practical methods for clinically evaluating the ability of response inhibition.

References

Alderson RM, Rapport MD, Kofler MJ (2007) Attention-deficit/hyperactivity disorder and behavioral inhibition: a meta-analytic review of the stop-signal paradigm. J Abnorm Child Psychol 35(5):745–758

Alderson RM, Rapport MD, Sarver DE, Kofler MJ (2008) ADHD and behavioral inhibition: a re-examination of the stop-signal task. J Abnorm Child Psychol 36(7):989–998

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders DSM-IV-TR fourth edition (text revision). Am Psychiatric Pub, Washington DC

Aron AR, Fletcher PC, Bullmore ET et al (2003) Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. Nat Neurosci 6(2):115–116

Aron AR, Poldrack RA (2006) Cortical and subcortical contributions to stop signal response inhibition: role of the subthalamic nucleus. J Neurosci 26(9):2424–2433

Band GP, van Boxtel GJ (1999) Inhibitory motor control in stop paradigms: review and reinterpretation of neural mechanisms. Acta Psychol 101(2–3):179–211

Band GPH, van der Molen MW, Logan GD (2003) Horse-race model simulations of the stop-signal procedure. Acta Psychol 112(2):105–142

Barkley RA (1997) Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychol Bull 121(1):65–94

Bechara A, Damasio AR, Damasio H, Anderson SW (1994) Insensitivity to future consequences following damage to human prefrontal cortex. Cognition 50(1–3):7–15

Bekker EM, Overtoom CC, Kenemans JL et al (2005) Stopping and changing in adults with ADHD. Psychol Med 35(6):807–816

Berg EA (1948) A simple objective technique for measuring flexibility in thinking. J Gen Psychol 39(1):15–22

Bidwell LC, Willcutt EG, Defries JC, Pennington BF (2007) Testing for neuropsychological endophenotypes in siblings discordant for attention-deficit/hyperactivity disorder. Biol Psychiatry 62(9):991–998

Bruce CJ, Goldberg ME (1985) Primate frontal eye fields. I. Single neurons discharging before saccades. J Neurophysiol 53(3):603–635

Bunge SA, Dudukovic NM, Thomason ME et al (2002) Immature frontal lobe contributions to cognitive control in children: evidence from fMRI. Neuron 33(2):301–311

Chambers CD, Bellgrove MA, Stokes MG et al (2006) Executive "brake failure" following deactivation of human frontal lobe. J Cogn Neurosci 18(3):444–455

Chevrier AD, Noseworthy MD, Schachar R (2007) Dissociation of response inhibition and performance monitoring in the stop signal task using event-related fMRI. Hum Brain Mapp 28(12):1347–1358

Chikazoe J (2010) Localizing performance of go/no-go tasks to prefrontal cortical subregions. Curr Opin Psychiatry 23(3):267–272
Damasio AR (1996) The somatic marker hypothesis and the possible functions of the prefrontal cortex. Philos Trans R Soc Lond B Biol Sci 351(1346):1413–1420
De Vito EE, Blackwell AD, Clark L et al (2009) Methylphenidate improves response inhibition but not reflection-impulsivity in children with attention deficit hyperactivity disorder (ADHD). Psychopharmacology 202(1–3):531–539
Drewe EA (1975a) An experimental investigation of Luria's theory on the effects of frontal lobe lesions in man. Neuropsychologia 13(4):421–429
Drewe EA (1975b) Go-no go learning after frontal lobe lesions in humans. Cortex 11(1):8–16
Duncan J, Owen AM (2000) Common regions of the human frontal lobe recruited by diverse cognitive demands. Trends Neurosci 23(10):475–483
Duque J, Labruna L, Verset S et al (2012) Dissociating the role of prefrontal and premotor cortices in controlling inhibitory mechanisms during motor preparation. J Neurosci 32(3):806–816
Durston S, Thomas KM, Worden MS et al (2002) The effect of preceding context on inhibition: an event-related fMRI study. Neuroimage 16(2):449–453
Eagle DM, Bari A, Robbins TW (2008) The neuropsychopharmacology of action inhibition: cross-species translation of the stop-signal and go/no-go tasks. Psychopharmacology 199(3):439–456
Faraone SV, Perlis RH, Doyle AE et al (2005) Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry 57(11):1313–1323
Feinberg TE, Schindler RJ, Flanagan NG, Haber LD (1992) Two alien hand syndromes. Neurology 42(1):19–24
Gagné RM, Smith EC (1962) A study of the effects of verbalization on problem solving. J Exp Psychol 63:12–18
Garavan H, Ross TJ, Murphy K et al (2002) Dissociable executive functions in the dynamic control of behavior: inhibition, error detection, and correction. Neuroimage 17(4):1820–1829
Garavan H, Ross TJ, Stein EA (1999) Right hemispheric dominance of inhibitory control: an event-related functional MRI study. Proc Natl Acad Sci U S A 96(14):8301–8306
Geurts HM, Vert S, Oosterlaan J et al (2005) ADHD subtypes: do they differ in their executive functioning profile? Arch Clin Neuropsychol 20(4):457–477
Gold JI, Shadlen MN (2007) The neural basis of decision making. Annu Rev Neurosci 30:535–574
Goldberg E (1986) Varieties of perseveration: a comparison of two taxonomies. J Clin Exp Neuropsychol 8(6):710–726
Gupta R, Kar BR (2009) Development of attentional processes in ADHD and normal children. Prog Brain Res 176:259–276
Hanes DP, Schall JD (1995) Countermanding saccades in macaque. Vis Neurosci 12(5):929–937
Hanes DP, Schall JD (1996) Neural control of voluntary movement initiation. Science 274(5286):427–430
Hanes DP, Patterson WF, Schall JD (1998) Role of frontal eye fields in countermanding saccades: visual, movement, and fixation activity. J Neurophysiol 79(2):817–834
Hodgson T, Chamberlain M, Parris B (2007) The role of the ventrolateral frontal cortex in inhibitory oculomotor control. Brain 130(Pt 6):1525–1537
Hoffmann MW, Bill PL (1992) The environmental dependency syndrome, imitation behaviour and utilisation behaviour as presenting symptoms of bilateral frontal lobe infarction due to moyamoya disease. S Afr Med J 81(5):271–273
Huster RJ, Eichele T, Enriquez-Geppert S et al (2011) Multimodal imaging of functional networks and event-related potentials in performance monitoring. Neuroimage 56(3):1588–1597
Ito S, Stuphorn V, Brown JW (2003) Performance monitoring by the anterior cingulate cortex during saccade countermanding. Science 302(5642):120–122

Karch S, Mulert C, Thalmeier T et al (2009) The free choice whether or not to respond after stimulus presentation. Hum Brain Mapp 30(9):2971–2985
Kashima H, Kato M (1993) Tests for frontal function-pattern of frontal dysfunction and its assessment. Shinkei Kenkyu no Shinpo 37:93–110
Kertesz A, Nicholson I, Cancelliere A et al (1985) Motor impersistence: a right-hemisphere syndrome. Neurology 35(5):662–666
Khan SA, Faraone SV (2006) The genetics of ADHD: a literature review of 2005. Curr Psychiatry Rep 8(5):393–397
Knight RT (1984) Decreased response to novel stimuli after prefrontal lesions in man. Electroencephalogr Clin Neurophysiol 59(1):9–20
Konishi S, Nakajima K, Uchida I et al (1998) No-go dominant brain activity in human inferior prefrontal cortex revealed by functional magnetic resonance imaging. Eur J Neurosci 10(3):1209–1213
Konishi S, Nakajima K, Uchida I et al (1999) Common inhibitory mechanism in human inferior prefrontal cortex revealed by event-related functional MRI. Brain 122(Pt 5):981–991
Kühn S, Bodammer NC, Brass M (2010) Dissociating mental states related to doing nothing by means of fMRI pattern classification. Neuroimage 53(4):1294–1300
Kühn S, Elsner B, Prinz W, Brass M (2009a) Busy doing nothing: evidence for nonaction–effect binding. Psychon Bull Rev 16(3):542–549
Kühn S, Gevers W, Brass M (2009b) The neural correlates of intending not to do something. J Neurophysiol 101(4):1913–1920
Lhermitte F, Pillon B, Serdaru M (1986) Human autonomy and the frontal lobes. Part I: Imitation and utilization behavior: a neuropsychological study of 75 patients. Ann Neurol 19(4):326–334
Li CSR, Huang C, Constable RT, Sinha R (2006) Imaging response inhibition in a stop-signal task: neural correlates independent of signal monitoring and post-response processing. J Neurosci 26(1):186–192
Li CSR, Yan P, Sinha R, Lee TW (2008) Subcortical processes of motor response inhibition during a stop signal task. Neuroimage 41(4):1352–1363
Liddle PF, Kiehl KA, Smith AM (2001) Event-related fMRI study of response inhibition. Hum Brain Mapp 12(2):100–109
Lijffijt M, Kenemans JL, Verbaten MN, van Engeland H (2005) A meta-analytic review of stopping performance in attention-deficit/hyperactivity disorder: deficient inhibitory motor control? J Abnorm Psychol 114(2):216–222
Liotti M, Pliszka SR, Perez R et al (2007) Electrophysiological correlates of response inhibition in children and adolescents with ADHD: influence of gender, age, and previous treatment history. Psychophysiology 44(6):936–948
Logan GD, Cowan WB (1984) On the ability to inhibit thought and action: a theory of an act of control. Psychol Rev 91(3):295–327
Luria AR (1966) Higher cortical functions in man. Basic Books, New York
Madras BK, Miller GM, Fischman AJ (2005) The dopamine transporter and attention-deficit/hyperactivity disorder. Biol Psychiatry 57(11):1397–1409
Majid DSA, Cai W, George JS et al (2012) Transcranial magnetic stimulation reveals dissociable mechanisms for global versus selective corticomotor suppression underlying the stopping of action. Cereb Cortex 22(2):363–371
Makris N, Biederman J, Monuteaux MC, Seidman LJ (2009) Towards conceptualizing a neural systems-based anatomy of attention-deficit/hyperactivity disorder. Dev Neurosci 31(1–2):36–49
Matsubara M, Yamaguchi S, Xu J, Kobayashi S (2004) Neural correlates for the suppression of habitual behavior: a functional MRI study. J Cogn Neurosci 16(6):944–954
Menon V, Adleman NE, White CD et al (2001) Error-related brain activation during a go/nogo response inhibition task. Hum Brain Mapp 12(3):131–143

Milner B (1963) Effects of different brain lesions on card sorting: The role of the frontal lobes. Arch Neurol 9(1):90–100
Nigg JT (2001) Is ADHD a disinhibitory disorder? Psychol Bull 127(5):571–598
Nigg JT, Blaskey LG, Huang-Pollock CL, Rappley MD (2002) Neuropsychological executive functions and DSM-IV ADHD subtypes. J Am Acad Child Adolesc Psychiatry 41(1):59–66
Oosterlaan J, Logan GD, Sergeant JA (1998) Response inhibition in AD/HD, CD, comorbid AD/HD + CD, anxious, and control children: a meta-analysis of studies with the stop task. J Child Psychol Psychiatry 39(3):411–425
Pelham WE, Fabiano GA, Massetti GM (2005) Evidence-based assessment of attention deficit hyperactivity disorder in children and adolescents. J Clin Child Adolesc Psychol 34(3):449–476
Perret E (1974) The left frontal lobe of man and the suppression of habitual responses in verbal categorical behaviour. Neuropsychologia 12(3):323–330
Reitan RM (1955) The relation of the trail making test to organic brain damage. J Consult Psychol 19(5):393–394
Renzi ED, Barbieri C (1992) The incidence of the grasp reflex following hemispheric lesion and its relation to frontal damage. Brain 115(Pt1):293–313
Renzi ED, Cavalleri F, Facchini S (1996) Imitation and utilisation behaviour. J Neurol Neurosurg Psychiatry 61(4):396–400
Rommelse NNJ, Altink ME, Oosterlaan J et al (2008) Support for an independent familial segregation of executive and intelligence endophenotypes in ADHD families. Psychol Med 38(11):1595–1606
Rubia K, Smith AB, Brammer MJ, Taylor E (2003) Right inferior prefrontal cortex mediates response inhibition while mesial prefrontal cortex is responsible for error detection. Neuroimage 20(1):351–358
Sandson J, Albert ML (1984) Varieties of perseveration. Neuropsychologia 22(6):715–732
Schachar R, Logan GD (1990) Impulsivity and inhibitory control in normal development and childhood psychopathology. Dev Psychol 26(5):710–720
Schachar R, Logan GD, Robaey P, Chen S, Ickowicz A, Barr C (2007) Restraint and cancellation: multiple inhibition deficits in attention deficit hyperactivity disorder. J Abnorm Child Psychol 35(2):229–238
Schachar RJ, Forget-Dubois N, Dionne G et al (2011) Heritability of response inhibition in children. J Int Neuropsychol Soc 17(2):238–247
Schall JD, Hanes DP, Thompson KG, King DJ (1995) Saccade target selection in frontal eye field of macaque. I. Visual and premovement activation. J Neurosci 15(10):6905–6918
Sharp DJ, Bonnelle V, Boissezon XD et al (2010) Distinct frontal systems for response inhibition, attentional capture, and error processing. Proc Natl Acad Sci U S A 107(13):6106–6111
Shaw P, Rabin C (2009) New insights into attention-deficit/hyperactivity disorder using structural neuroimaging. Curr Psychiatry Rep 11(5):393–398
Simmonds DJ, Pekar JJ, Mostofsky SH (2008) Meta-analysis of go/no-go tasks demonstrating that fMRI activation associated with response inhibition is task-dependent. Neuropsychologia 46(1):224–232
Stroop JR (1935) Studies of interference in serial verbal reactions. J Exp Psychol 18(6):643–662
Stuphorn V, Schall JD (2006) Executive control of countermanding saccades by the supplementary eye field. Nat Neurosci 9(7):925–931
Stuphorn V, Taylor TL, Schall JD (2000) Performance monitoring by the supplementary eye field. Nature 408(6814):857–860
Stuss DT, Benson DF (1984) Neuropsychological studies of the frontal lobes. Psychol Bull 95(1):3–28
Tabu H, Mima T, Aso T et al (2011) Functional relevance of pre-supplementary motor areas for the choice to stop during stop signal task. Neurosci Res 70(3):277–284
Vendrell P, Junqu C, Pujol J et al (1995) The role of prefrontal regions in the Stroop task. Neuropsychologia 33(3):341–352

Verbruggen F, Logan GD (2008) Response inhibition in the stop-signal paradigm. Trends Cogn Sci 12(11):418–424
Vink M, Kahn RS, Raemaekers M et al (2005) Function of striatum beyond inhibition and execution of motor responses. Hum Brain Mapp 25(3):336–344
Watanabe M (1986a) Prefrontal unit activity during delayed conditional go/no-go discrimination in the monkey. I. Relation to the stimulus. Brain Res 382(1):1–14
Watanabe M (1986b) Prefrontal unit activity during delayed conditional go/no-go discrimination in the monkey. II. Relation to go and no-go responses. Brain Res 382(1):15–27
Weiss M, Worling D, Wasdell M (2003) A chart review study of the inattentive and combined types of ADHD. J Atten Disord 7(1):1–9

Chapter 8
Fear Conditioning and Extinction

Bronwyn M. Graham and Mohammed R. Milad

List of Abbreviations

CS	Conditioned stimulus, an initially neutral stimulus that is paired with an aversive stimulus during fear conditioning;
US	Unconditioned stimulus, an innately aversive stimulus that is paired with the to-be-conditioned stimulus during fear conditioning;
CR	Conditioned response, a species-specific defensive reaction induced by the non-reinforced presentation of a conditioned stimulus;
SCR	Skin conductance response, a psychophysiological index of arousal in humans;
BLA	Basolateral nucleus of the amygdala;
CEA	Central nucleus of the amygdala;
DH	Dorsal hippocampus;
vmPFC	Ventromedial prefrontal cortex;
PL	Prelimbic division of the ventromedial prefrontal cortex;
IL	Infralimbic division of the ventromedial prefrontal cortex;
BDNF	Brain-derived neurotrophic factor;
dAC	Dorsal anterior cingulate;
PTSD	Post-traumatic stress disorder, an anxiety disorder that affects 15–20% of people exposed to a traumatic event;
EMG	Electromyography, the measure of electrical activity produced by skeletal muscles; an index of startle in humans.

B.M. Graham (✉)
School of Psychology, University of New South Wales, Sydney, Australia
e-mail: bgraham@psy.unsw.edu.au

M.R. Milad
Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, USA
e-mail: milad@nmr.mgh.harvard.edu

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_8

8.1 Fear Conditioning: Definition and Overview

8.1.1 Acquisition of Fear: Cued and Contextual Fear Conditioning

Pavlovian fear conditioning is a commonly used laboratory procedure in both non-human animals and humans (Milad et al. 2006). Typically in this procedure, an initially neutral to-be-conditioned stimulus (CS; e.g., a light or a tone) is paired with an aversive unconditioned stimulus (US; e.g., a mild electrical shock). After several pairings, the subject starts to exhibit conditioned fear responses (CRs) to presentations of the CS itself, having learnt that the CS predicts the US. This type of fear conditioning is known as "cued conditioning"; however, fear conditioning can also occur to distinct environments. This type of conditioning, known as "contextual fear conditioning," involves the presentation of an unsignaled US in a specific context. Fear conditioning is a robust phenomenon in the laboratory in human subjects (Hofmann et al. 2010), who can be conditioned to fear ecologically relevant stimuli (such as images of negative or fearful faces, and innately fear-provoking animals) or completely neutral stimuli (e.g., shapes, neutral images).

Fear conditioning produces both overlapping and species-specific fear responses in non-human animals and humans. In rodents, conditioned freezing (defined as the absence of all movements except that used for respiration; Fanselow 1980), fear-potentiated startle responses (defined as an increase in the reflexive startle response that occurs in the presence of the CS versus a neutral stimulus; Davis 1990), and conditioned suppression of feeding (where food intake decreases in the presence of the CS; Bodnoff et al. 1988) are among the most commonly used measures of fear (see Cryan and Holmes 2005, for review). In humans, skin-conductance responses (SCRs) and fear-potentiated startle responses are the most commonly used psychophysiological measures of fear (Milad et al. 2006). Fear conditioning has been viewed as a valid model of the symptoms of anxiety, as it induces similar fear responses to those seen in humans with anxiety disorders (Cryan and Holmes 2005).

8.1.2 Inhibition of Fear: Extinction

Once conditioned, fear responses to both a conditioned context and a discrete CS can be reduced via a procedure known as extinction training. During such training, the subject is repeatedly exposed to the feared CS in the absence of any reinforcement. After several presentations, fear responses gradually decline as the subject learns that the stimulus no longer predicts the aversive outcome. The diminution of fear responses during extinction training is known as "within-session extinction training," or "extinction learning" (Myers and Davis 2007). At a later time point (usually the next day), subjects can also be tested for long-term maintenance

of extinction, known as "extinction recall" or "extinction retention" (Graham and Milad 2011). Good extinction recall is indexed by low levels of conditioned responding, whereas poor extinction recall is indexed by recovered conditioned fear responses. Exposure therapy, which is a commonly used and empirically validated treatment for anxiety disorders, is based on the extinction procedure (Foa 2011; Wolpe 1954). During exposure therapy, the individual is exposed to fear-eliciting cues, situations, and outcomes, in the absence of any danger, which challenges unrealistic cognitions about the probability and actual cost of negative events, and ultimately results in a reduction in anxiety (Otto et al. 2004).

8.2 Cognitive and Neural Mechanisms of Fear Conditioning and Extinction

8.2.1 Cognitive and Behavioral Theories of Conditioning and Extinction

In addition to its utility in modeling the symptoms of anxiety, fear conditioning is just as often used as a task with which to examine the cognitive, behavioral, and neurobiological mechanisms behind memory formation. Cued fear conditioning is most commonly conceptualized as involving the formation of an associative memory, dependent on an understanding of the temporal relationship between the CS and US (Maren 2001). Contextual fear conditioning also relies on the formation of an association between the context and the US; however, it is different from cued conditioning in the sense that a context does not provide temporal information regarding the onset of the US and requires the integration of information from multiple senses (e.g., hearing, sight, smell) to form a contextual representation (Maren et al. 1998; Rudy et al. 2004). In this way, cued and contextual fear conditioning rely on somewhat different neurobiological mechanisms (see below).

Although extinction causes reductions in conditioned responding, it is a process distinct from forgetting as it depends on the animal being presented the non-reinforced cue. If the animal receives no such presentations, its fear for the cue will remain across weeks, and even years (Gale et al. 2004). Fear extinction was originally thought to reflect unlearning of the fear conditioning memory (Rescorla and Wagner 1972). However, several lines of evidence have led to the now commonly accepted view that, like fear conditioning, fear extinction also appears to depend on the formation of a new extinction memory that coexists with the original fear memory (reviewed in Myers and Davis 2007; Quirk and Mueller 2008). The main evidence that the fear conditioning memory still exists following extinction is that several manipulations have been shown to lead to recovery of fear responses. For example, fear responses often recover when the subject presented an extinguished cue in a different context to that in which extinction training took place, a phenomenon known as "renewal" (Bouton and Bolles 1979a). In addition,

fear responses often recover following the occurrence of a mildly stressful event, such as an unsignaled footshock, known as "reinstatement" (Bouton and Bolles 1979b). Finally, fear responses also have been shown to recover with increasing intervals between extinction training and test, known as "spontaneous recovery" (Bouton 1993). Together, these findings have prompted the theory that extinction involves the formation of a new memory that is contextually gated (Bouton 2002). According to this account, during extinction, the subject learns that the CS no longer predicts the US in that specific context. Therefore, when the cue is presented in a context other than that in which it was extinguished, fear of the cue returns. The change in the context that precipitates relapse may be the physical environment in which the extinguished cue is presented, as occurs in renewal, or it may reflect changes in the temporal context as occurs in spontaneous recovery (Bouton 1993). Finally, it may reflect changes in the affective value of the extinction context itself, as in the case of reinstatement, where an unsignaled stressor only elicits recovered fear responses when it occurs in the same context as extinction (Bouton and Bolles 1979b).

Although it is well accepted that fear extinction at least partly occurs via new learning, more recently some researchers have proposed that extinction may also lead to partial erasure of the original fear conditioning memory. For one thing, relapse following fear extinction is rarely complete—subsequent to renewal, reinstatement, and spontaneous recovery procedures, subjects typically express a level of fear that is less than that expressed following fear conditioning (Delamater 2004). For another, the mechanisms underlying fear extinction appear to change across development. In contrast to adult rodents, young rodents exhibit a relapse-resistant form of extinction that does not depend on the same neural or molecular substrates of extinction that occurs during adulthood, and some have suggested that extinction during early development involves fear erasure (Gogolla et al. 2009; Kim and Richardson 2010). It is therefore possible that extinction in adulthood retains some of the qualities of extinction during development, but that the relative contribution of the mechanisms switches such that new learning is now dominant. Indeed, neurobiological evidence supports the notion that extinction during adulthood reverses some of the changes caused by fear conditioning, in line with an erasure hypothesis (see below). These findings have led some researchers to propose "hybrid" models of extinction, which purport that extinction results from multiple mechanisms, most likely a combination of erasure and new learning (Quirk et al. 2010).

8.2.2 Neurobiological Models of Conditioning and Extinction

The neurobiological mechanisms by which fear is acquired have been extensively studied in the rodent. Such research has revealed that following the processing of sensory information about the CS and the US by the thalamus, the basolateral

nucleus of the amygdala (BLA) converges this information to produce a specific representation of the CS–US association (LeDoux 2007; Orsini and Maren 2012). The expression of fear responses depends on BLA activation of the central nucleus of the amygdala (CEA), which in turn activates downstream structures involved in species-specific defensive responses (e.g., the periaqueductal gray). Disruption of BLA functioning through lesions, inactivation, or administration of drug antagonists have all been shown to cause specific impairments in fear conditioning (see review by Maren and Quirk 2004). Although originally thought to be primarily involved in the expression of fear, more recent evidence has suggested that the CEA is also involved in the acquisition of fear memories, as functional inactivation of the CEA prior to fear conditioning disrupts the formation of such memories (Ciocchi et al. 2010; Wilensky et al. 2006).

Contextual fear conditioning, like cued conditioning, also depends on the amygdala (Goosens and Maren 2001). In addition, the hippocampus appears to have a specific role in contextual, but not cued, fear conditioning. Lesions to the dorsal hippocampus immediately after cued conditioning spared memory for the cue, but impaired memory for the context in which the cued conditioning took place (Anagnostaras et al. 1999). This suggests that the hippocampus is necessary for conditioning to diffuse, but not discrete, stimuli. On the basis of these and other findings, it has been suggested that the hippocampus is responsible for integrating the various sensory information about the context into one unified representation, which is then converged with the US representation in the BLA (Matus-Amat et al. 2004).

There is also evidence that the prelimbic (PL) division of the ventromedial prefrontal cortex (vmPFC) regulates amygdala activation during recall of fear conditioning. Expression of both contextual and cued fear conditioning is disrupted following PL inactivation (Laurent and Westbrook 2009; Sierra-Mercado et al. 2011), and microstimulation of PL increases conditioned fear responses and hinders extinction (Corcoran and Quirk 2007; Vidal-Gonzalez et al. 2006). In addition, freezing responses to a conditioned tone during conditioning and extinction training are positively correlated with tone responses in the PL, and persistent tone responses in the PL during recall are associated with failure to extinguish conditioned freezing (Burglos-Robles et al. 2009). Finally, disrupted consolidation of cued fear has also been reported to occur in mice with virally mediated brain-derived neurotrophic factor (BDNF) gene deletion in the PL. Viral-infected mice exhibit normal acquisition and expression of fear during conditioning, but impaired recall when tested one day later, suggesting that in addition to regulating the expression of learned fear, BDNF activity in the PL may also mediate its consolidation (Choi et al. 2010).

Rodent studies have also established that similar to fear conditioning, fear extinction involves interactions between the vmPFC and limbic structures. Specifically, it is purported that during extinction consolidation and recall the infralimbic (IL) region of the vmPFC inhibits conditioned responding by activating the inhibitory interneurons of the BLA, which in turn prevent activation of the output neurons of the CEA, thus preventing downstream activation of specific

fear responses (see Quirk and Mueller 2008, for an extensive review). Again, the hippocampus is thought to be involved in the contextual regulation of extinction memories, activating the IL only when the extinguished CS is presented in the extinction context (Corcoran and Maren 2001). The involvement of the dorsal hippocampus (DH) in the expression of extinction is supported by studies showing that temporary inactivation of the DH prior to retrieval test eliminates the renewal effect (Corcoran and Maren 2001, 2004). The DH may also be involved in the acquisition and retention of extinction, as inactivation of the DH prior to extinction training slows the rate of extinction and leads to reduced recall the following day (Corcoran et al. 2005). Finally, more recent evidence implicates the ventral hippocampus in the acquisition of extinction, as inactivation prior to extinction training, but not immediately after, causes deficits in recall the following day (Sierra-Mercado et al. 2011).

Studies using neuroimaging tools in humans have demonstrated remarkable preservation of the neural circuitry regulating both conditioning and extinction across species. The human amygdala increases activity during acquisition and recall of fear conditioning (Knight et al. 2004; Phelps et al. 2004), and decreases activity across extinction training (La Bar et al. 1998). The dorsal anterior cingulate (dAC) has also been shown to increase activity during acquisition and recall of conditioning, and there is some evidence to suggest that the thickness of the dAC cortex is correlated with fear conditioning strength (Milad et al. 2007, b; but see Hartley et al. 2011). This may suggest that the human dAC is functionally analogous to the rodent PL.

The human vmPFC has also been shown to play a specific role in extinction; hence, it may be viewed as functionally analogous to the rodent IL. vmPFC activity has been shown to increase over the course of extinction training (Gottfried and Dolan 2004). Studies examining the neurocircuitry involved in long-term recall of extinction memories have shown that vmPFC activity and thickness are both correlated with levels of extinction recall (Milad et al. 2005; 2007, b). Finally, just as in the rodent, the human hippocampus also appears to be involved in the contextual gating of extinction memories. Hippocampal activity increases during extinction recall (Knight et al. 2004; Milad et al. 2007, b), and one study has shown that hippocampus activity increases only when the CS is presented in the extinction context, and no changes in hippocampal activity occur when the CS is presented outside of the extinction context (Kalisch et al. 2006), supporting the notion that the hippocampus gates when and where extinction memories are expressed on the basis of contextual cues.

It was noted above that more recent theories of extinction postulate partial erasure of the fear memory. Research in rodents has supported at least two neurobiological mechanisms by which this could occur. The first is depotentiation, which refers to a reversal of the long-term, synaptic changes associated with long-term memory. Lin et al. (2003) demonstrated that low-frequency stimulation to the amygdala of adult rodents applied after fear conditioning induced depotentiation and reduced conditioned fear expression (i.e., caused “extinction” of fear). Kim et al. (2007) subsequently demonstrated that fear extinction caused depotentiation

of auditory fear conditioning-induced synaptic changes at thalamic input synapses onto the lateral amygdala. Second, it has recently been demonstrated that fear conditioning causes elimination of dendritic spines in the frontal cortex of mice, and that extinction causes spine formation in the same location of the eliminated spines, suggesting that extinction reverses the changes in dendritic remodeling induced by conditioning. Erasure of fear memory has also been reported to occur in humans if the inter-trial interval between the first and second CS presentations during extinction is extended; however, the neural correlates of this finding are yet to be identified (Schiller et al. 2010).

8.3 Do Fear Conditioning and Extinction Constitute Behavioral Phenotypes or Endophenotypes?

Part of the attraction to research on fear conditioning and extinction is that, as noted in the first section of this chapter, these procedures model the symptoms of anxiety along with the reductions in anxiety observed following successful treatment. The advantage of having robust laboratory models of psychiatric disorders is that they foster the development of novel treatments that can be easily tested in a preclinical context (Graham et al. 2011). However, there are concerns that laboratory models of clinical phenotypes that do not reflect the etiology of psychiatric disorders may potentially stunt progress in determining the genetic basis for such disorders (Hettema et al. 2003). In the following section, we review existing evidence examining whether fear conditioning and extinction processes extend beyond mere models of anxiety/treatment to also represent the underlying etiology and mechanisms of dysfunction in pathological anxiety. Specifically, we will examine whether certain conditioning and extinction profiles may be behavioral phenotypes or EPs that represent the genetic basis for anxiety, according to the criteria for EPs delineated by Gottesman and Gould (2003).

8.3.1 Fear Conditioning and Extinction as Behavioral Phenotypes or Endophenotypes: Evidence for Reliability

At a very basic level, a useful behavioral phenotype must be reliably measured and relatively stable. As noted previously, most studies examining fear conditioning and extinction processes in humans use psychophysiological measures of fear responses, such as potentiated startle or SCRs. These measures have the advantage of eliminating concerns about inter-rater reliability, and also circumvent the subjectivity associated with self-report regarding participants' knowledge of the CS–US contingencies (particularly as controversy exists as to whether or not

explicit awareness of such contingencies is necessary for conditioning; Lovibond and Shanks 2002). Accepting that conditioned fear responses can be reliably and objectively measured using psychophysiology, is there any evidence that conditioning and extinction abilities are stable traits? Animal research exploiting the observation of large individual differences in conditioning and extinction abilities in rodents supports the notion that specific phenotypes reflecting conditioning and/or extinction ability can be identified, and that these phenotypes are stable across testing sessions. For example, using the measure of conditioned freezing, Bush et al. (2007) separated Sprague-Dawley rats into high and low reactivity, or fast and slow recovery phenotypes, according to freezing levels exhibited during fear conditioning and extinction training, respectively. They reported that these phenotypes were consistent across subsequent tests that took place in both the conditioning and extinction contexts. Moreover, the "recovery" phenotype persisted at the follow-up time points despite both groups exhibiting comparable extinction learning by the end of extinction training. This suggests the presence of two distinct, relatively stable behavioral phenotypes in rats with respect to conditioning and extinction.

To the best of our knowledge, only one study has examined the test–retest reliability of psychophysiological indices of conditioning and extinction across time in humans. We examined conditioning and extinction ability in a population of healthy adults across three test sessions, each separated by an interval of 8–12 weeks (Zeidan et al. 2011). SCRs were used as a measure of conditioned responses. No significant differences in average fear acquisition, extinction learning, or extinction recall were found across the three time points, and responses during these phases were correlated within subjects across the three time points. This suggests that conditioning and extinction abilities can be reliably measured using SCRs and that, at least in the healthy adult population, these abilities remain stable across a course of around 24 weeks.

8.3.2 Fear Conditioning and Extinction as Behavioral Phenotypes or Endophenotypes: Evidence for Heritability

In addition to being reliable, Gottesman and Gould (2003) stipulate that behavioral phenotypes should be heritable. Animal studies have provided some evidence to suggest that fear conditioning and extinction abilities are heritable traits. Such studies have reported the existence of strain differences in conditioning and/or extinction profiles, suggesting that these phenotypes can be selectively bred. For example, Hefner et al. (2008) reported significant differences in extinction recall between two inbreeds of mice, despite there being no differences in fear acquisition or extinction learning. Moreover, extinction in the impaired breed was associated with reduced activity in the IL and BLA, and was unresponsive to treatments

that normally enhance extinction recall (e.g., increased extinction training trials or pharmacological adjuncts). Similar findings have been reported for Wistar rats selectively bred for high- and low-anxiety-related behavior (Muigg et al. 2008). Despite showing comparable fear acquisition to low-anxiety rats, high-anxiety rats exhibited impaired extinction learning and recall, and reduced activity in IL and lateral amygdala.

The few studies that have examined heritability of fear conditioning and extinction in humans have revealed similar results to those reported in rodents. For example, Hettema et al. (2003) examined fear conditioning and extinction learning in a population of healthy monozygotic and dizygotic twins. There were higher correlations in conditioning, and extinction rates between monozygotic than dizygotic twins, and the authors reported that genetic heritability accounted for 35–45% of the variance associated with these rates. Thus, this study supports the idea of conditioning and extinction being moderately heritable traits in humans. Furthermore, this study also reported evidence suggesting that heritability of conditioning and extinction to ecologically relevant fear stimuli (e.g., snakes and spiders) may be greater than that to neutral fear stimuli (e.g., shapes). Given that many phobias occur to ecologically relevant stimuli and that humans preferentially condition to stimuli that were ecologically relevant to the pre-technical man (Mineka and Öhman 2002), this might suggest that the use of such stimuli in laboratory tasks may be optimally suited to detect genetic substrates of conditioning/extinction processes that are relevant to the etiology of anxiety. The findings from Hettema et al. (2003) also fit with previous reports that correlations between eyeblink conditioning rates are higher in monozygotic than dizygotic twins (Merrill et al. 1999). Although eyeblink conditioning is not strictly considered "fear" conditioning, it is mediated by an associative learning process. Together, these studies do support the notion that associative learning, the theorized cognitive mechanism underlying conditioning and extinction, is at least somewhat heritable.

8.3.3 Fear Conditioning and Extinction as Behavioral Phenotypes or Endophenotypes: Association with Anxiety Disorders

In order to be considered as behavioral phenotypes for anxiety disorders, specific conditioning and extinction phenotypes should be implicated in the etiology of anxiety. Earlier behavioral/learning accounts of anxiety disorders were subject to the criticism that they failed to account for the complexity of individual differences regarding the psychological ramifications of traumatic events. That is, not everyone who experiences a conditioning episode (i.e., a trauma) develops anxiety, and not everyone with an anxiety disorder can recall a specific conditioning episode that precipitated the disorder (Rachman 1990). In the last two decades, however, more contemporary learning models of anxiety have been developed that

consider factors such as conditioning through vicarious rather than directly experiential means, the nature of the event (i.e., controllable versus uncontrollable), and the impact of pre- and post-event variables (such as learning history), to better account for the complexity of individual differences in the development of anxiety. As a result, the view is now well accepted that learning processes underlying fear conditioning and extinction, combined with temperamental/personality vulnerabilities, can at least partly account for the development and maintenance of anxiety disorders. An extensive review on the evidence supporting this account is beyond the scope of this chapter; however, the interested reader should refer to Mineka and Zinbarg (2006) for an excellent review on this topic.

Accepting the relevance of conditioning and extinction processes in the etiology and course of anxiety, it next needs to be determined whether people with clinical anxiety exhibit specific conditioning and extinction phenotypes. Indeed, there is much evidence to suggest that clinical anxiety is associated with heightened conditionability and/or impaired extinction. For example, a recent meta-analysis that reviewed 20 studies of laboratory conditioning and extinction tasks in a range of anxiety disorders demonstrated moderately enhanced conditioned responding during conditioning and extinction in people with anxiety disorders relative to healthy controls (Lissek et al. 2005). This analysis mainly included studies that required participants to learn about and subsequently extinguish fear to simple, single cues. Other studies comparing responses to a conditioned cue versus a "safety" cue (i.e., a cue that was never reinforced) have revealed that people with post-traumatic stress disorder (PTSD) tend to exhibit higher levels of conditioned responding to both the conditioned cue as well as the non-reinforced cue, suggesting a diminished ability among people with PTSD to discriminate between dangerous and safe cues (Blechert et al. 2007; Norrholm et al. 2011; Orr et al. 2000; Peri et al. 2000). These latter studies also reported delays in subsequent extinction, which may merely be a reflection of heightened acquisition during conditioning, or may reflect an additional impairment in fear extinction.

It does appear that anxiety is also associated with deficient extinction, beyond its association with initial conditioning strength. Recent studies have reported specific failures in extinction learning or extinction recall, despite there being no differences in fear conditioning, in anxious populations. This has been demonstrated in people with panic disorder using both SCRs and valence ratings as indices of conditioned fear (Michael et al. 2007). We have reported that people with PTSD exhibit impairments extinction recall, despite there being no differences in conditioning or extinction learning (Milad et al. 2008, 2009).

PTSD impairment in safety learning has also been reported in a different model of fear inhibition that examines the ability to suppress fear responses when a CS is shown in the presence of a conditioned inhibitor (i.e., a safety signal). Compared to healthy controls, PTSD participants exhibited reduced suppression of potentiated startle in trials that included the conditioned inhibitor (Jovanovich et al. 2009). This finding was recently replicated in different cohorts of participants with PTSD, and moreover, the impairment in conditioned inhibition was not detected in participants with major depressive disorder (Jovanovic et al. 2010). This suggests

that impaired fear inhibition may be specific to anxiety disorders, rather than a reflection of psychiatric distress in general.

The alterations in conditioning and extinction appear to be related to symptom severity, where the greater the severity the more heightened the conditioning, and/or the more impaired the extinction ability (Milad et al. 2009; Norrholm et al. 2011). In addition, these alterations are associated with differences in the neural circuitry underlying fear conditioning and extinction. For example, using positron emission tomography, Bremner et al. (2005) demonstrated that people with PTSD exhibited heightened behavioral responses during fear acquisition and extinction that were associated with increased resting metabolic activity in the left amygdala, and decreased resting metabolic activity in the ventromedial prefrontal cortex, respectively, compared to healthy controls. We reported that the impaired extinction recall observed in PTSD populations is associated with reduced activity in the vmPFC and hippocampus, but heightened dAC activity to conditioned cues (Milad et al. 2009) and contexts (Rougemont-Bücking et al. 2011). This suggests that behavioral or psychophysiological measures of conditioning and extinction ability in anxious populations may tap into underlying dysfunctions in cortical and limbic regions that mediate emotion regulation.

8.3.4 Fear Conditioning and Extinction as Behavioral Phenotypes or Endophenotypes: Issues of Co-segregation and State-Independency

The previous section described evidence that dysfunctions in acquisition and extinction of fear are associated with anxiety disorders, and that these dysfunctions are captured in a variety of laboratory tasks across different anxiety subtypes. The question remains, however, whether these dysfunctions represent genetic vulnerabilities to the development of anxiety, or whether they are merely epiphenomenal to the general pathology. One way to assess this is to determine whether fear conditioning/extinction phenotypes and anxiety disorders "co-segregate" in family members (Gottesman and Gould 2003). The first study to examine this compared the genetic covariation between psychophysiological measures of conditioning/extinction profiles and self-reported phobic fears in monozygotic and dizygotic twins (Hettema et al. 2008). A surprising negative correlation was found between psychophysiological fear responses and self-reported phobic fears, and genetic factors underlying fear conditioning/extinction accounted for only 9% of individual differences in self-reported phobic fears. The authors suggested that their data should caution against the use of fear conditioning as a behavioral phenotype for specific phobia.

Likewise, in our examination of a population of monozygotic twins discordant for trauma exposure and PTSD, we observed that extinction recall was impaired in PTSD participants but not their non-trauma exposed co-twin, relative to non-PTSD twins discordant for trauma exposure (Milad et al. 2008). Of course, both

our and Hettema et al.'s (2008) findings do not preclude the possibility of a gene-by-environment interaction, whereby the non-affected co-twin may be genetically vulnerable, but that this vulnerability will only manifest after exposure to trauma. These studies do suggest that impaired extinction may not be a reflection of a pre-existing genetic factor, and further, that it is not a consequence of trauma exposure per se. Rather, impaired extinction may be a specific consequence of the development of PTSD.

Gottesman and Gould (2003) have also stipulated that a psychiatric behavioral phenotype should be present in an individual regardless of whether or not the illness is active (i.e., it should be state-independent). As successful treatment of anxiety may eventually alter the conditioning/extinction behavioral phenotype (even if it is the underlying cause of the disorder), a prudent way to assess this criterion would be to examine whether the phenotype exists *prior to* symptom development, and thus may be predictive of the eventual development of anxiety. Such prospective studies are difficult to conduct; however, Guthrie and Bryant (2006) examined fear conditioning and extinction learning in firefighters during cadet training using SCRs and corrugator electromyography (EMG) responses as indices of conditioned responses. Participants were reassessed for PTSD 24 months post-training following trauma exposure. Heightened EMG responses during extinction training at the time of cadet training accounted for 31% of the variance associated with subsequent PTSD symptomatology two years post-cadet training. This study suggests that impairments in extinction may be moderately predictive of vulnerability to anxiety and challenges the previously described studies suggesting that impaired extinction is merely a consequence of anxiety.

8.4 Conclusion

Considerable research over the past decades has explored the behavioral, cognitive, and neurobiological mechanisms underlying conditioning and extinction in rodents, and more recently, in humans. Evidence suggests that conditioning and extinction abilities are altered in clinically anxious populations, and that these alterations are reflected by changes in the neural circuitry that mediates such abilities (Milad et al. 2009; Rougemont-Bücking et al. 2011). In addition, it is accepted that learning processes underlying conditioning and extinction at least partly mediate the development and maintenance of anxiety disorders. Despite this, there is a dearth of research that has examined whether the conditioning and extinction profiles observed in anxiety are genetically acquired. Thus, it is difficult to draw firm conclusions as to whether current models of conditioning and extinction measure behavioral phenotypes that reflect the genetic factors underlying anxiety. The few studies that have examined whether deficits in fear extinction associated with anxiety are also seen in first degree; unaffected relatives have indicated that the deficits are specific to those inflicted with the disorder (Hettema et al. 2008; Milad et al. 2008). The one study that has examined fear extinction ability as a

predictor of future anxiety has revealed that impairments in extinction can account for a considerable amount of the variance associated with subsequent PTSD symptoms (Guthrie and Bryant 2006). There are at least two explanations for these apparently inconsistent findings: First, it is possible that heightened conditioning/impaired extinction profiles are consequences of anxiety disorders, and thus do not constitute true behavioral phenotypes. This explanation would account for the lack of co-segregation of conditioning/extinction profiles and anxiety disorders within monozygotic twins (Hettema et al. 2008; Milad et al. 2008). This explanation would also be consistent with the postulated role for conditioning/extinction processes in the *maintenance* of anxiety disorders, in the sense that once an anxiety disorder is acquired, the consequent impaired extinction ability would serve to prevent natural extinction of the anxiety and potentially impede the impact of exposure-based treatments. However, this explanation does not account for the finding that extinction impairments precede PTSD symptom onset (Guthrie and Bryant 2006). Moreover, it is inconsistent with evidence that learning processes prior to, during, and subsequent to traumatic events contribute to the initial development of anxiety (Mineka and Zinbarg 2006).

A second possible explanation is that specific conditioning/extinction profiles are predisposing vulnerabilities to anxiety, but that these vulnerabilities are acquired (e.g., through early-life experiences) rather than genetic in origin. This explanation would account for Guthrie and Bryant's (2006) report of pre-existing deficiencies in extinction in people who develop PTSD symptoms, but would also account for the apparent lack of such deficiencies in non-affected monozygotic co-twins (Hettema e al. 2008; Milad et al. 2008). On the face of it, this explanation may appear to be contrary to reports that conditioning/extinction phenotypes are heritable (Hettema et al. 2003). However, when it is considered that all phenotypes will represent a combination of genetic and environmental factors, it is feasible to consider the proposition that in some cases, environmental experience may overshadow the impact of genetics on conditioning/extinction profiles, hence leading to null effects in co-segregation studies. Indeed, the idea that conditioning/extinction profiles can be modified by life events is supported by preclinical studies in rodents that have demonstrated that early-life maternal deprivation (Callaghan and Richardson 2011, 2012), early-life exposure to neurotrophic factor (Graham and Richardson 2010), or chronic stress (Izquierdo et al. 2006; Miracle et al. 2006) all impact conditioning and/or extinction abilities later in life.

Another potential reason for the apparently discrepant findings regarding whether conditioning/extinction traits are acquired versus pre-existing may be that some studies have focused on conditioning and extinction learning, and others have focused on longer-term retention of the extinction memory. Animal studies support the idea that the three phases of the model (conditioning, extinction learning, and extinction recall) may be distinct phenotypes controlled by discrete neurocircuitry. It is possible that not all of these subphases are equally relevant to/informative about the origin and maintenance of anxiety disorders. In fact, a recent review of exposure processes in clinical anxiety has demonstrated that there is little evidence for correlations between initial fear response or within-session

extinction (i.e., extinction learning) and between-session extinction, referring to the maintenance of the extinction memory across repeated sessions (Craske et al. 2008). This notion has also been supported by preclinical studies in rodents (Plendl and Wotjak 2010). Given that preserved between-session extinction is clearly necessary to maintain treatment gains over the longer term, it may be the case that deficient extinction recall is the more relevant phenotype of anxiety rather than initial conditioning strength or within-session extinction learning. It is possible that if future studies focus on the extinction recall phase, more consistent findings regarding the contribution of conditioning/extinction processes to the genetic factors underlying anxiety will emerge.

References

Anagnostaras SG, Maren S, Fanselow MS (1999) Temporally-graded retrograde amnesia of contextual fear after hippocampal damage in rats: within subjects examination. J Neurosci 19:1106–1114

Blechert J, Michael T, Vriends N et al (2007) Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic experiential and behavioural responses. Behav Res Ther 45:2019–2033

Bodnoff SR, Suranyi-Cadotte B, Aitken DH et al (1988) The effects of chronic antidepressant treatment in an animal model of anxiety. Psychopharmacology 95:298–302

Bouton ME (1993) Context time and memory retrieval in the interference paradigms of Pavlovian conditioning. Psychol Bull 114:80–99

Bouton ME (2002) Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. Biol Psych 52(10):976–986

Bouton ME, Bolles RC (1979a) Contextual control of the extinction of conditioned fear. Learn Motiv 10:445–466

Bouton ME, Bolles RC (1979b) Role of conditioned contextual stimuli in reinstatement of extinguished fear. J Exp Psychol Anim Behav Process 5:368–378

Bremner JD, Vermetten E, Schmahl et al (2005) Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse related post-traumatic stress disorder. Psychol Med 35:791–806

Burglos-Robles A, Vidal-Gonzalez I, Quirk GJ (2009) Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. J Neurosci 29:8474–8482

Bush DEA, Sotres-Bayon F, LeDoux JE (2007) Individual differences in fear: isolating fear reactivity and fear recovery phenotypes. J Trauma Stress 20:413–422

Callaghan BL, Richardson R (2011) Maternal separation results in early emergence of adult-like fear and extinction learning in infant rats. Behav Neurosci 125:20–28

Callaghan BL, Richardson R (2012) Early-life stress affects extinction during critical periods of development: an analysis of the effects of maternal-separation on extinction in adolescent rats. Stress 15(6):671–679

Choi DC, Maguschak KA, Ye K et al (2010) Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. Proc Natl Acad Sci U S A 107:2675–2680

Ciocchi S, Herry C, Grenier F et al (2010) Encoding of conditioned fear in central amygdala inhibitory circuits. Nature 468:277–284

Corcoran KA, Desmond TJ, Frey KA, Maren S (2005) Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction. J Neurosci 25:8978–8987

Corcoran KA, Quirk GJ (2007) Activity in prelimbic cortex is necessary for the expression of learned but not innate fears. J Neurosci 27:840–844
Corcoran KA, Maren S (2001) Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. J Neurosci 21:1720–1726
Corcoran KA, Maren S (2004) Factors regulating the effects of hippocampal inactivation on renewal of conditional fear after extinction. Learn Mem 11:598–603
Craske MG, Kircanski K, Zelikowsky M et al (2008) Optimizing inhibitory learning during exposure therapy. Behav Res Ther 46:5–27
Cryan JF, Holmes A (2005) The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4:775–790
Delamater AR (2004) Experimental extinction in Pavlovian conditioning: behavioural and neuroscience perspectives. Q J Exp Psychol 57B:97–132
Davis M (1990) Animal models of anxiety based on classical conditioning: the conditioned emotional response (CER) and the fear-potentiated startle effect. Pharmacol Ther 47:147–165
Fanselow MS (1980) Conditional and unconditional components of postshock freezing. Pavlov J Biol Sci 15:177–182
Foa EB (2011) Prolonged exposure therapy: past present future. Depress Anxiety 28:1043–1047
Gale GD, Anagnostaras SG, Gosdsil BP et al (2004) Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. J Neurosci 24:3810–3815
Gogolla N, Caroni P, Lüthi A, Herry C (2009) Perineuronal nets protect fear memories from erasure. Science 325:1258–1261
Goosens KA, Maren S (2001) Contextual and auditory fear conditioning are mediated by the lateral basal and central amygdaloid nuclei in rats. Learn Mem 8:148–155
Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160:636–645
Gottfried JA, Dolan RJ (2004) Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. Nat Neurosci 7:1145–1153
Graham BM, Langton JM, Richardson R (2011) Pharmacological enhancement of fear reduction: preclinical models. Br J Pharmacol 164:1230–1247
Graham BM, Milad MR (2011) The study of fear extinction: implications for anxiety disorders. Am J Psychiatry 168:1255–1265
Graham BM, Richardson R (2010) Early-life exposure to fibroblast growth factor-2 facilitates context-dependent long-term memory in developing rats. Behav Neurosci 124:337–345
Guthrie RM, Bryant RA (2006) Extinction learning before trauma and subsequent posttraumatic stress. Psychosom Med 68:307–311
Hartley CA, Fischl B, Phelps EA (2011) Brain structure correlates of individual differences in the acquisition and inhibition of conditioned fear. Cerebr Cortex 21:1954–1962
Hefner K, Whittle N, Juhasz J et al (2008) Impaired fear extinction learning and corticoamygdala circuit abnormalities in a common genetic mouse strain. J Neurosci 28:8074–8085
Hettema JM, Annas P, Neale MC et al (2008) The genetic covariation between fear conditioning and self-report fears. Biol Psychiatry 63:587–593
Hettema JM, Annas P, Neale MC et al (2003) A twin study of the genetics of fear conditioning. Arch Gen Psychiatry 60:702–708
Izquierdo A, Wellman CL, Homes A (2006) Brief uncontrollable stress causes dendritic retraction in infralimbic cortex and resistance to fear extinction in mice. J Neurosci 26:5733–5738
Jovanovic T, Norrholm SD, Fennell JE et al (2009) Posttraumatic stress disorder may be associated with impaired fear inhibition: relation to symptom severity. Psychiatry Res 167:151–160
Jovanovic T, Norrholm SD, Blanding NQ et al (2010) Impaired fear inhibition is a biomarker of PTSD but not depression. Depress Anxiety 27:244–251
Kalisch R, Korenfeld E, Stephan KE et al (2006) Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. J Neurosci 26:9503–9511

Kim J, Lee S, Park K et al (2007) Amygdala depotentiation and fear extinction. Proc Natl Acad Sci U S A 104:20955–20960
Kim JH, Richardson R (2010) New findings on extinction of conditioned fear early in development: theoretical and clinical implications. Biol Psychiatry 67:297–303
Knight DC, Smith CN, Cheng DT et al (2004) Amygdala and hippocampal activity during acquisition and extinction of human fear conditioning. Cogn Affect Behav Neurosci 4:317–325
LaBar KS, Gatenby JC, Gore JC et al (1998) Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. Neuron 20:937–945
Laurent V, Westbrook RF (2009) Inactivation of the infralimbic but not the prelimbic cortex impairs conolidation and retrieval of fear extinction. Learn Mem 16:520–529
LeDoux JE (2007) The amygdala. Curr Op. Biol 17:868–874
Lin C, Lee C, Gean P (2003) Involvement of a calcineurin cascade in amygdala depotentiation and quenching of fear memory. Mol Pharmacol 63:44–52
Lissek S, Powers AS, McClure EB et al (2005) Classical fear conditioning in the anxiety disorders: a meta-analysis. Behav Res Ther 43:1391–1424
Lovibond PF, Shanks DR (2002) The role of awareness in Pavlovian conditioning: empirical evidence and theoretical implications. J Exp Psychol Anim Behav Process 28:3–26
Maren S (2001) Neurobiology of Pavlovian fear conditioning. Ann Rev Neurosci 24:897–931
Maren S, Anagnostaras SG, Fanselow MS (1998) The startled seahorse: is the hippocampus necessary for contextual fear conditioning? Trends Cogn Sci 2:39–42
Maren S, Quirk GJ (2004) Neuronal signalling of fear memory. Nature 5:844–852
Matus-Amat P, Higgins EA, Barrientos RM, Rudy JW (2004) The role of the dorsal hippocampus in the acquisition and retrieval of context memory representations. J Neurosci 24:2431–2439
Merrill KA, Steinmetz JE, Viken RJ, Rose RJ (1999) Genetic influences on human conditionability: a twin study of the conditioned eyeblink response. Behav Genet 29:95–102
Michael T, Blechert J, Vriends N et al (2007) Fear conditioning in panic disorder: enhanced resistance to extinction. J Abnorm Psychol 116:612–617
Milad MR, Rauch SL, Pitman RK, Quirk GJ (2006) Fear extinction in rats: implications for human brain imaging and anxiety disorders. Biol Psychiatry 73:61–71
Milad MR, Quinn BT, Pitman RK et al (2005) Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. Proc Natl Acad Sci U S A 102:10706–10711
Milad MR, Quirk GJ, Pitman RK et al (2007a) A role for the human dorsal anterior cingulate cortex in fear expression. Biol Psychiatry 62:1191–1194
Milad MR, Wright CI, Orr SP et al (2007b) Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol Psychiatry 62:446–454
Milad MR, Orr SP, Lasko NB et al (2008) Presence and acquired origin of reduced recall for fear extinction in PTSD: results of a twin study. J Psychiatric Res 42:515–520
Milad MR, Pitman RK, Ellis CB et al (2009) Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. Biol Psychiatry 66:1075–1082
Hofmann W, De Houwer J, Perugini M et al (2010) Evaluative conditioning in humans: a meta-analysis. Psychol Bull 136:390–421
Mineka S, Öhman A (2002) Phobias and preparedness: the selective automatic and encapsulated nature of fear. Biol Psychiatry 52:927–937
Mineka S, Zinbarg R (2006) A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. Am Psychol 61:10–26
Miracle AD, Brace MF, Huyck KD et al (2006) Chronic stress impairs recall of extinction of conditioned fear. Neurobiol Learn Mem 85:213–218
Muigg P, Hetzenauer A, Hauer G et al (2008) Impaired extinction of learned fear in rats selectively bred for high anxiety—evidence of altered neuronal processing in prefrontal-amygdala pathways. Eur J Neurosci 28:2299–2309
Myers KM, Davis M (2007) Mechanisms of fear extinction. Mol Psychiatry 12:120–150

Norrholm SD, Jovanovic T, Olin IW et al (2011) Fear extinction in traumatized civilians with posttraumatic stress disorder: relation to symptom severity. Biol Psychiatry 69:556–563
Orr SP, Metzger LJ, Lasko NB et al (2000) De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. J Abnorm Psychol 109:290–298
Orsini CA, Maren S (2012) Neural and cellular mechanisms of fear and extinction memory formation. Neurosci Biobehav Rev 36(7):1773–1802
Otto MW, Smits JAJ, Reese HE (2004) Cognitive-behavioral therapy for the treatment of anxiety disorders. J Clin Psychiatry 65:s34–s41
Peri T, Ben-Shakhar G, Orr SP, Shalev AY (2000) Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. Biol Psychiatry 47:512–519
Phelps EA, Delgado MR, Nearing KI, LeDoux JE (2004) Extinction learning in humans: role of the amygdala and vmPFC. Neuron 43:897–905
Plendl W, Wotjak CT (2010) Dissociation of within- and between-session extinction of conditioned fear. J Neurosci 30:4990–4998
Quirk GJ, Mueller D (2008) Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 33:56–72
Quirk GJ, Parè D, Richardson R et al (2010) Erasing fear memories with extinction training. J Neurosci 30:14993–14997
Rachman S (1990) Fear and Courage, 2nd edn. Freeman, New York
Rescorla RA, Wagner AR (1972) A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and non-reinforcement. In: Prokasy AH (ed) Classical conditioning II: current research and theory. Appleton-Century-Croft, New York, pp 64–99
Rougemont-Bücking A, Linnman C, Zeffiro TA et al (2011) Altered processing of contextual information during fear extinction in PTSD: an fMRI study. CNS Neurosci Ther 17:227–236
Rudy JW, Nuff NC, Matus-Amat P (2004) Understanding contextual fear conditioning: insights from a two-process model. Neurosci Biobehav Rev 28:675–685
Schiller D, Monfils MH, Raio CM et al (2010) Preventing the return of fear in humans using reconsolidation update mechanisms. Nature 463:49–54
Sierra-Mercado D, Padilla-Coreano N, Quirk GJ (2011) Dissociable roles of prelimbic and infralimbic cortices ventral hippocampus and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology 36:529–538
Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ (2006) Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learn Mem 13:728–733
Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE (2006) Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition consolidation and expression of pavlovian fear conditioning. J Neurosci 26:12387–12396
Wolpe J (1954) Reciprocal inhibition as the main basis of psychotherapeutic effects. Arch Neurol Psychiatry 72:205–226
Zeidan MA, Lebron-Milad K, Thompson-Hollands J et al (2011) Test-retest reliability during fear acquisition and fear extinction in humans. CNS Neurosci Ther 18(4):313–317

Chapter 9
Neural Markers of Errors as Endophenotypes in Neuropsychiatric Disorders

Dara S. Manoach and Yigal Agam

9.1 Introduction

Learning from errors is fundamental to adaptive human behavior. It requires detecting errors, evaluating what went wrong, and adjusting behavior accordingly. These dynamic adjustments are at the heart of behavioral flexibility and accumulating evidence suggests that deficient error processing contributes to maladaptively rigid and repetitive behavior in a range of neuropsychiatric disorders. Neuroimaging and electrophysiological studies reveal highly reliable neural markers of error processing. In this review, we evaluate the evidence that abnormalities in these neural markers can serve as sensitive endophenotypes of neuropsychiatric disorders. We describe the behavioral and neural hallmarks of error processing, their mediation by common genetic polymorphisms, and impairments

Support: National Institute for Mental Health: R01 MH67720 (DSM); F32 MH088081 (YA).
Note from Volume Editors:
This chapter was published in the open source journal 'Frontiers in Human Neuroscience,' July 2013, Vol. 7, Article 350. In view of the topical and thematic overlap between the invited chapter and the journal article that the chapter authors had submitted at the time of the invitation, the authors kindly granted permission to reproduce the article in this volume.

D.S. Manoach · Y. Agam (✉)
Department of Psychiatry, Massachusetts General Hospital,
Harvard Medical School, Boston, USA
e-mail: yigal@nmr.mgh.harvard.edu

D.S. Manoach
e-mail: dara@nmr.mgh.harvard.edu

D.S. Manoach · Y. Agam
Athinoula A. Martinos Center for Biomedical Imaging, Boston, USA

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_9

in schizophrenia, obsessive–compulsive disorder, and autism spectrum disorders. We conclude that neural markers of errors meet several important criteria as endophenotypes including heritability, established neuroanatomical and neurochemical substrates, association with neuropsychiatric disorders, presence in syndromally unaffected family members, and evidence of genetic mediation. Understanding the mechanisms of error processing deficits in neuropsychiatric disorders may provide novel neural and behavioral targets for treatment and sensitive surrogate markers of treatment response. Treating error processing deficits may improve functional outcome since error signals provide crucial information for flexible adaptation to changing environments. Given the dearth of effective interventions for cognitive deficits in neuropsychiatric disorders, this represents a promising approach.

To adapt to the environment, human beings must learn from the consequences of their behavior. Understanding the nature of the brain mechanisms that flexibly modify behavior based on its consequences is a fundamental goal of neuroscience. These mechanisms are also of considerable clinical importance since a number of neuropsychiatric disorders are strongly associated with maladaptively rigid and repetitive behaviors that are not optimally responsive to outcomes. One approach to understanding the neural basis of learning from consequences is to study error processing. Errors provide critical information for adjusting behavior to optimize outcomes. Error processing, which is also referred to as 'response monitoring' or 'performance monitoring,' involves detecting errors during task performance, evaluating what went wrong, and adjusting behavior accordingly. These dynamic adjustments of responses are at the heart of behavioral flexibility. They enable individuals to optimize function in complex, uncertain, and constantly changing environments. Since learning from errors is impaired in several neuropsychiatric disorders, understanding the neural and genetic mechanisms of error processing has important clinical implications. Identifying specific deficits can illuminate the pathophysiology of these disorders and provide novel targets for treatment. Below, we selectively review the behavioral and neural hallmarks of error processing; impairments in schizophrenia, obsessive–compulsive disorder, and autism spectrum disorders; and genetic contributions. The goal is to evaluate the potential of the neural markers of errors to serve as endophenotypes. Endophenotypes are biologically based heritable dysfunctions that are thought to be a closer reflection of the effects of the genes that predispose to illness than either the diagnosis itself, or the symptoms that define it (Gottesman and Gould 2003). The identification of clinically relevant endophenotypes can facilitate the discovery of susceptibility genes, mechanisms of illness, and targets for intervention (Hariri et al. 2006).

9.2 Behavioral Indices of Error Processing

Both the behavioral and neural markers of error processing are considered to be 'generic' in that they are elicited by a wide range of tasks regardless of response modality (Holroyd and Coles 2002). Many experimental tasks used to study error

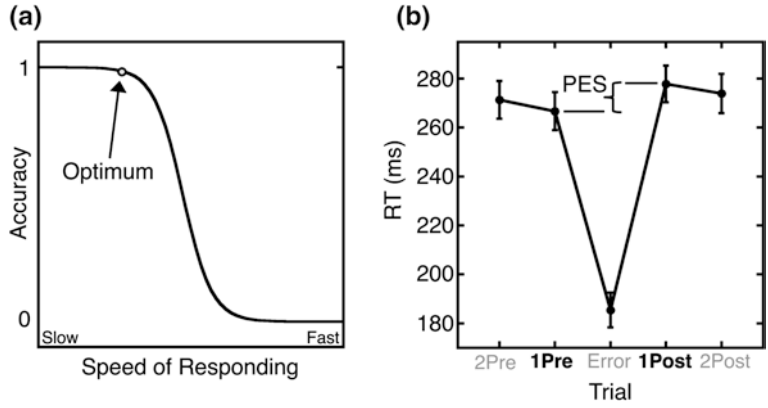

Fig. 9.1 Trial-by-trial adjustments of reaction time (RT). **a** A schematic depiction of the speed-accuracy trade-off (SATO) function. The *circle* denotes the optimum: the point at which the highest accuracy is achieved at the fastest possible speed. Beyond this point, speedier responses entail a cost (trade-off) in reduced accuracy. **b** Mean saccadic RT during an antisaccade task as a function of trial position relative to an error trial. Post-error slowing (PES) is defined as the difference in RT between the trial following the error (1Post) and the trial preceding the error (1Pre). *Error bars* represent the standard error of the mean

processing in humans require response inhibition, or the suppression of prepotent but contextually inappropriate responses. These include variations of go/no-go, antisaccade (Hallett 1978), countermanding, or stop signal (Logan and Cowan 1984), Stroop (1935), Simon (1969), and perhaps most commonly, Eriksen flanker (Eriksen and Eriksen 1974) tasks.

Errors give rise to both immediate and longer-term remedial adjustments of behavior. Short-term or trial-by-trial adjustments include the immediate self-correction of errors and the slowing of reaction time (RT) in trials that follow an error (i.e., post-error slowing) (Rabbitt 1966). These trial-by-trail adjustments of RT based on the error history are well described by the Speed-Accuracy Trade-Off (SATO) function. The SATO function depicts the nonlinear relation between speed and accuracy such that faster responding does not affect accuracy, but only up to a point. Beyond that point, speed and accuracy are inversely related, with slower responses having a greater probability of being correct (Fig. 9.1). This transition point can be regarded as an optimum, where the best accuracy is achieved at the fastest possible speed. Over trials, responses speed up until an error is committed (Ridderinkhof et al. 2003), and following an error, RT slows, and the probability of an error decreases (Fig. 9.1). This pattern can be interpreted as a progression to riskier positions on the SATO function culminating in an error. The error is followed by a shift back to a safer position on the function that has a greater likelihood of a correct response.

Reinforcement learning theory (Thorndike 1911) can be invoked to account for longer-term behavioral changes in response to errors. Its main principle is that rewarded actions are more likely to be repeated, while actions with negative

consequences are less likely to recur. In behavioral terms, reinforcement learning involves the strengthening or weakening of stimulus–response mappings based on behavioral outcomes. While reinforcement learning has traditionally been studied using explicit rewards and punishments, recent theory extends it to errors (Holroyd and Coles 2002). Errors on cognitive tasks are both salient (in that they are often unexpected) and aversive (representing the non-achievement of a goal). As failures of performance they often have negative consequences. For these reasons, errors prompt reinforcement learning.

9.3 Neural Markers of Errors, Their Functional Significance, and Relations to One Another

Electrophysiological and neuroimaging studies have identified two highly reliable neural markers of error commission—the error-related negativity (ERN) and functional MRI (fMRI) activation of the dorsal anterior cingulate cortex (dACC; (Taylor et al. 2007)—that are the focus of the present review. Although these error markers have been extensively studied, their functional significance and relations to one another are incompletely understood.

9.3.1 The Error-Related Negativity (ERN)

The ERN or error negativity (Ne) is an event-related potential that peaks ~100 ms following an error (Fig. 9.2, Dehaene et al. 1994; Falkenstein et al. 1991; Gehring et al. 1993; van Veen and Carter 2002) and is usually measured on the scalp with

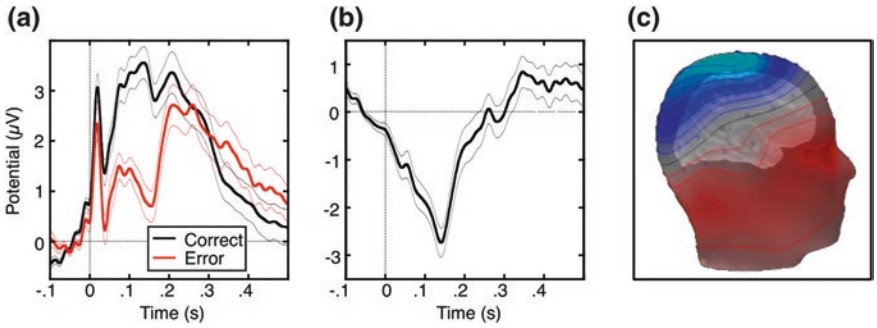

Fig. 9.2 The error-related negativity (ERN). **a** Grand average waveforms for correct (*black*) and error (*red*) antisaccade trials, time-locked to the onset of the saccade. **b** Difference waveform, obtained by subtracting the correct waveform from the error waveform. **c** Scalp distribution of the ERN, displayed on a template head model. Adapted from Agam et al. (2011)

electroencephalography (EEG), magnetoencephalography (MEG; Keil et al. 2010), or a combination of both techniques (Agam et al. 2011). The ERN is usually defined as the peak of the difference between the averaged waveforms of error and correct trials time-locked to the onset of the response. The ERN is the earliest error marker and is 'generic' in that it is seen across a variety of behavioral paradigms and response modalities. Comparisons of ERNs time-locked to button presses, saccadic eye movements, or foot presses, reveal a similar morphology, amplitude, and scalp topography (Holroyd et al. 1998; Van't Ent and Apkarian 1999). ERN latency, however, varies based on the measurement technique. Button presses elicit shorter latencies than ERNs locked to the electromyography (EMG) or saccadic responses as measured by electrooculography (EOG). This reflects that EMG and EOG measure the onset of movement, which occurs earlier than its outcome (e.g., a button press). The ERN is usually maximal at electrode Cz on the scalp (e.g., Agam et al. 2011; van Schie et al. 2004; van Veen and Carter 2002), but the peak location can be more anterior (e.g., Endrass et al. 2005; Gehring and Fencsik 2001; Nieuwenhuis et al. 2003) or posterior (e.g., Hajcak et al. 2004; Ladouceur et al. 2007; van Boxtel et al. 2005) and factors such as response modality and task fail to provide a convincing account of this variability.

The ERN has been proposed to reflect error detection and reinforcement learning (Holroyd and Coles 2002; Holroyd et al. 2004b; Paus et al. 1993). Its amplitude is greater when accuracy is emphasized over speed (Gehring et al. 1993), when errors are corrected (Scheffers and Coles 2000), when errors incur greater loss (Holroyd et al. 2004a), and when errors are less frequent and therefore also less expected (Gehring et al. 1993; Hajcak et al. 2003). Larger ERNs are associated with greater post-error slowing of responses (Debener et al. 2005), and ERN latency predicts the speed of self-corrections (Fiehler et al. 2005). These findings suggest that the ERN detects errors, is sensitive to both the predictability and value of outcomes, and contributes to dynamic, trial-by-trial adjustments of performance.

9.3.2 Error Positivity (Pe)

A second EEG error marker warrants consideration given its relevance to neuropsychiatric disorders. The error positivity or Pe (van Veen and Carter 2002) is an event-related potential that occurs approximately 300–500 ms following an error (for review see, Overbeek et al. 2005). The Pe has been localized to the rostral anterior cingulate cortex (van Boxtel et al. 2005; van Veen and Carter 2002), though one study reported a dACC source (Herrmann et al. 2004). The Pe is not as well characterized and is less consistently observed than the ERN, which may reflect that it is a later and more variable component of error processing. While the ERN is present regardless of whether an error was perceived, the Pe is present only for perceived errors and is thought to index error awareness (Endrass et al. 2007; Nieuwenhuis et al. 2001). The Pe has also been associated with the

subjective or emotional appraisal of errors (van Veen and Carter 2002) and with short-term performance adjustments such as error correction and post-error slowing (Nieuwenhuis et al. 2001).

9.3.3 *Error-Related FMRI Activation of the Anterior Cingulate Cortex (ACC)*

Error commission is also reliably associated with increased fMRI activation of the ACC on error compared with correct trials (i.e., error-related activation, Fig. 9.3; (for review, see Taylor et al. 2007). The ACC can be divided into a dorsal region (dACC) that extends caudally from the genu of the corpus callosum to the vertical

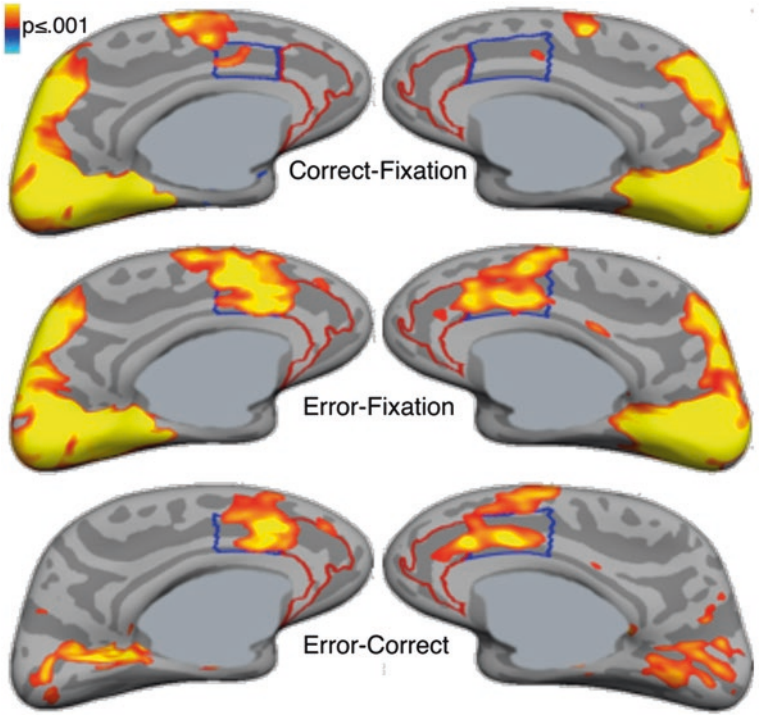

Fig. 9.3 Error-related activation in the anterior cingulate cortex (ACC). Statistical maps, displayed on medial cortical surface templates, show activation on correct trials versus a fixation baseline (*top*), error versus fixation (*middle*), and error versus correct (*bottom*). *Gray masks* cover subcortical regions in which activation is displaced in a surface rendering. The dACC and rACC are outlined in *blue* and *red*, respectively. Adapted from Polli et al. (2005)

plane of the anterior commissure, and interacts with the striatum and other cortical regions to mediate motor and cognitive processing, and a rostral region (rACC) that lies anterior and ventral to the genu of the corpus callosum and interacts with other paralimbic and limbic regions, including the amygdala and insula, to mediate emotional processing (Bush et al. 1998, 2000; Devinsky et al. 1995; Phillips et al. 2003; Whalen et al. 1998). Like the Pe, error-related rACC activation is thought to reflect appraisal of the affective or motivational significance of errors (Luu et al. 2003; Taylor et al. 2006; van Veen and Carter 2002). Such appraisal may also involve the insula and amygdala, both of which are densely interconnected with the rACC (van Hoesen et al. 1993) and show increased activity with errors (Brazdil et al. 2002; Garavan et al. 2002; Menon et al. 2001; Polli et al. 2009). While both dACC and rACC show error-related activation (Luu et al. 2003; Taylor et al. 2006; van Veen and Carter 2002), dACC activation is more consistently observed. Like the ERN, greater error-related dACC activation is associated with lower error rates (Fitzgerald et al. 2010; Polli et al. 2008) and increased post-error slowing (Garavan et al. 2002; Kerns et al. 2004; Klein et al. 2007a).

9.3.4 Modulation of Default Network Activation in Relation to Errors

The brain's default network is thought to mediate self-referential and affective processing and is usually deactivated during effortful cognitive tasks (Buckner et al. 2008; Raichle et al. 2001). During error trials (Polli et al. 2005) and trials immediately preceding errors (Eichele et al. 2008; Li et al. 2007), however, the default network shows relatively increased activation, which may reflect increased focus on the internal milieu at the expense of attention to the task (Drevets and Raichle 1998). In trials that follow errors, task-induced deactivation is re-established (Eichele et al. 2008). This cyclical pattern of default network activation in trials including and surrounding errors correlates with speed-accuracy trade-off-based changes in RT (i.e., pre-error speeding, faster errors, and post-error slowing, Agam et al., in press) and suggests that interference from internally directed thought culminates in an error, which, in turn prompts renewed attention to the task in the subsequent trial. These changes in activation are not strictly error markers (i.e., they are not specific to errors nor do they necessarily indicate that an error has occurred), but they may contribute to error commission and to behavioral adjustments following errors such as post-error slowing. Several reviews have addressed the role of default network function in neuropsychiatric disorders (e.g., Broyd et al. 2009; Buckner et al. 2008; Sandrone 2012; Whitfield-Gabrieli and Ford 2012). Whether changes of default network activity in relation to errors are affected in neuropsychiatric disorders, however, is largely unexplored.

9.3.5 Error-Based Reinforcement Learning

Error-related dACC activation is often assumed to be the hemodynamic correlate of the ERN. This assumption is consistent with both EEG and MEG studies that have reported a dACC source for the ERN and with models that attribute both error markers to a specific neural mechanism that implements error-based reinforcement learning (Holroyd and Coles 2002; Ridderinkhof et al. 2004; Taylor et al. 2007). Consistent with animal neurophysiology and human neuroimaging findings, these models view the neural sequelae of error commission as indices of error-based reinforcement learning (Holroyd and Coles 2002; Schultz 2002). When an error occurs, the striatum detects a mismatch between the intended (correct) outcome and actual (error) outcome. This mismatch or 'prediction error' results in a phasic decrease in mesencephalic dopamine (DA) release that results in the disinhibition of neurons in the dACC. These neurons generate the ERN. According to this theory, both increased dACC activation and the ERN reflect the use of DA-dependent error signals to modify the associative strength of stimulus–response mappings in the service of optimizing behavioral outcomes (Holroyd et al. 2003, 2004b). Thus, both error-related dACC activation and ERN can be conceptualized as DA-dependent training signals that are used to learn from errors (Brown and Braver 2005; Holroyd and Coles 2002). Similar neural mechanisms of error processing have been observed across species for a variety of learning tasks. For example, the songbird uses input from a basal ganglia—thalamocortical circuit to recognize and correct vocal errors while learning its distinctive song (Andalman and Fee 2009). Such findings suggest that this neural circuitry represents an evolutionarily conserved mechanism for learning from errors.

9.3.6 Relation of the ERN to DACC Activation

Despite the many studies that report a dACC source for the ERN, the location of the neural generator of the ERN is still a topic of debate. When compared across studies, the dACC source loci of the ERN show considerable variation (for review, see Agam et al. 2011) and all are posterior to the mean location of error-related fMRI activation (based on a meta-analysis of 13 fMRI studies, Ridderinkhof et al. 2004). Some ERN loci also fall in the posterior cingulate cortex (PCC) according to standard anatomical definitions that place the ACC/PCC border between $y = -2$ and $y = -12$ mm in Talairach space (Bush et al. 2000). The PCC is also a plausible generator of the ERN. It shows error-related fMRI activation (Fassbender et al. 2004; Menon et al. 2001; Wittfoth et al. 2008), though not nearly as consistently as the dACC, and like the ERN, its activity is modulated by the value of behavioral outcomes (Fujiwara et al. 2009; McCoy et al. 2003; Smith et al. 2009). An MEG study reported a PCC source for the feedback-related negativity, which is thought to be generated by the same generic mechanism as the

ERN (Donamayor et al. 2011). Further, a study from Agam and colleagues that combined data from EEG and MEG localized the source of the ERN to the PCC (Agam et al. 2011). This PCC region was clearly distinct from error-related dACC activation measured in the same participants performing the same task during fMRI.

These findings challenge the view that dACC activation and the ERN are different measurements of the same underlying neural mechanism. Instead, they indicate that the ERN and fMRI activation of the dACC reflect distinct neural responses to errors. In the combined MEG/EEG, fMRI, and diffusion tensor imaging (DTI) study of Agam and colleagues, ERN amplitude correlated with fMRI activation in both the PCC and dACC, and these two regions showed coordinated activity based on functional connectivity MRI. This suggests that the dACC and PCC are components of a functional network that mediates error processing. The PCC and ACC have direct anatomical connections through the cingulum bundle (Schmahmann et al. 2007) and increased microstructural integrity of the posterior cingulum bundle (as indexed by DTI measurements of fractional anisotropy) predicted faster error self-correction. To the degree that fractional anisotropy reflects myelination, increased myelination along the cingulum bundle may speed the conduction of the message that an error has occurred, thereby resulting in faster corrective responses. Taken together, these findings are consistent with the theory that the PCC detects errors, gives rise to the ERN, and then relays error information to the dACC via the cingulum bundle to implement corrective behavior. Refinements of this working model will likely follow given that the mechanisms of error processing remain a highly active area of research.

9.4 Error Processing Impairments in Neuropsychiatric Disorders

Although the present review focuses on schizophrenia, obsessive–compulsive disorder (OCD) and autism spectrum disorder (ASD), accumulating evidence suggests that error processing deficits contribute to rigid, repetitive behavior in a range of disorders. For example, a previous review described ERN abnormalities in anxiety disorders, depression, and substance abuse and their relations to symptoms (Olvet and Hajcak 2008). Emerging evidence also indicates that error processing deficits differ by diagnosis suggesting distinct neural mechanisms and genetic contributions. This has important implications for understanding pathophysiology and for the treatment of associated cognitive and behavioral dysfunction. Below, we evaluate evidence that neuroimaging-based markers of deficient error processing can serve as sensitive endophenotypes of neuropsychiatric disorders.

9.4.1 Schizophrenia

Perseveration, or the contextually inappropriate and unintentional repetition of responses, is a classic behavioral abnormality in schizophrenia. At least some forms of perseveration may reflect a failure to use error feedback to guide behavior. A classic example is continuing to make a previously reinforced response to the Wisconsin Card Sort Test even though feedback indicates that it is no longer correct (e.g., Goldberg et al. 1987). These perseverative errors reflect both motivational and cognitive factors (Summerfelt et al. 1991) and exemplify the behavioral rigidity despite changing contingencies that is often observed in schizophrenia.

Both neuroimaging and electrophysiological studies consistently report blunted neural responses to errors in schizophrenia. fMRI studies show reduced error-related dACC and rACC activations (Carter et al. 2001; Kerns et al. 2005; Laurens et al. 2003). Reduced error-related activation extends to 'reinforcement learning circuitry,' comprising the dACC, substantia nigra, caudate, and putamen, and to 'affective appraisal circuitry' comprising the rACC, insula, and amygdala, in which reduced activation may reflect diminished concern regarding behavioral outcomes (Polli et al. 2008). These reductions remain after statistically controlling for the effects of antipsychotic medication dose and error rate, the latter indicating that the blunted neural response to errors in schizophrenia is not simply a reflection of more frequent, and therefore more predictable errors.

Patients with schizophrenia also consistently show a blunted ERN (Alain et al. 2002; Bates et al. 2002; Foti et al. 2012; Kopp and Rist 1999; Mathalon et al. 2002; Morris et al. 2006; Perez et al. 2012). Even in the context of an abnormal ERN, however, the Pe is intact in patients in many (Alain et al. 2002; Mathalon et al. 2002; Morris et al. 2006; Simmonite et al. 2012) but not all studies (Foti et al. 2012; Perez et al. 2012). Immediate error-related performance adjustments such as post-error slowing and error self-correction are also often intact (Kopp and Rist 1994, 1999; Laurens et al. 2003; Levy et al. 1998; Mathalon et al. 2002; Polli et al. 2006, 2008), although impaired performance adjustments have also been reported (Carter et al. 2001; Malenka et al. 1982, 1986; Turken et al. 2003). Dissociations between intact performance adjustments and reduced ACC activity and ERN amplitude are often seen within single studies (Kopp and Rist 1999; Laurens et al. 2003; Mathalon et al. 2002; Polli et al. 2008) and suggest that error processing deficits in schizophrenia are selective.

Findings of blunted ERN and dACC activation in schizophrenia are remarkably consistent and may reflect a more general problem with reinforcement learning, which is impaired in schizophrenia (Waltz et al. 2007, 2010; Waltz and Gold 2007). They may also reflect functional and structural abnormalities of the cingulate cortex. There is overwhelming evidence of abnormal ACC function and structure in schizophrenia including gray matter abnormalities (e.g., Goldstein et al. 1999; Ha et al. 2004; Kuperberg et al. 2003; Mitelman et al. 2005; Ohnuma et al. 1997; Sigmundsson et al. 2001; Suzuki et al. 2002; Yamasue et al. 2004), volume reductions in the white matter underlying the ACC (McDonald et al. 2005;

Mitelman et al. 2005) and reduced fractional anisotropy of white matter underlying the cingulate cortex in many (Ardekani et al. 2003; Hao et al. 2006; Kubicki et al. 2003; Manoach et al. 2007; Sun et al. 2003; Wang et al. 2004) but not all studies (Agartz et al. 2001; Buchsbaum et al. 1998; Burns et al. 2003; Foong et al. 2002). Histopathological studies give evidence of disturbances in ACC micro- and macrocircuitry that might alter communication with connected regions (e.g., Benes 1993, 2000), consistent with reports of reduced functional and structural connectivity of the ACC in schizophrenia (e.g., Kyriakopoulos et al. 2012; Manoach et al. 2007; Tu et al. 2010; Yan et al. 2012).

Treatment with antipsychotic drugs is an important confound in this literature given their effects on dopamine neurotransmission and indices of error processing (e.g., Zirnheld et al. 2004). Several lines of evidence suggest that deficient error processing is not merely a side effect of treatment. Functional and structural ACC abnormalities, which predict the onset of psychosis (Fornito et al. 2008), are also seen in never-medicated high-risk youth (Whalley et al. 2006), and in never-medicated children experiencing psychotic symptoms (Jacobson et al. 2009). In addition, a blunted ERN, similar to that observed in schizophrenia, is seen in syndromally unaffected siblings (Simmonite et al. 2012), in never-medicated children with putative antecedents to schizophrenia (Laurens et al. 2009) and in antipsychotic naïve patients at high clinical risk for psychosis (Perez et al. 2012). These studies suggest that antipsychotic drugs do not fully account for blunted error processing or other functional and structural ACC abnormalities in schizophrenia. Instead, this literature suggests that ACC abnormalities and error processing deficits are trait markers of genetic vulnerability to schizophrenia that predate the onset of illness. Impairments in evaluating and learning from errors in schizophrenia may substantially contribute to the rigid, perseverative, and maladaptive patterns of thought and behavior that characterize schizophrenia and compromise social and occupational function (Kim et al. 2006). In support of this possibility, a recent study reported that a blunted ERN was associated with more severe negative symptoms and poorer real-world function as indicated by unemployment and rehospitalization (Foti et al. 2012).

9.4.2 Obsessive–Compulsive Disorder (OCD)

OCD is characterized by uncontrollable, unwanted thoughts (i.e., obsessions) and repetitive, ritualized behaviors that individuals feel compelled to perform (compulsions). In contrast to the blunted neural responses to errors in schizophrenia, OCD is often associated with exaggerated error responses including increased error-related ACC activation (Fitzgerald et al. 2005, 2010; Maltby et al. 2005; Ursu et al. 2003) and increased ERN amplitude not only on error trials (Endrass et al. 2008, 2010; Gehring et al. 2000; Johannes et al. 2001; Ruchsow et al. 2005; Santesso et al. 2006; Xiao et al. 2011) but also on correct trials in some (Maltby et al. 2005; Ursu et al. 2003), but not all studies (Fitzgerald et al. 2005;

Gehring et al. 2000). One study reported a normal ERN to errors in OCD (e.g., Nieuwenhuis et al. 2005) and recent findings (Kaczkurkin 2013) including those of a meta-analysis (Mathews et al. 2012) suggest that while the ERN is generally increased, this varies based on the type of task, the level of difficulty and the symptoms present. A recent study of children with OCD found an increased ERN in both patients and their unaffected siblings relative to controls suggesting that the ERN is a marker of genetic risk for OCD (Carrasco et al. 2013). Both increased ERN amplitude (Gehring et al. 2000) and error-related ACC activation (Fitzgerald et al. 2005; Ursu et al. 2003) have been associated with the severity of obsessions and compulsions in OCD suggesting that hyperactive error processing contributes to the defining features of behavioral and cognitive repetition and rigidity. This hypothesis is consistent with a long-standing theory of OCD that inappropriate and exaggerated error signals in response to behavioral outcomes lead to a pervasive sense of incompleteness and self-doubt (Pitman 1987) that triggers the compulsion to repeat behaviors, even if they were already successfully completed (Maltby et al. 2005). In this scenario, an individual suffering from OCD may remember correctly that they locked the door, but inappropriate and persistent error signals may indicate that something is 'not quite right' and compel them to check repeatedly that the door is indeed locked. Findings that the ACC and connected regions show increased activation during symptom provocation in OCD (Breiter et al. 1996) and that cingulotomy relieves obsessions and compulsions (Dougherty et al. 2002) also support the link between hyperactivity in ACC circuitry and rigid, repetitive behaviors.

Measurements of obsessive-compulsive behavior have also been related to indices of error processing in non-clinical samples. Obsessive characteristics are related to the amplitudes of the ERN and Pe in children (Santesso et al. 2006) and to the amplitude of the ERN in college undergraduates (Hajcak and Simons 2002). These findings suggest that obsessive–compulsive traits in the general population are also mediated by error processing mechanisms.

9.4.3 Autism Spectrum Disorders (ASDs)

ASDs are neurodevelopmental disorders that are characterized by three core features: impaired social interaction; impaired communication; and restricted, repetitive, and stereotyped patterns of behavior, interests, and activities. Although repetitive and restricted behaviors are often the most disabling feature of ASD (Bishop et al. 2007), they have received the least research attention. They are present as early as 18 months, predict outcome independently of social and communication deficits, and may interfere with the development of social and communication skills that are deficient in ASD (Morgan et al. 2008; Watt et al. 2008). The hypothesis that error processing deficits characterize ASD and contribute to behavioral repetition and rigidity receives only mixed support from the literature.

Several studies have reported a blunted ERN in ASD (Santesso et al. 2011; Sokhadze et al. 2010, 2012b; South et al. 2010; Vlamings et al. 2008), one has reported normal ERN (Groen et al. 2008), and yet another found an increased latency (and amplitude in a high-functioning subset of participants) of the ERN (Henderson et al. 2006). The finding that repetitive low-frequency transcranial magnetic stimulation (rTMS) to bilateral dorsolateral prefrontal cortex in high-functioning children with ASD was associated with an increased ERN (but also a decreased error rate) suggests the possibility of intervention to modulate error processing (Sokhadze et al. 2012a).

Behaviorally, reduced error self-correction (Russell and Jarrold 1998), normal rates of error self-correction (Thakkar et al. 2008), and reduced post-error slowing (Bogte et al. 2007) have all been observed. Two fMRI studies reported exaggerated error-related ACC activation in ASD (Goldberg et al. 2011; Thakkar et al. 2008) and in one of these, increased ACC activation on correct trials that correlated with higher clinical ratings of restricted, repetitive behavior in ASD, thus linking abnormal error processing to a core symptom (Thakkar et al. 2008). This relation may reflect that reduced discrimination between correct and error outcomes interferes with adjusting behavior to obtain the most favorable outcome. Another compatible possibility is that like OCD, in ASD uncomfortable error signals following correct responses compel repetitive behavior. In ASD, these abnormal signals on correct trials were maximal in the rACC, which is thought to contribute to an appraisal of the affective or motivational salience of errors (Luu et al. 2003; Taylor et al. 2006; van Veen and Carter 2002). Finally, three studies, including the one reporting increased ACC activation on both error and correct trials, have reported reduced fractional anisotropy (FA) in ACC white matter as measured by DTI (Barnea-Goraly et al. 2004; Noriuchi et al. 2010; Thakkar et al. 2008), but not a fourth, which reported increased FA in ACC white matter (Cheng et al. 2010).

In summary, the literature provides only preliminary support for the hypothesis that cingulate cortex abnormalities impair error processing in ASD and contribute to restricted, repetitive behavior. At present, repetitive behaviors in ASD are incompletely understood and neurobiologically valid dimensions have not been delineated. Efforts to understand the contribution of error processing to specific dimensions of repetitive behavior and to identify the underlying mechanisms can guide the development of targeted treatments.

9.5 Rationale for the Use of Neuroimaging-Based Cognitive Endophenotypes

Although Diagnostic and Statistical Manual (DSM) Axis I psychiatric disorders are highly heritable, their genetic origins remain elusive. A major obstacle to identifying genetic risk factors is the difficulty defining neurobiologically valid

phenotypes for inclusion in studies. Current DSM criteria for disorders such as schizophrenia and autism define phenotypes that are so broad that it is possible for two study samples with the same diagnosis to bear little resemblance to one another. This phenotypic heterogeneity suggests etiological and genetic heterogeneity, and reliance on such overly broad diagnostic categories can lead to inconsistent findings across genetic studies. Within studies, relatively large effects may be obscured because they only characterize a subset of the sample. While phenotypic heterogeneity is expected in complex genetic disorders such as schizophrenia and autism, subdivision based on the phenotype has not led to neurobiologically valid subtyping schemes. In schizophrenia, for example, most subtyping schemes have been based on the symptoms (e.g., positive vs. negative, deficit vs. non-deficit, and paranoid vs. non-paranoid), but symptom definitions are broad and imprecise and their assessment is heavily dependent on the self-report of individuals whose disorder often robs them of insight. In addition, symptoms often lack temporal stability and predictive validity (i.e., they do not provide an adequate account of variability in other important measures such as brain structure or function, disease course, or functional outcome). Moreover, neither diagnosis nor symptoms can identify syndromally unaffected relatives who carry susceptibility genes. Finally, the substantially shared genetic liability for neuropsychiatric disorders such as schizophrenia, major depressive disorder, autism spectrum disorder, attention-deficit/hyperactivity disorder and bipolar disorder (e.g., Craddock et al. 2006a; Crespi et al. 2009; Cross-Disorder Group of the Psychiatric Genomics et al. 2013; Purcell et al. 2009) reinforces the fact that our present diagnostic categories and symptom definitions do not map onto distinct underlying genetic etiologies. To the extent that genes *cause* psychiatric disorders and their signs and symptoms, they do so via their effects on brain function (Tan et al. 2008). Given the heterogeneity of present diagnostic categories, alternate phenotyping strategies are needed to understand the genetic origins of psychiatric disorders and to facilitate the development of more valid psychiatric nosology and more effective interventions. This imperative spurred the National Institute of Mental Health (NIMH) to implement a Research Domain Criteria Project, or 'RDoC' (see http://www.nimh.nih.gov/research-funding/rdoc/index.shtml) strategy. The RDoC strategy involves developing, '…for research purposes, new ways of classifying mental disorders based on the dimensions of observable behavior and neurobiological measures.' RDoC encourages researchers to base their selection of subjects on neurobiologically valid dimensions that can be characterized along the causal chain from genes to molecules to circuits to behavior, rather than relying on DSM categories (Fig. 9.4 illustrates a theoretical causal chain for error processing). 'Cognitive Systems' is one of the broad domains identified by RDoC for study, and below, we argue that neuroimaging-based measures of cognition are more sensitive indices of genetic mechanisms than behavior.

While it is well accepted that genetic variation influences brain function and contributes to cognitive deficits in neuropsychiatric disorders, genetically

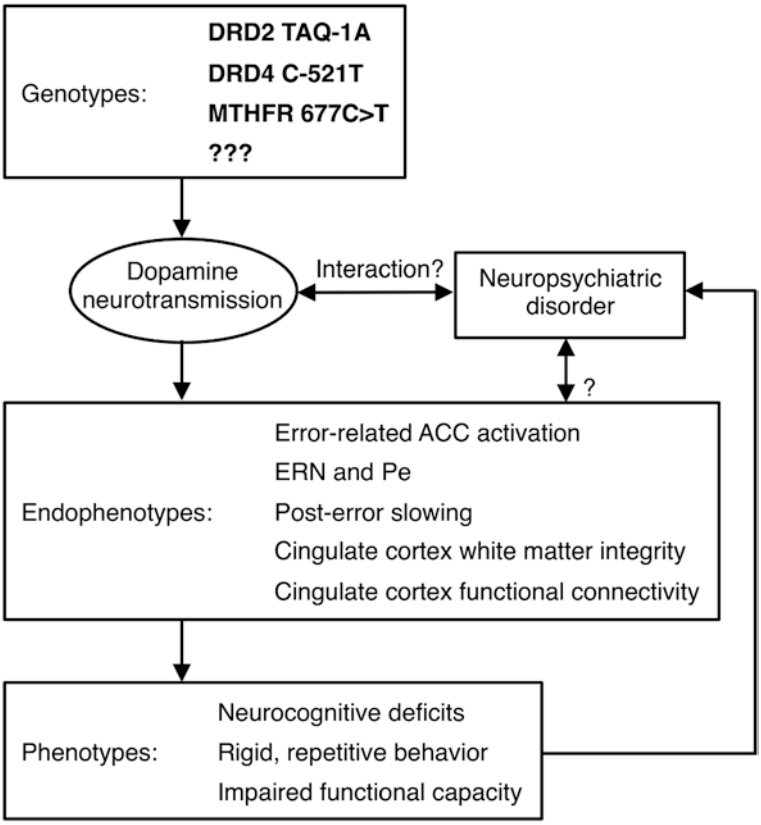

Fig. 9.4 Model of a causal pathway for error processing. Specific genetic polymorphisms affect dopamine neurotransmission, which may interact with a neuropsychiatric disorder to affect neuroimaging-based endophenotypes. These endophenotypes, in turn, contribute to the expression of phenotypes, which may influence whether a psychiatric diagnosis is given

mediated alterations in brain function are not always manifest at the level of behavior. Preserved behavior may reflect the use of an alternate strategy and/or the recruitment of compensatory neural circuitry. Conversely, disordered behavior may reflect not only the brain function of interest, but deficits of other systems, including of the motor output systems that are required to produce the behavior. Thus, behavior is an indirect and possibly unreliable index of genetic effects on brain function. Because brain function is a more direct index of genetic mechanisms than behavior, neuroimaging-based endophenotypes can result in increased effect sizes in studies of genetic variation. Gene effects on functional and structural neuroimaging phenotypes are often highly penetrant (e.g., Canli et al. 2005) and can be surprisingly large (e.g., Roffman et al. 2008a).

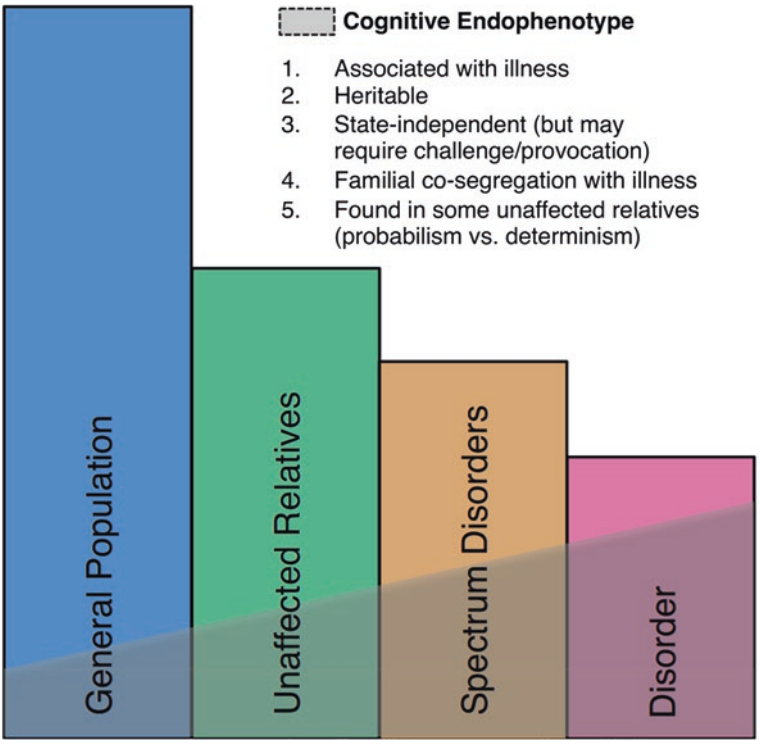

Fig. 9.5 A schematic illustration of the endophenotype concept. *Shaded areas* indicate the presence of the endophenotype in affected patients, individuals with spectrum disorders, syndromally unaffected family members and the general population. Criteria taken from Gould and Gottesman (2006)

This allows the investigation of substantially smaller sample sizes and makes it possible to detect significant genotype effects in the absence of overt behavioral differences (e.g., Roffman et al. 2008a). For these reasons, the study of genetic mediation using neuroimaging-based endophenotypes holds promise for uncovering susceptibility genes, mechanisms of illness, and targets for intervention (Hariri et al. 2006).

Neural markers of errors, such as the ERN, meet several important criteria as endophenotypes (Gottesman and Gould 2003) including high heritability based on both sibling (Albrecht et al. 2008) and twin (Anokhin et al. 2008) studies, established neuroanatomical and neurochemical substrates, and association with psychiatric disorders, though they are also seen in the general population (Fig. 9.5). There is also growing evidence of genetic mediation of neural error markers both in health and psychopathology.

9.6 Genetic Variation Influences Error Processing in Health and Neuropsychiatric Disorders (See Table 9.1 for a Summary)

9.6.1 The Role of Dopamine in Error Processing

Empirical work and theory document a critical role for the dopaminergic system, particularly D2-like DA receptors, in reinforcement learning (Schultz et al. 1997). Reinforcement learning theory has been extended to encompass error-based reinforcement learning and, as described above, both the ERN and error-related dACC activation are seen to arise from this DA-dependent mechanism (Holroyd and Coles 2002). Converging lines of evidence support a role for DA in error processing. Individuals with Parkinson's disease, which is caused by a loss of midbrain DA neurons, have a blunted ERN (Falkenstein et al. 2001; Ito and Kitagawa 2006; Willemssen et al. 2009). Pharmacological manipulation of DA affects neural responses to errors. Haloperidol, a DA D2 receptor antagonist, blunted ERN amplitude in two studies (de Bruijn et al. 2006; Zirnheld et al. 2004), while D-amphetamine, an indirect DA agonist, increased it (de Bruijn et al. 2004). Additional support for a DA-dependent mechanism of error processing comes from findings that genetic polymorphisms affecting DA neurotransmission influence error markers in both health and neuropsychiatric disorders.

Table 9.1 Genetic polymorphisms affecting EEG and fMRI error markers

Polymorphism	Effect on error markers
DRD2-TAQ-IA (rs1800497)	Reduced dACC activation in A1-allele carriers (Klein et al. 2007b), increased ERN in A1 carriers (Meyer et al. 2012), no effect on the ERN (Althaus et al. 2009)
DRD4 C-521T (rs1800955)	Increased ERN in T-allele carriers (Kramer et al. 2007)
DRD4 exon 3 VNTR	Reduced ERN in 7R-allele carriers (Biehl et al. 2011)
DAT1 3′-UTR VNTR	Increased ERN (Meyer et al. 2012), increased Pe (Althaus et al. 2010), and decreased Pe (Biehl et al. 2011) in 9R-allele carriers
COMT Val^{158}Met (rs4680)	In *Val*-allele carriers increased ERN (Osinsky et al. 2012) or a trend to an increased ERN (Kramer et al. 2007), no effect on the ERN but increased Pe in *Met* homozygotes (Frank et al. 2007)
MTHFR 677C > T (rs1801133)	Reduced dACC activation in T-allele carriers (Roffman et al. 2011a, c)
Serotonin transporter 5-HTTLPR	Increased ERN in short allele homozygotes (Fallgatter et al. 2004), no effect on the ERN (Olvet et al. 2010)
5-HT1A receptor C-1019G (rs6295)	Reduced ERN in G-allele carriers (Beste et al. 2010).
BDNF Val^{66}Met (rs6265)	Reduced ERN and post-error slowing in *Met*-allele carriers (Beste et al. 2012)
NPSR Asn^{107}Ile (rs324981)	Increased ERN and post-error slowing in Ile carriers (Beste et al. 2013)

DRD2 TAQ-IA: The DA D2 receptor gene is a risk gene for schizophrenia (Shi et al. 2008) and the polymorphism, *TAQ-1A* (rs1800497), which is associated with schizophrenia (Parsons et al. 2007), predicts response to treatment with risperidone (Ikeda et al. 2008) and aripiprazole (Kwon et al. 2008). An fMRI study of healthy individuals (Klein et al. 2007b) found that *A1*-allele carriers, with putatively reduced striatal DA receptor density (Jonsson et al. 1999; Pohjalainen et al. 1998; Ritchie and Noble 2003), showed decreased dACC activation in response to errors and decreased avoidance learning, suggesting that they were less efficient in learning from errors. *A1*-allele carriers also showed decreased functional connectivity of the dACC and striatum. With regard to the ERN, there are conflicting reports of no association with *DRD2 TAQ-IA* (Althaus et al. 2009) and an increased ERN amplitude in *A1*-allele carriers (Meyer et al. 2012).

DRD4 C-521T: The DA D4 receptor gene (*DRD4*) is also a candidate gene for schizophrenia (Shi et al. 2008) and the -521 single nucleotide polymorphism (SNP) refers to a *C*-to-*T* substitution in the *DRD4* promoter region (rs1800955) with the *T* allele resulting in 40% less transcriptional efficiency (Okuyama et al. 1999). The *DRD4-521C* allele has been associated with schizophrenia (Allen et al. 2008; Okuyama et al. 1999; Xing et al. 2003) and healthy individuals homozygous for the *C* allele showed a decreased ERN and decreased post-error slowing compared to *T* homozygotes (Kramer et al. 2007).

DRD4 exon 3 VNTR: Another *DRD4* polymorphism linked to error processing consists of a variable number of tandem repeats of a 48-base-pair sequence in the third exon (Van Tol et al. 1992). The most frequently occurring numbers of repeats are 4 (*4R*; 70%), 7 (*7R*; 20%), and 2 (*2R*; 5%) (Asghari et al. 1995). The *7R* allele has been associated with higher risk of OCD (Taj et al. 2013), with tics in OCD (Cruz et al. 1997), and with reduced ERN amplitude, but comparable Pe (Biehl et al. 2011).

The DA transporter *(DAT1) 3′-UTR VNTR*: DAT1 plays a key role in regulating DA neurotransmission by facilitating reuptake of DA in the synaptic cleft (Jaber et al. 1997). A polymorphism in the 3′-untranslated region (3′-UTR) of this gene consists of a variable number of tandem repeats of a 40-base-pair sequence, ranging from 3 to 11 copies of the repeated sequence, with the most common variants being 9 (*9R*; 24%) and 10 (*10R*; 70%) repeats (Vandenbergh et al. 1992). Carriers of the *9R* allele have increased levels of DAT1 in the striatum (van de Giessen et al. 2009; van Dyck et al. 2005) and a trend for increased risk of OCD based on the meta-analysis (Liu et al. 2012). The *9R* allele has also been associated with a larger ERN (referred to as ΔERN in Meyer et al. 2012), and a larger Pe in one study (Althaus et al. 2010), but a smaller Pe in a second study (Biehl et al. 2011).

COMT Val^{158}*Met*: A G-to-A SNP in the catechol-*O*-methyltransferase (COMT) gene leads to a valine-to-methionine substitution (*COMT Val*^{158}*Met, rs4680*). COMT metabolizes released DA and the *Met* allele significantly reduces COMT activity, leading to higher DA. The *COMT Val*^{158}*Met* polymorphism has been studied extensively in relation to schizophrenia, and several meta-analyses have argued against association (Fan et al. 2005; Munafo et al. 2005; Okochi et al. 2009). While *COMT Val*^{158}*Met* primarily affects DA availability in the prefrontal cortex (Craddock et al. 2006b; Egan et al. 2001), it may also have downstream effects on midbrain DA

(Meyer-Lindenberg et al. 2005). Studies of error processing have yielded inconsistent findings, showing an increased amplitude of the ERN in *Val*-allele carriers (Osinsky et al. 2012), only a trend-level enhancement of the ERN in *Val* compared to *Met* homozygotes (Kramer et al. 2007), and no effect of *COMT* $Val^{158}Met$ on the ERN but an increased Pe in *Met* homozygotes compared to *Val* carriers (Frank et al. 2007).

MTHFR 677C>T: The hypofunctional *677T* variant in the methylenetetrahydrofolate reductase gene (*MTHFR 677C>T, rs1801133*) has been associated with increased risk for schizophrenia (Allen et al. 2008; Gilbody et al. 2007), executive dysfunction (Roffman et al. 2008b), and negative symptoms (Roffman et al. 2008c). Several steps in the DA life cycle rely on methylation reactions regulated by MTHFR (Friso et al. 2002) and each copy of the *T* allele reduces MTHFR activity by 35% (Frosst et al. 1995). The *T* allele has been shown to reduce dorsolateral prefrontal cortex fMRI activation during working memory performance in schizophrenia, both on its own, and via epistatic interactions with the low-DA *COMT 158Val* allele, supporting a role of *MTHFR* in prefrontal DA signaling (Roffman et al. 2008a). There is also indirect evidence linking *MTHFR* to striatal DA. MTHFR is a key enzyme in the metabolism of homocysteine, which has toxic effects on DA neurons in the striatum of rats (Imamura et al. 2007). In alcohol-dependent individuals, *MTHFR 677T* has been associated with higher plasma levels of homocysteine and increased risk of withdrawal seizures, which were interpreted to reflect the neurotoxic effects of homocysteine on the mesencephalic DA system (Lutz 2008; Lutz et al. 2006, 2007).

In a prior study of executive function in schizophrenia (Roffman et al. 2008b), *MTHFR 677T* was specifically related to a behavioral index of error processing, namely increased perseverative errors on the Wisconsin Card Sort Test, which reflect a failure to use feedback to adjust behavior. Recent work has demonstrated significant *677T*-allele-related reductions in error-related fMRI activation of the dACC in healthy individuals and in two independent samples of patients with schizophrenia (Roffman et al. 2011a, b). The reductions in dACC activation were linearly related to allele dose regardless of diagnosis (Roffman et al. 2011a). This suggests that *MTHFR 677T* mediates error processing in both health and schizophrenia.

9.6.2 Other Genetic Variation Related to Error Processing

The serotonin transporter gene (5-HTTLPR): Evidence linking serotonin to ACC function and structure comes from studies of a functional length variation in the transcriptional control region of the serotonin transporter gene in healthy individuals. This polymorphism was associated with differences in the anatomy and function of the amygdala-rACC circuit in healthy individuals (Pezawas et al. 2005), which has been implicated in generating and learning from negative affect (for review, see Baxter and Murray 2002; Drevets 2000; Zald 2003). This learning may extend to errors since both rACC and amygdala respond to errors, and together, activation in these structures predicts error rate (Polli et al. 2008, 2009).

More direct evidence of a role for this polymorphism in error processing are findings of a significantly increased ERN amplitude and a trend to increased Pe amplitude in short allele homozygotes, who presumably produce less serotonin transporter transcript, compared to long allele homozygotes (Fallgatter et al. 2004). A larger study, however, failed to replicate the association of *5-HTTLPR* genotype with ERN amplitude (Olvet et al. 2010).

5-HT1A receptor gene C-1019G: A SNP present in about a third of the population consisting of an extra base pair in the promoter region of the *5-HT1A* receptor gene (*C-1019G*, rs6295) has been associated with reduced ERN and post-error slowing (Beste et al. 2010). The presence of a guanine nucleotide prevents binding of repressor proteins, which leads to enhanced gene expression and reduced serotonergic transmission (Lemonde et al. 2003). The *G* allele has been linked to increased risk of schizophrenia (Huang et al. 2004) and to worse treatment outcomes (Mossner et al. 2009; Reynolds et al. 2006), but a meta-analysis reported no association with schizophrenia (Kishi et al. 2011).

BDNF Val^{66}*Met*: The brain-derived neurotrophic factor (BDNF) is a nerve growth factor thought to facilitate synaptic connections in the brain (Cohen-Cory et al. 1996). A SNP in the eponymous gene, which encodes for BDNF, results in valine-to-methionine substitution in the prodomain of the protein (*BDNF Val*^{66}*Met*, rs6265) that leads to reduced activity-dependent secretion of BDNF (Egan et al. 2003). One meta-analysis found an elevated risk for schizophrenia in homozygous *Met* carriers (Gratacos et al. 2007), but another did not (Kanazawa et al. 2007). The *Met* allele has been associated with earlier onset of schizophrenia (Chao et al. 2008) and reductions of ERN amplitude and post-error slowing (Beste et al. 2012).

NPSR Asn^{107}*Ile*: Neuropeptide S (NPS) is a 20 amino acid peptide that modulates stress and arousal (Okamura and Reinscheid 2007). An A-to-T substitution at position 107 of the gene encoding for the NPS receptor (NPSR) leads to an amino acid exchange from Asn to Ile (Asn^{107}Ile, rs324981) and increases the efficacy of NPS about tenfold (Reinscheid et al. 2005). The T allele is thought to be related to anxiety disorders, particularly panic disorder (Domschke et al. 2011), and is associated with an increased ERN and more pronounced post-error slowing (Beste et al. 2013).

9.7 Challenges to the Study of Neural Indices Error Processing as Endophenotypes

9.7.1 Failures of Replication in Imaging-Genetics Studies

Failures of replication are extremely common in imaging-genetics studies and represent a major challenge. Imaging-genetics findings are often based on relatively small samples, and negative results are much less likely to be published. Smaller

samples are often justified based on the evidence that neuroimaging-based endophenotypes result in increased effect sizes in studies of genetic variation than behavior or diagnosis. The pragmatic justification is that neuroimaging studies are costly and require considerable infrastructure to accomplish. Relatively small and comprehensive studies can identify the most promising cognitive constructs and endophenotypes, which can then be exported for use in larger multisite studies of patients, relatives, and racially and ethnically homogeneous groups as has been done for studies of other putative cognitive endophenotypes (e.g., Radant et al. 2010; Turetsky et al. 2008). Studies in developing countries such as China can complement and extend these efforts by identifying overlapping and distinct genetic contributions in non-Western populations (e.g., Chan et al. 2010). To protect against false-positive associations in smaller studies, it is often advisable to investigate the effects of only a limited set of polymorphisms that are selected based on stringent criteria and to seek convergence in the data. This strategy can maximize scientific yield while minimizing the risk of spurious findings by focusing on a hypothesis-driven set of loci that affect specific neural mechanisms and are most likely to affect a particular endophenotype or set of related endophenotypes given the current state of knowledge. A limitation to this approach is that it will not represent the full complement of genes that influence the phenotypes of interest.

9.7.2 Methodological Differences Across Studies May Lead to Conflicting Findings

A major challenge in the error processing literature is that the definition and measurement of neural indices of error processing vary across studies. The ERN, for example, can be defined based on the peak of negativity in either the error waveform alone or in the difference (error vs. correct) waveform. It is arguable which method is more valid. Such measurement differences can affect study outcomes as can be illustrated in OCD, which is characterized by exaggerated neural responses on both correct and error trials. Several studies reporting an increased ERN in OCD, or in non-clinical populations with OCD symptoms defined it using only the error trial (Endrass et al. 2008, 2010; Gehring et al. 2000; Hajcak and Simons 2002; Johannes et al. 2001). In at least two of these studies, the waveform for correct trials was also more negative in OCD participants than controls (referred to as the correct-related negativity or CRN). Consequently, had the ERN been defined as the difference waveform, it might not have been greater in OCD patients than controls.

Methodological differences may also contribute to discrepancies in fMRI results. For example, most standard fMRI analysis techniques assume a shape to the hemodynamic response. While this is a statistically powerful technique when the models are correct, a single assumed model is unlikely to be valid across all

brain regions and stimulus types (Duann et al. 2002) and, importantly, model inaccuracies may lead to the misattribution of activity to adjacent events (Manoach et al. 2003). Thus, it is possible that in some studies, increased ACC activation on error trials may reflect greater activation while planning or preparing the response rather than an exaggerated response to the error. Finite impulse response (FIR, Burock and Dale 2000; Jansma et al. 2013) or other models that make no a priori assumptions about the shape of the hemodynamic response may more accurately distinguish preparatory activation from error-related activation and can also be used to evaluate the temporal characteristics of the hemodynamic response, which may differ between the study groups (e.g., Dyckman et al. 2011).

Conflicting findings of error processing deficits in particular disorders may also arise from the use of different tasks and levels of difficulty. The characteristics of the samples studied such as whether certain symptoms are present, treatment with medications, and task performance also matter (e.g., Mathews et al. 2012). By affecting neurotransmitter systems that mediate error responses, medications such as antipsychotics, selective serotonin reuptake inhibitors, and antidepressants may affect outcome measures and obscure group differences and effects of genetic variation. Task performance is also important to consider in evaluating error indices. More frequent errors are also more predictable, and ERN amplitude is thought to code the degree to which errors are unexpected (Brown and Braver 2005; Holroyd and Coles 2002), consistent with findings of inverse correlations between error rate and ERN amplitude (Agam et al. 2011; Gehring et al. 1993; Hajcak et al. 2003). The same may be true for dACC activation, which also correlates with error rate in some studies (e.g., Polli et al. 2008). Thus, different error rates in patient and control samples or in pre- and post-treatment conditions (e.g., Sokhadze et al. 2012a) represent a potential confound that could be statistically controlled (e.g., Polli et al. 2008). For example, in several ERN studies, ASD participants had a higher error rate than controls (e.g., Sokhadze et al. 2010, 2012b; South et al. 2010), making it unclear whether the blunted ERN reflected more frequent errors or deficient error recognition and signaling.

9.7.3 Limitations to the Clinical Utility of Error Processing Endophenotypes

Unlike neurodegenerative disorders such as Alzheimer's disease, which are associated with specific neuropathologies, neuropsychiatric disorders likely have multiple overlapping etiologies and neuropathologies. Consequently, neuropsychiatric disorders lack sensitive and specific pathophysiological markers such as amyloid beta-protein, which is specific to Alzheimer's disease, is thought to cause the associated dementia, and can be measured in vivo to assess the risk of developing symptoms and response to therapy (Klunk 2011). Error processing endophenotypes, in contrast to amyloid beta-protein, do not index a known, specific

neuropathology, rather they may indicate cognitive dysfunction and genetic vulnerability to illness and their diagnostic specificity remains to be established. Also, unlike amyloid beta-protein, whose presence is usually associated with pathology, neural error markers are normally present and abnormality is defined as statistical deviation of their parameters from the norm, which varies from study to study. Measurement variability, the lack of consensus definitions of error markers, and the absence of large-scale studies make it difficult to define clear cutoffs for 'normality.' In addition, error processing endophenotypes, such as a blunted or exaggerated ERN or error-related dACC activation, are only probabilistically associated with illness, they do not determine illness. The cognitive dysfunction that they index may make illness more probable, but is likely just one of a number of cumulative hits of relatively small effect. Given that we lack a comprehensive understanding of the genetic contributions to these markers, it is difficult to distinguish 'false positives' (i.e., abnormal error markers in the absence of genetic risk in an otherwise healthy individual) from valid genetic vulnerability for a disorder that has not manifested itself for environmental reasons or due to other, protective, epigenetic, or genetic factors. Similarly, because endophenotypes are only probabilistically related to illness and current diagnostic categories are heterogeneous, they may only be present in a subset of individuals within a given diagnostic group.

Establishing the clinical relevance of error processing deficits: While there is clear evidence that deficient error processing is associated with symptoms and functional outcome in neuropsychiatric disorders, further research is required to fully elaborate the bases of these relations both within and across the disorders. If, as we and others have proposed, deficient error processing mediates the pathway between genetic predisposition and illness by interfering with adaptive responses to outcomes (e.g., Olvet and Hajcak 2008), early intervention and prevention may be possible, for example, in individuals at high risk for schizophrenia who show a blunted ERN (e.g., Laurens et al. 2009; Perez et al. 2012; Simmonite et al. 2012). It may also be possible to intervene to prevent relapse. Two recent studies demonstrate that error-related dACC activation predicts relapse and time to relapse in cocaine-dependent individuals (Luo et al. 2013) and recidivism in criminal offenders (Aharoni et al. 2013). These findings provide a rationale for the development of interventions to ameliorate error processing deficits in neuropsychiatric disorders as well as other populations characterized by repetitive, maladaptive behaviors.

9.8 Conclusion

The existing literature on error processing allows the generation of biologically plausible hypotheses concerning the effects of genetic variation on well-validated and heritable indices of error processing that are abnormal in neuropsychiatric disorders, show evidence of diagnostic specificity, contribute to disability, and are

thought to be mediated by specific neural mechanisms. Understanding the genetic mediation and mechanisms of error processing deficits in neuropsychiatric disorders may eventually lead to the development of specifically targeted interventions and enable the use genetic information to identify individuals most likely to benefit from these treatments. This can substantially reduce outcome variability, thereby increasing power, and reducing the required sample size and cost of treatment trials. The findings of imaging-genetics investigations may also provide novel neural and behavioral targets for treatment and sensitive surrogate markers of treatment response. Treating error processing deficits may significantly affect functional outcome in neuropsychiatric disorders or possibly even prevent onset or relapse since error signals provide crucial information for flexible adaptation to changing environments, and deficits in learning from errors, as indexed by abnormal neural responses and reduced behavioral adaptation, likely substantially contribute to rigid, perseverative, and maladaptive patterns of behavior. Given the dearth of effective interventions for cognitive deficits in neuropsychiatric disorders, this represents a promising approach.

Acknowledgements The chapter authors are grateful to Jordan Smoller and Daniel Z. Press for consultation.

References

Agam Y, Hamalainen MS, Lee AK et al (2011) Multimodal neuroimaging dissociates hemodynamic and electrophysiological correlates of error processing. Proc Natl Acad Sci U S A 108(42):17556–17561

Agam Y, Carey C, Barton JJS et al (in press) Network dynamics underlying speed-accuracy trade-offs in response to errors. PLoS One 12;8(9):e73692

Agartz I, Andersson JL, Skare S (2001) Abnormal brain white matter in schizophrenia: a diffusion tensor imaging study. NeuroReport 12(10):2251–2254

Aharoni E, Vincent GM, Harenski CL et al (2013) Neuroprediction of future rearrest. Proc Natl Acad Sci U S A 9;110(15):6223–6228

Alain C, McNeely HE, He Y et al (2002) Neurophysiological evidence of error-monitoring deficits in patients with schizophrenia. Cereb Cortex 12(8):840–846

Albrecht B, Brandeis D, Uebel H et al (2008) Action monitoring in boys with attention-deficit/hyperactivity disorder, their nonaffected siblings, and normal control subjects: evidence for an endophenotype. Biol Psychiatry 64(7):615–625

Allen NC, Bagade S, McQueen MB et al (2008) Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat Genet 40(7):827–834

Althaus M, Groen Y, Wijers AA et al (2009) Differential effects of 5-HTTLPR and DRD2/ANKK1 polymorphisms on electrocortical measures of error and feedback processing in children. Clin Neurophysiol 120(1):93–107

Althaus M, Groen Y, Wijers AA et al (2010) Variants of the SLC6A3 (DAT1) polymorphism affect performance monitoring-related cortical evoked potentials that are associated with ADHD. Biol Psychol 85(1):19–32

Andalman AS, Fee MS (2009) A basal ganglia-forebrain circuit in the songbird biases motor output to avoid vocal errors. Proc Natl Acad Sci U S A 106(30):12518–12523

Anokhin AP, Golosheykin S, Heath AC (2008) Heritability of frontal brain function related to action monitoring. Psychophysiology 45(4):524–534

Ardekani BA, Nierenberg J, Hoptman MJ et al (2003) MRI study of white matter diffusion anisotropy in schizophrenia. NeuroReport 14(16):2025–2029
Asghari V, Sanyal S, Buchwaldt S et al (1995) Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. J Neurochem 65(3):1157–1165
Barnea-Goraly N, Kwon H, Menon V et al (2004) White matter structure in autism: preliminary evidence from diffusion tensor imaging. Biol Psychiatry 55(3):323–326
Bates AT, Kiehl KA, Laurens KR et al (2002) Error-related negativity and correct response negativity in schizophrenia. Clin Neurophysiol 113(9):1454–1463
Baxter MG, Murray EA (2002) The amygdala and reward. Nat Rev Neurosci 3(7):563–573
Benes FM (1993) Neurobiological investigations in cingulate cortex of schizophrenic brain. Schizophr Bull 19(3):537–549
Benes FM (2000) Emerging principles of altered neural circuitry in schizophrenia. Brain Res Brain Res Rev 31(2–3):251–269
Beste C, Domschke K, Kolev V et al (2010) Functional 5-HT1a receptor polymorphism selectively modulates error-specific subprocesses of performance monitoring. Hum Brain Mapp 31(4):621–630
Beste C, Kolev V, Yordanova J et al (2012) The role of the BDNF Val66Met polymorphism for the synchronization of error-specific neural networks. J Neurosci 30(32):10727–10733
Beste C, Konrad C, Uhlmann C et al (2013) Neuropeptide S receptor (NPSR1) gene variation modulates response inhibition and error monitoring. Neuroimage 71:1–9
Biehl SC, Dresler T, Reif A et al (2011) Dopamine transporter (DAT1) and dopamine receptor D4 (DRD4) genotypes differentially impact on electrophysiological correlates of error processing. PLoS ONE 6(12):e28396
Bishop SL, Richler J, Cain AC et al (2007) Predictors of perceived negative impact in mothers of children with autism spectrum disorder. Am J Ment Retard 112(6):450–461
Bogte H, Flamma B, van der Meere J et al (2007) Post-error adaptation in adults with high functioning autism. Neuropsychologia 45(8):1707–1714
Brazdil M, Roman R, Falkenstein M et al (2002) Error processing–evidence from intracerebral ERP recordings. Exp Brain Res 146(4):460–466
Breiter HC, Rauch SL, Kwong KK et al (1996) Functional magnetic resonance imaging of symptom provocation in obsessive-compulsive disorder. Arch Gen Psychiatry 53(7):595–606
Brown JW, Braver TS (2005) Learned predictions of error likelihood in the anterior cingulate cortex. Science 307(5712):1118–1121
Broyd SJ, Demanuele C, Debener S et al (2009) Default-mode brain dysfunction in mental disorders: a systematic review. Neurosci Biobehav Rev 33(3):279–296
Buchsbaum MS, Tang CY, Peled S et al (1998) MRI white matter diffusion anisotropy and PET metabolic rate in schizophrenia. NeuroReport 9(3):425–430
Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci 1124:1–38
Burns J, Job D, Bastin ME et al (2003) Structural disconnectivity in schizophrenia: a diffusion tensor magnetic resonance imaging study. Br J Psychiatry 182:439–443
Burock MA, Dale AM (2000) Estimation and detection of event-related fMRI signals with temporally correlated noise: a statistically efficient and unbiased approach. Hum Brain Mapp 11(4):249–260
Bush G, Whalen PJ, Rosen BR et al (1998) The counting stroop: an interference task specialized for functional neuroimaging—validation study with functional MRI. Hum Brain Mapp 6(4):270–282
Bush G, Luu P, Posner MI (2000) Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 4(6):215–222
Canli T, Omura K, Haas BW et al (2005) Beyond affect: a role for genetic variation of the serotonin transporter in neural activation during a cognitive attention task. Proc Natl Acad Sci U S A 102(34):12224–12229
Carrasco M, Harbin SM, Nienhuis JK et al (2013) Increased error-related brain activity in youth with obsessive-compulsive disorder and unaffected siblings. Depress Anxiety 30(1):39–46

Carter CS, MacDonald AW 3rd, Ross LL et al (2001) Anterior cingulate cortex activity and impaired self-monitoring of performance in patients with schizophrenia: an event-related fMRI study. Am J Psychiatry 158(9):1423–1428

Chan RC, Gottesman II, Ge X et al (2010) Strategies for the study of neuropsychiatric disorders using endophenotypes in developing countries: a potential databank from China. Front Hum Neurosci 4:207

Chao HM, Kao HT, Porton B (2008) BDNF Val66Met variant and age of onset in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 147B(4):505–506

Cheng Y, Chou KH, Chen IY et al (2010) Atypical development of white matter microstructure in adolescents with autism spectrum disorders. Neuroimage 50(3):873–882

Cohen-Cory S, Escandon E, Fraser SE (1996) The cellular patterns of BDNF and trkB expression suggest multiple roles for BDNF during Xenopus visual system development. Dev Biol 179(1):102–115

Craddock N, O'Donovan MC, Owen MJ (2006a) Genes for schizophrenia and bipolar disorder? Implications for psychiatric nosology. Schizophr Bull 32(1):9–16

Craddock N, Owen MJ, O'Donovan MC (2006b) The catechol-*O*-methyl transferase (COMT) gene as a candidate for psychiatric phenotypes: evidence and lessons. Mol Psychiatry 11(5):446–458

Crespi B, Stead P, Elliot M (2009) Evolution in health and medicine Sackler colloquium: comparative genomics of autism and schizophrenia. Proc Natl Acad Sci U S A 26(107 Suppl 1):1736–1741

Cross-Disorder Group of the Psychiatric Genomics Consortium, Smoller JW, Craddock N et al (2013) Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet 381(9875):1371–1379

Cruz C, Camarena B, King N et al (1997) Increased prevalence of the seven-repeat variant of the dopamine D4 receptor gene in patients with obsessive-compulsive disorder with tics. Neurosci Lett 231(1):1–4

de Bruijn ER, Hulstijn W, Verkes RJ et al (2004) Drug-induced stimulation and suppression of action monitoring in healthy volunteers. Psychopharmacology 177(1–2):151–160

de Bruijn ER, Sabbe BG, Hulstijn W et al (2006) Effects of antipsychotic and antidepressant drugs on action monitoring in healthy volunteers. Brain Res 1105(1):122–129

Debener S, Ullsperger M, Siegel M et al (2005) Trial-by-trial coupling of concurrent electroencephalogram and functional magnetic resonance imaging identifies the dynamics of performance monitoring. J Neurosci 25(50):11730–11737

Dehaene S, Posner MI, Tucker DM (1994) Localization of a neural system for error detection and compensation. Psychol Sci 5(5):303–305

Devinsky O, Morrell MJ, Vogt BA (1995) Contributions of anterior cingulate cortex to behaviour. Brain 118(Pt 1):279–306

Domschke K, Reif A, Weber H et al (2011) Neuropeptide S receptor gene—converging evidence for a role in panic disorder. Mol psychiatry 16(9):938–948

Donamayor N, Marco-Pallares J, Heldmann M et al (2011) Temporal dynamics of reward processing revealed by magnetoencephalography. Hum Brain Mapp 32(12):2228–2240

Dougherty DD, Baer L, Cosgrove GR et al (2002) Prospective long-term follow-up of 44 patients who received cingulotomy for treatment-refractory obsessive-compulsive disorder. Am J Psychiatry 159(2):269–275

Drevets WC (2000) Neuroimaging studies of mood disorders. Biol Psychiatry 48(8):813–829

Drevets WC, Raichle ME (1998) Reciprocal suppression of regional cerebral blood flow during emotional versus higher cognitive processes: implications for interactions between emotion and cognition. Cognit Emot 12(3):353–385

Duann JR, Jung TP, Kuo WJ et al (2002) Single-trial variability in event-related BOLD signals. Neuroimage 15(4):823–835

Dyckman KA, Lee AKC, Agam Y et al (2011) Abnormally persistent fMRI activation during antisaccades in schizophrenia: a neural correlate of perseveration? Schizophr Res 132(1):62–68

Egan MF, Goldberg TE, Kolachana BS et al (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A 98(12):6917–6922

Egan MF, Kojima M, Callicott JH et al (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112(2):257–269

Eichele T, Debener S, Calhoun VD et al (2008) Prediction of human errors by maladaptive changes in event-related brain networks. Proc Natl Acad Sci U S A 105(16):6173–6178

Endrass T, Franke C, Kathmann N (2005) Error awareness in a saccade countermanding task. J Psychophysiol 19(4):275–280

Endrass T, Reuter B, Kathmann N (2007) ERP correlates of conscious error recognition: aware and unaware errors in an antisaccade task. Eur J Neurosci 26(6):1714–1720

Endrass T, Klawohn J, Schuster F et al (2008) Overactive performance monitoring in obsessive-compulsive disorder: ERP evidence from correct and erroneous reactions. Neuropsychologia 46(7):1877–1887

Endrass T, Schuermann B, Kaufmann C et al (2010) Performance monitoring and error significance in patients with obsessive-compulsive disorder. Biol Psychol 84(2):257–263

Eriksen BA, Eriksen CW (1974) Effects of noise letters upon the identification of a target letter in a nonsearch task. Percept Psychophys 16:143–149

Falkenstein M, Hohnsbein J, Hoormann J et al (1991) Effects of crossmodal divided attention on late ERP components. II. Error processing in choice reaction tasks. Electroencephalogr Clin Neurophysiol 78(6):447–455

Falkenstein M, Hielscher H, Dziobek I et al (2001) Action monitoring, error detection, and the basal ganglia: an ERP study. NeuroReport 12(1):157–161

Fallgatter AJ, Herrmann MJ, Roemmler J et al (2004) Allelic variation of serotonin transporter function modulates the brain electrical response for error processing. Neuropsychopharmacology 29(8):1506–1511

Fan JB, Zhang CS, Gu NF et al (2005) Catechol-*O*-methyltransferase gene Val/Met functional polymorphism and risk of schizophrenia: a large-scale association study plus meta-analysis. Biol Psychiatry 57(2):139–144

Fassbender C, Murphy K, Foxe JJ et al (2004) A topography of executive functions and their interactions revealed by functional magnetic resonance imaging. Brain Res Cogn Brain Res 20(2):132–143

Fiehler K, Ullsperger M, von Cramon DY (2005) Electrophysiological correlates of error correction. Psychophysiology 42(1):72–82

Fitzgerald KD, Welsh RC, Gehring WJ et al (2005) Error-related hyperactivity of the anterior cingulate cortex in obsessive-compulsive disorder. Biol Psychiatry 57(3):287–294

Fitzgerald KD, Perkins SC, Angstadt M et al (2010) The development of performance-monitoring function in the posterior medial frontal cortex. Neuroimage 49(4):3463–3473

Foong J, Symms MR, Barker GJ et al (2002) Investigating regional white matter in schizophrenia using diffusion tensor imaging. NeuroReport 13(3):333–336

Fornito A, Yung AR, Wood SJ et al (2008) Anatomic abnormalities of the anterior cingulate cortex before psychosis onset: an MRI study of ultra-high-risk individuals. Biol Psychiatry 64(9):758–765

Foti D, Kotov R, Bromet E et al (2012) Beyond the broken error-related negativity: functional and diagnostic correlates of error processing in psychosis. Biol Psychiatry 71(10):864–872

Frank MJ, D'Lauro C, Curran T (2007) Cross-task individual differences in error processing: neural, electrophysiological, and genetic components. Cogn Affect Behav Neurosci 7(4):297–308

Friso S, Choi SW, Girelli D et al (2002) A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A 99(8):5606–5611

Frosst P, Blom HJ, Milos R et al (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 10(1):111–113
Fujiwara J, Tobler PN, Taira M et al (2009) Segregated and integrated coding of reward and punishment in the cingulate cortex. J Neurophysiol 101(6):3284–3293
Garavan H, Ross TJ, Murphy K et al (2002) Dissociable executive functions in the dynamic control of behavior: inhibition, error detection, and correction. NeuroImage 17(4):1820–1829
Gehring WJ, Fencsik DE (2001) Functions of the medial frontal cortex in the processing of conflict and errors. J Neurosci 21(23):9430–9437
Gehring WJ, Goss B, Coles MG et al (1993) A neural system for error detection and compensation. Psychol Sci 4(6):385–390
Gehring WJ, Himle J, Nisenson LG (2000) Action-monitoring dysfunction in obsessive-compulsive disorder. Psychol Sci 11(1):1–6
Gilbody S, Lewis S, Lightfoot T (2007) Methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms and psychiatric disorders: a HuGE review. Am J Epidemiol 165(1):1–13
Goldberg TE, Weinberger DR, Berman KF et al (1987) Further evidence for dementia of the prefrontal type in schizophrenia? A controlled study of teaching the Wisconsin Card Sorting Test [see comments]. Arch Gen Psychiatry 44(11):1008–1014
Goldberg MC, Spinelli S, Joel S et al (2011) Children with high functioning autism show increased prefrontal and temporal cortex activity during error monitoring. Dev Cogn Neurosci 1(1):47–56
Goldstein JM, Goodman JM, Seidman LJ et al (1999) Cortical abnormalities in schizophrenia identified by structural magnetic resonance imaging. Arch Gen Psychiatry 56(6):537–547
Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160(4):636–645
Gould TD, Gottesman II (2006) Psychiatric endophenotypes and the development of valid animal models. Genes Brain Behav 5(2):113–119
Gratacos M, Gonzalez JR, Mercader JM et al (2007) Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. Biol Psychiatry 61(7):911–922
Groen Y, Wijers AA, Mulder LJ et al (2008) Error and feedback processing in children with ADHD and children with Autistic Spectrum Disorder: an EEG event-related potential study. Clin Neurophysiol 119(11):2476–2493
Ha TH, Youn T, Ha KS et al (2004) Gray matter abnormalities in paranoid schizophrenia and their clinical correlations. Psychiatry Res 132(3):251–260
Hajcak G, Simons RF (2002) Error-related brain activity in obsessive-compulsive undergraduates. Psychiatry Res 110(1):63–72
Hajcak G, McDonald N, Simons RF (2003) To err is autonomic: error-related brain potentials, ANS activity, and post-error compensatory behavior. Psychophysiology 40(6):895–903
Hajcak G, McDonald N, Simons RF (2004) Error-related psychophysiology and negative affect. Brain Cogn 56(2):189–197
Hallett PE (1978) Primary and secondary saccades to goals defined by instructions. Vision Res 18(10):1279–1296
Hao Y, Liu Z, Jiang T et al (2006) White matter integrity of the whole brain is disrupted in first-episode schizophrenia. NeuroReport 17(1):23–26
Hariri AR, Drabant EM, Weinberger DR (2006) Imaging genetics: perspectives from studies of genetically driven variation in serotonin function and corticolimbic affective processing. Biol Psychiatry 59(10):888–897
Henderson H, Schwartz C, Mundy P et al (2006) Response monitoring, the error-related negativity, and differences in social behavior in autism. Brain Cogn 61(1):96–109
Herrmann MJ, Rommler J, Ehlis AC et al (2004) Source localization (LORETA) of the error-related-negativity (ERN/Ne) and positivity (Pe). Brain Res Cogn Brain Res 20(2):294–299
Holroyd CB, Coles MG (2002) The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. Psychol Rev 109(4):679–709

Holroyd CB, Dien J, Coles MG (1998) Error-related scalp potentials elicited by hand and foot movements: evidence for an output-independent error-processing system in humans. Neurosci Lett 242(2):65–68

Holroyd CB, Nieuwenhuis S, Yeung N et al (2003) Errors in reward prediction are reflected in the event-related brain potential. NeuroReport 14(18):2481–2484

Holroyd CB, Larsen JT, Cohen JD (2004a) Context dependence of the event-related brain potential associated with reward and punishment. Psychophysiology 41(2):245–253

Holroyd CB, Nieuwenhuis S, Yeung N et al (2004b) Dorsal anterior cingulate cortex shows fMRI response to internal and external error signals. Nat Neurosci 7(5):497–498

Huang YY, Battistuzzi C, Oquendo MA et al (2004) Human 5-HT1A receptor C(-1019)G polymorphism and psychopathology. Int J Neuropsychoph 4:441–451

Ikeda M, Yamanouchi Y, Kinoshita Y et al (2008) Variants of dopamine and serotonin candidate genes as predictors of response to risperidone treatment in first-episode schizophrenia. Pharmacogenomics 9(10):1437–1443

Imamura K, Takeshima T, Nakaso K et al (2007) Homocysteine is toxic for dopaminergic neurons in primary mesencephalic culture. NeuroReport 18(13):1319–1322

Ito J, Kitagawa J (2006) Performance monitoring and error processing during a lexical decision task in patients with Parkinson's disease. J Geriatr Psychiatry Neurol 19(1):46–54

Jaber M, Jones S, Giros B et al (1997) The dopamine transporter: a crucial component regulating dopamine transmission. Mov Disord 12(5):629–633

Jacobson S, Kelleher I, Harley M et al (2009) Structural and functional brain correlates of subclinical psychotic symptoms in 11–13 year old schoolchildren. Neuroimage

Jansma JM, de Zwart JA, van Gelderen P et al (2013) In vivo evaluation of the effect of stimulus distribution on FIR statistical efficiency in event-related fMRI. J Neurosci Methods 215(2):190–195

Johannes S, Wieringa BM, Nager W et al (2001) Discrepant target detection and action monitoring in obsessive-compulsive disorder. Psychiatry Res 108(2):101–110

Jonsson EG, Nothen MM, Grunhage F et al (1999) Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. Mol Psychiatry 4(3):290–296

Kaczkurkin AN (2013) The effect of manipulating task difficulty on error-related negativity in individuals with obsessive-compulsive symptoms. Biol Psychol 93(1):122–131

Kanazawa T, Glatt SJ, Kia-Keating B et al (2007) Meta-analysis reveals no association of the Val66Met polymorphism of brain-derived neurotrophic factor with either schizophrenia or bipolar disorder. Psychiatric Genet 17(3):165–170

Keil J, Weisz N, Paul-Jordanov I et al (2010) Localization of the magnetic equivalent of the ERN and induced oscillatory brain activity. Neuroimage 51(1):404–411

Kerns JG, Cohen JD, MacDonald AW 3rd et al (2004) Anterior cingulate conflict monitoring and adjustments in control. Science 303:1023–1026

Kerns JG, Cohen JD, MacDonald AW 3rd et al (2005) Decreased conflict- and error-related activity in the anterior cingulate cortex in subjects with schizophrenia. Am J Psychiatry 162(10):1833–1839

Kim MS, Kang SS, Shin KS et al (2006) Neuropsychological correlates of error negativity and positivity in schizophrenia patients. Psychiatry Clin Neurosci 60(3):303–311

Kishi T, Okochi T, Tsunoka T et al (2011) Serotonin 1A receptor gene, schizophrenia and bipolar disorder: an association study and meta-analysis. Psychiatry Res 185(1–2):20–26

Klein TA, Endrass T, Kathmann N et al (2007a) Neural correlates of error awareness. Neuroimage 34(4):1774–1781

Klein TA, Neumann J, Reuter M et al (2007b) Genetically determined differences in learning from errors. Science 318(5856):1642–1645

Klunk WE (2011) Amyloid imaging as a biomarker for cerebral beta-amyloidosis and risk prediction for Alzheimer dementia. Neurobiol Aging 32(Suppl 1):S20–S36

Kopp B, Rist F (1994) Error-correcting behavior in schizophrenic patients. Schizophr Res 13(1):11–22

Kopp B, Rist F (1999) An event-related brain potential substrate of disturbed response monitoring in paranoid schizophrenic patients. J Abnorm Psychol 108(2):337–346

Kramer UM, Cunillera T, Camara E et al (2007) The impact of catechol-*O*-methyltransferase and dopamine D4 receptor genotypes on neurophysiological markers of performance monitoring. J Neurosci 27(51):14190–14198

Kubicki M, Westin CF, Nestor PG et al (2003) Cingulate fasciculus integrity disruption in schizophrenia: a magnetic resonance diffusion tensor imaging study. Biol Psychiatry 54(11):1171–1180

Kuperberg GR, Broome MR, McGuire PK et al (2003) Regionally localized thinning of the cerebral cortex in schizophrenia. Arch Gen Psychiatry 60(9):878–888

Kwon JS, Kim E, Kang DH et al (2008) Taq1A polymorphism in the dopamine D2 receptor gene as a predictor of clinical response to aripiprazole. Eur Neuropsychopharmacol 18(12):897–907

Kyriakopoulos M, Dima D, Roiser JP et al (2012) Abnormal functional activation and connectivity in the working memory network in early-onset schizophrenia. J Am Acad Child Adolesc Psychiatry 51(9):911–920 e912

Ladouceur CD, Dahl RE, Carter CS (2007) Development of action monitoring through adolescence into adulthood: ERP and source localization. Dev Sci 10(6):874–891

Laurens KR, Ngan ET, Bates AT et al (2003) Rostral anterior cingulate cortex dysfunction during error processing in schizophrenia. Brain 126(Pt 3):610–622

Laurens KR, Hodgins S, Mould GL et al (2009) Error-related processing dysfunction in children aged 9 to 12 years presenting putative antecedents of schizophrenia. Biol Psychiatry 1;67(3):238–245

Lemonde S, Turecki G, Bakish D et al (2003) Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J Neurosci 23(25):8788–8799

Levy DL, Mendell NR, LaVancher CA et al (1998) Disinhibition in antisaccade performance in schizophrenia. In: Lenzenweger MF, Dworkin RH (eds) Origins and development of schizophrenia. American Psychological Association, Washington DC, pp 185–210

Li CS, Yan P, Bergquist KL et al (2007) Greater activation of the "default" brain regions predicts stop signal errors. Neuroimage 38(3):640–648

Liu Y, Gehring WJ, Weissman DH et al (2012) Trial-by-trial adjustments of cognitive control following errors and response conflict are altered in pediatric obsessive compulsive disorder. Front Psychiatry 3:41

Logan GD, Cowan WB (1984) On the ability to inhibit thought and action: a theory of an act of control. Psychol Rev 91:295–327

Luo X, Zhang S, Hu S et al (2013) Error processing and gender-shared and -specific neural predictors of relapse in cocaine dependence. Brain 136(Pt 4):1231–1244

Lutz UC (2008) Alterations in homocysteine metabolism among alcohol dependent patients—clinical, pathobiochemical and genetic aspects. Curr Drug Abuse Rev 1(1):47–55

Lutz UC, Batra A, Kolb W et al (2006) Methylenetetrahydrofolate reductase C677T-polymorphism and its association with alcohol withdrawal seizure. Alcohol Clin Exp Res 30(12):1966–1971

Lutz UC, Batra A, Wiatr G et al (2007) Significant impact of MTHFR C677T polymorphism on plasma homovanillic acid (HVA) levels among alcohol-dependent patients. Addict Biol 12(1):100–105

Luu P, Tucker DM, Derryberry D et al (2003) Electrophysiological responses to errors and feedback in the process of action regulation. Psychol Sci 14(1):47–53

Malenka RC, Angel RW, Hampton B et al (1982) Impaired central error-correcting behavior in schizophrenia. Arch Gen Psychiatry 39(1):101–107

Malenka RC, Angel RW, Thiemann S et al (1986) Central error-correcting behavior in schizophrenia and depression. Biol Psychiatry 21(3):263–273

Maltby N, Tolin DF, Worhunsky P et al (2005) Dysfunctional action monitoring hyperactivates frontal-striatal circuits in obsessive-compulsive disorder: an event-related fMRI study. Neuroimage 24(2):495–503

Manoach DS, Greve DN, Lindgren KA et al (2003) Identifying regional activity associated with temporally separated components of working memory using event-related functional MRI. Neuroimage 20(3):1670–1684

Manoach DS, Ketwaroo GA, Polli FE et al (2007) Reduced microstructural integrity of the white matter underlying anterior cingulate cortex is associated with increased saccadic latency in schizophrenia. Neuroimage 37(2):599–610

Mathalon DH, Fedor M, Faustman WO et al (2002) Response-monitoring dysfunction in schizophrenia: an event-related brain potential study. J Abnorm Psychol 111(1):22–41

Mathews CA, Perez VB, Delucchi KL et al (2012) Error-related negativity in individuals with obsessive-compulsive symptoms: toward an understanding of hoarding behaviors. Biol Psychol 89(2):487–494

McCoy AN, Crowley JC, Haghighian G et al (2003) Saccade reward signals in posterior cingulate cortex. Neuron 40(5):1031–1040

McDonald C, Bullmore E, Sham P et al (2005) Regional volume deviations of brain structure in schizophrenia and psychotic bipolar disorder: computational morphometry study. Br J Psychiatry 186:369–377

Menon V, Adleman NE, White CD et al (2001) Error-related brain activation during a Go/NoGo response inhibition task. Hum Brain Mapp 12(3):131–143

Meyer A, Klein DN, Torpey DC et al (2012) Additive effects of the dopamine D2 receptor and dopamine transporter genes on the error-related negativity in young children. Genes Brain Behav 11(6):695–703

Meyer-Lindenberg A, Kohn PD, Kolachana B et al (2005) Midbrain dopamine and prefrontal function in humans: interaction and modulation by COMT genotype. Nat Neurosci 8(5):594–596

Mitelman SA, Shihabuddin L, Brickman AM et al (2005) Volume of the cingulate and outcome in schizophrenia. Schizophr Res 72(2–3):91–108

Morgan L, Wetherby AM, Barber A (2008) Repetitive and stereotyped movements in children with autism spectrum disorders late in the second year of life. J Child Psychol Psychiatry 49(8):826–837

Morris SE, Yee CM, Nuechterlein KH (2006) Electrophysiological analysis of error monitoring in schizophrenia. J Abnorm Psychol 115(2):239–250

Mossner R, Schuhmacher A, Kuhn KU et al (2009) Functional serotonin 1A receptor variant influences treatment response to atypical antipsychotics in schizophrenia. Pharmacogenet Genomics 19(1):91–94

Munafo MR, Bowes L, Clark TG et al (2005) Lack of association of the COMT (Val158/108 Met) gene and schizophrenia: a meta-analysis of case-control studies. Mol Psychiatry 10(8):765–770

Nieuwenhuis S, Ridderinkhof KR, Blom J et al (2001) Error-related brain potentials are differentially related to awareness of response errors: evidence from an antisaccade task. Psychophysiology 38(5):752–760

Nieuwenhuis S, Yeung N, van den Wildenberg W et al (2003) Electrophysiological correlates of anterior cingulate function in a go/no-go task: effects of response conflict and trial type frequency. Cogn Affect Behav Neurosci 3(1):17–26

Nieuwenhuis S, Nielen MM, Mol N et al (2005) Performance monitoring in obsessive-compulsive disorder. Psychiatry Res 134(2):111–122

Noriuchi M, Kikuchi Y, Yoshiura T et al (2010) Altered white matter fractional anisotropy and social impairment in children with autism spectrum disorder. Brain Res 1362:141–149

Ohnuma T, Kimura M, Takahashi T et al (1997) A magnetic resonance imaging study in first-episode disorganized-type patients with schizophrenia. Psychiatry Clin Neurosci 51(1):9–15

Okamura N, Reinscheid RK (2007) Neuropeptide S: a novel modulator of stress and arousal. Stress 10(3):221–226
Okochi T, Ikeda M, Kishi T et al (2009) Meta-analysis of association between genetic variants in COMT and schizophrenia: an update. Schizophr Res 110(1–3):140–148
Okuyama Y, Ishiguro H, Toru M et al (1999) A genetic polymorphism in the promoter region of DRD4 associated with expression and schizophrenia. Biochem Biophys Res Commun 258(2):292–295
Olvet DM, Hajcak G (2008) The error-related negativity (ERN) and psychopathology: toward an endophenotype. Clin Psychol Rev 28(8):1343–1354
Olvet DM, Hatchwell E, Hajcak G (2010) Lack of association between the 5-HTTLPR and the error-related negativity (ERN). Biol Psychol 85(3):504–508
Osinsky R, Hewig J, Alexander N et al (2012) COMT Val158Met genotype and the common basis of error and conflict monitoring. Brain Res 1452:108–118
Overbeek TJM, Nieuwenhuis S, Ridderinkhof KR (2005) Dissociable components of error processing. J Psychophysiology 19(4):319–329
Parsons MJ, Mata I, Beperet M et al (2007) A dopamine D2 receptor gene-related polymorphism is associated with schizophrenia in a Spanish population isolate. Psychiatr Genet 17(3):159–163
Paus T, Petrides M, Evans AC et al (1993) Role of the human anterior cingulate cortex in the control of oculomotor, manual, and speech responses: a positron emission tomography study. J Neurophysiol 70(2):453–469
Perez VB, Ford JM, Roach BJ et al (2012) Error monitoring dysfunction across the illness course of schizophrenia. J Abnorm Psychol 121(2):372–387
Pezawas L, Meyer-Lindenberg A, Drabant EM et al (2005) 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. Nat Neurosci 8(6):828–834
Phillips ML, Drevets WC, Rauch SL et al (2003) Neurobiology of emotion perception I: the neural basis of normal emotion perception. Biol Psychiatry 54(5):504–514
Pitman RK (1987) A cybernetic model of obsessive-compulsive psychopathology. Compr Psychiatry 28(4):334–343
Pohjalainen T, Rinne JO, Nagren K et al (1998) The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psychiatry 3(3):256–260
Polli FE, Barton JJ, Cain MS et al (2005) Rostral and dorsal anterior cingulate cortex make dissociable contributions during antisaccade error commission. Proc Natl Acad Sci U S A 102(43):15700–15705
Polli FE, Barton JJ, Vangel M et al (2006) Schizophrenia patients show intact immediate error-related performance adjustments on an antisaccade task. Schizophr Res 82(2–3):191–201
Polli FE, Barton JJ, Thakkar KN et al (2008) Reduced error-related activation in two anterior cingulate circuits is related to impaired performance in schizophrenia. Brain 131(4):971–986
Polli FE, Wright CI, Milad MR et al (2009) Hemispheric differences in amygdala contributions to response monitoring. NeuroReport 20(4):398–402
Purcell SM, Wray NR, Stone JL et al (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460(7256):748–752
Rabbitt PM (1966) Errors and error correction in choice-response tasks. J Exp Psychol 71(2):264–272
Radant AD, Dobie DJ, Calkins ME et al (2010) Antisaccade performance in schizophrenia patients, their first-degree biological relatives, and community comparison subjects: data from the COGS study. Psychophysiology 47(5):846–856
Raichle ME, MacLeod AM, Snyder AZ et al (2001) A default mode of brain function. Proc Natl Acad Sci U S A 98(2):676–682
Reinscheid RK, Xu YL, Okamura N et al (2005) Pharmacological characterization of human and murine neuropeptide s receptor variants. J Pharmacol Exp Ther 3:1338–1345

Reynolds GP, Arranz B, Templeman LA et al (2006) Effect of 5-HT1A receptor gene polymorphism on negative and depressive symptom response to antipsychotic treatment of drug-naive psychotic patients. Am J Psychiatry 163(10):1826–1829

Ridderinkhof KR, Nieuwenhuis S, Bashore TR (2003) Errors are foreshadowed in brain potentials associated with action monitoring in cingulate cortex in humans. Neurosci Lett 348(1):1–4

Ridderinkhof KR, Ullsperger M, Crone EA et al (2004) The role of the medial frontal cortex in cognitive control. Science 306(5695):443–447

Ritchie T, Noble EP (2003) Association of seven polymorphisms of the D2 dopamine receptor gene with brain receptor-binding characteristics. Neurochem Res 28(1):73–82

Roffman JL, Gollub RL, Calhoun VD et al (2008a) MTHFR 677C⇒T genotype disrupts prefrontal function in schizophrenia through an interaction with COMT 158Val⇒Met. Proc Natl Acad Sci U S A 105(45):17573–17578

Roffman JL, Weiss AP, Deckersbach T et al (2008b) Interactive effects of COMT Val108/158Met and MTHFR C677T on executive function in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 5;147B(6):990–995

Roffman JL, Weiss AP, Purcell S et al (2008c) Contribution of methylenetetrahydrofolate reductase (MTHFR) polymorphisms to negative symptoms in schizophrenia. Biol Psychiatry 63(1):42–48

Roffman JL, Brohawn DG, Friedman JS et al (2011a) MTHFR 677C>T effects on anterior cingulate structure and function during response monitoring in schizophrenia: a preliminary study. Brain Imaging Behav 5(1):65–75

Roffman JL, Nitenson AZ, Agam Y et al (2011b) A hypomethylating variant of MTHFR, 677C>T, blunts the neural response to errors in patients with schizophrenia and healthy individuals. PLoS ONE 6(9):e25253

Ruchsow M, Spitzer M, Gron G et al (2005) Error processing and impulsiveness in normals: evidence from event-related potentials. Brain Res Cogn Brain Res 24(2):317–325

Russell J, Jarrold C (1998) Error-correction problems in autism: evidence for a monitoring impairment? J Autism Dev Disord 28(3):177–188

Sandrone S (2012) The brain as a crystal ball: the predictive potential of default mode network. Front Hum Neurosci 6:261

Santesso DL, Segalowitz SJ, Schmidt LA (2006) Error-related electrocortical responses are enhanced in children with obsessive-compulsive behaviors. Dev Neuropsychol 29(3):431–445

Santesso DL, Drmic IE, Jetha MK et al (2011) An event-related source localization study of response monitoring and social impairments in autism spectrum disorder. Psychophysiology:241–251

Scheffers MK, Coles MG (2000) Performance monitoring in a confusing world: error-related brain activity, judgments of response accuracy, and types of errors. J Exp Psychol Hum Percept Perform 26(1):141–151

Schmahmann JD, Pandya DN, Wang R et al (2007) Association fibre pathways of the brain: parallel observations from diffusion spectrum imaging and autoradiography. Brain 130(3):630–653

Schultz W (2002) Getting formal with dopamine and reward. Neuron 36(2):241–263

Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593–1598

Shi J, Gershon ES, Liu C (2008) Genetic associations with schizophrenia: meta-analyses of 12 candidate genes. Schizophr Res 104(1–3):96–107

Sigmundsson T, Suckling J, Maier M et al (2001) Structural abnormalities in frontal, temporal, and limbic regions and interconnecting white matter tracts in schizophrenic patients with prominent negative symptoms. Am J Psychiatry 158(2):234–243

Simmonite M, Bates AT, Groom MJ et al (2012) Error processing-associated event-related potentials in schizophrenia and unaffected siblings. Int J Psychophysiol 84(1):74–79

Simon JR (1969) Reactions towards the source of stimulation. J Experimental Psychol 81:174–176
Smith BW, Mitchell DG, Hardin MG et al (2009) Neural substrates of reward magnitude, probability, and risk during a wheel of fortune decision-making task. Neuroimage 44(2):600–609
Sokhadze E, Baruth J, El-Baz A et al (2010) Impaired error monitoring and correction function in autism. J Neurother 14(2):79–95
Sokhadze EM, Baruth JM, Sears L et al (2012a) Prefrontal neuromodulation using rTMS improves error monitoring and correction function in autism. Appl Psychophysiol Biofeedback 37(2):91–102
Sokhadze EM, Baruth JM, Sears L et al (2012b) Event-related potential study of attention regulation during illusory figure categorization task in Adhd, autism spectrum disorder, and typical children. J Neurother 16(1):12–31
South M, Larson MJ, Krauskopf E et al (2010) Error processing in high-functioning autism spectrum disorders. Biol Psychol 85(2):242–251
Stroop JR (1935) Studies of interference in serial verbal reaction. J Exp Psychol 18:643–662
Summerfelt AT, Alphs LD, Wagman AM et al (1991) Reduction of perseverative errors in patients with schizophrenia using monetary feedback. J Abnorm Psychol 100(4):613–616
Sun Z, Wang F, Cui L et al (2003) Abnormal anterior cingulum in patients with schizophrenia: a diffusion tensor imaging study. NeuroReport 14(14):1833–1836
Suzuki M, Nohara S, Hagino H et al (2002) Regional changes in brain gray and white matter in patients with schizophrenia demonstrated with voxel-based analysis of MRI. Schizophr Res 55(1–2):41–54
Taj MJR, Viswanath B, Purushottam M et al (2013) DRD4 gene and obsessive compulsive disorder: do symptom dimensions have specific genetic correlates? Prog Neuropsychopharmacol Biol Psychiatry 41:18–23
Tan HY, Callicott JH, Weinberger DR (2008) Intermediate phenotypes in schizophrenia genetics redux: is it a no brainer? Mol Psychiatry 13(3):233–238
Taylor SF, Martis B, Fitzgerald KD et al (2006) Medial frontal cortex activity and loss-related responses to errors. J Neurosci 26(15):4063–4070
Taylor SF, Stern ER, Gehring WJ (2007) Neural systems for error monitoring: recent findings and theoretical perspectives. Neuroscientist 13(2):160–172
Thakkar KN, Polli FE, Joseph RM et al (2008) Response monitoring, repetitive behaviour and anterior cingulate abnormalities in autism spectrum disorders (ASD). Brain 131(Pt 9):2464–2478
Thorndike E (1911) Animal intelligence. Macmillan, New York
Tu P, Buckner RL, Zollei L et al (2010) Reduced functional connectivity in a right-hemisphere network for volitional ocular motor control in schizophrenia. Brain 133(2):625–637
Turetsky BI, Greenwood TA, Olincy A et al (2008) Abnormal auditory N100 amplitude: a heritable endophenotype in first-degree relatives of schizophrenia probands. Biol Psychiatry 64(12):1051–1059
Turken AU, Vuilleumier P, Mathalon DH et al (2003) Are impairments of action monitoring and executive control true dissociative dysfunctions in patients with schizophrenia? Am J Psychiatry 160(10):1881–1883
Ursu S, Stenger VA, Shear MK et al (2003) Overactive action monitoring in obsessive-compulsive disorder: evidence from functional magnetic resonance imaging. Psychol Sci 14(4):347–353
van Boxtel GJM, van der Molen MW, Jennings JR (2005) Differential involvement of the anterior cingulate cortex in performance monitoring during a stop-signal task. J Psychophysiol 19(1):1–10
van de Giessen E, de Win MM, Tanck MW et al (2009) Striatal dopamine transporter availability associated with polymorphisms in the dopamine transporter gene SLC6A3. J Nucl Med 50(1):45–52
van Dyck CH, Malison RT, Jacobsen LK et al (2005) Increased dopamine transporter availability associated with the 9-repeat allele of the SLC6A3 gene. J Nucl Med 46(5):745–751

van Hoesen GW, Morecraft RJ, Vogt BA (1993) Connections of the monkey cingulate cortex. In: Vogt BA, Gabriel M (eds) Neurobiology of cingulate cortex and limbic thalamus: a comprehensive handbook. Birkhauser, Boston, pp 249–284

van Schie HT, Mars RB, Coles MG et al (2004) Modulation of activity in medial frontal and motor cortices during error observation. Nat Neurosci 7(5):549–554

Van Tol HH, Wu CM, Guan HC et al (1992) Multiple dopamine D4 receptor variants in the human population. Nature 358(6382):149–152

van Veen V, Carter CS (2002) The timing of action-monitoring processes in the anterior cingulate cortex. J Cogn Neurosci 14(4):593–602

Van't Ent D, Apkarian P (1999) Motoric response inhibition in finger movement and saccadic eye movement: a comparative study. Clin Neurophysiol 110(6):1058–1072

Vandenbergh DJ, Persico AM, Hawkins AL et al (1992) Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics 14(4):1104–1106

Vlamings PH, Jonkman LM, Hoeksma MR et al (2008) Reduced error monitoring in children with autism spectrum disorder: an ERP study. Eur J Neurosci 28(2):399–406

Waltz JA, Gold JM (2007) Probabilistic reversal learning impairments in schizophrenia: further evidence of orbitofrontal dysfunction. Schizophr Res 93(1–3):296–303

Waltz JA, Frank MJ, Robinson BM et al (2007) Selective reinforcement learning deficits in schizophrenia support predictions from computational models of striatal-cortical dysfunction. Biol Psychiatry 62(7):756–764

Waltz JA, Schweitzer JB, Ross TJ et al (2010) Abnormal responses to monetary outcomes in cortex, but not in the Basal Ganglia, in schizophrenia. Neuropsychopharmacology

Wang F, Sun Z, Cui L et al (2004) Anterior cingulum abnormalities in male patients with schizophrenia determined through diffusion tensor imaging. Am J Psychiatry 161(3):573–575

Watt N, Wetherby AM, Barber A et al (2008) Repetitive and stereotyped behaviors in children with autism spectrum disorders in the second year of life. J Autism Dev Disord 38(8):1518–1533

Whalen PJ, Bush G, McNally RJ et al (1998) The emotional counting Stroop paradigm: a functional magnetic resonance imaging probe of the anterior cingulate affective division. Biol Psychiatry 44(12):1219–1228

Whalley HC, Simonotto E, Moorhead W et al (2006) Functional imaging as a predictor of schizophrenia. Biol Psychiatry 60(5):454–462

Whitfield-Gabrieli S, Ford JM (2012) Default mode network activity and connectivity in psychopathology. Annu Rev Clin Psychol 8:49–76

Willemssen R, Muller T, Schwarz M et al (2009) Response monitoring in de novo patients with Parkinson's disease. PLoS One 4(3):e4898

Wittfoth M, Kustermann E, Fahle M et al (2008) The influence of response conflict on error processing: evidence from event-related fMRI. Brain Res 1194:118–129

Xiao Z, Wang J, Zhang M et al (2011) Error-related negativity abnormalities in generalized anxiety disorder and obsessive-compulsive disorder. Prog Neuropsychopharmacol Biol Psychiatry 35(1):265–272

Xing QH, Wu SN, Lin ZG et al (2003) Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene in schizophrenia. Schizophr Res 65(1):9–14

Yamasue H, Iwanami A, Hirayasu Y et al (2004) Localized volume reduction in prefrontal, temporolimbic, and paralimbic regions in schizophrenia: an MRI parcellation study. Psychiatry Res 131(3):195–207

Yan H, Tian L, Yan J et al (2012) Functional and anatomical connectivity abnormalities in cognitive division of anterior cingulate cortex in schizophrenia. PLoS One 7(9):e45659

Zald DH (2003) The human amygdala and the emotional evaluation of sensory stimuli. Brain Res Brain Res Rev 41(1):88–123

Zirnheld PJ, Carroll CA, Kieffaber PD et al (2004) Haloperidol impairs learning and error-related negativity in humans. J Cogn Neurosci 16(6):1098–1112

Chapter 10
Reward Dependence and Reward Deficiency

Marlene Oscar-Berman and Kenneth Blum

In this chapter, we outline the neural network involved with reward value. We emphasize the prominent role of dopamine (DA) within the mesocorticolimbic system of the network in mediating the reinforcing effects of drugs. The cascade of neuronal events leading to the net release of DA at the nucleus accumbens is detailed, followed by a discussion of the evolutionary genetics of dopamine and the dopamine D_2 receptor gene.

Based on the scientific support sampled, we posit a common underlying mechanism of action for the powerful effects that all addictions have on human

M. Oscar-Berman (✉)
Departments of Behavioral Neuroscience, Psychiatry, Neurology, and Anatomy & Neurobiology, Boston University School of Medicine, and VA Healthcare System, Boston, MA, USA
e-mail: oscar@bu.edu

K. Blum
Department of Psychiatry & McKnight Brain Institute, University of Florida College of Medicine, Gainesville, USA
e-mail: drd2gene@gmail.com

K. Blum
Dominion Diagnostics, Inc, North Kingstown, RI, USA

K. Blum
G&G Holistic Addiction Treatment Center, Miami, FL, USA

K. Blum
Malibu Beach Recovery Center, Malibu, CA, USA

K. Blum
Path Foundation, New York, NY, USA

K. Blum
Center for Genomics and Applied Gene Technology, Institute of Integrative Omics and Applied Biotechnology, Purba Medinipur, India

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_10

motivation. That is, biological drives may have common molecular genetic antecedents, which, if impaired, lead to aberrant behaviors. We further hypothesize that "reward genes," especially dopaminergic genes and other candidate neurotransmitter-related gene polymorphisms, affect both hedonic and anhedonic behavioral outcomes. Reward genes are important common determinants of a generalized set of behaviors as a phenotype. We refer to this phenotype as *Reward Deficiency Syndrome* (RDS). RDS refers to the breakdown of a cascade of neurotransmitters in the brain in which one reaction triggers another—the reward cascade—and resultant deviant conduct. RDS is a genetic phenotype; it does not represent any one single addictive behavior. Genetic association studies have amassed strong evidence implicating the DRD2 gene in harmful conditions such as alcoholism. Additionally, the dopamine D_2 receptor gene (DRD2) has been found to be involved in other substance use disorders including cocaine, nicotine, and opioid dependence, as well as obesity, poor impulse control, sex addiction, gambling, Internet gaming, and certain neuropsychiatric conditions.

We hypothesize that there is a common neurochemical mechanism of action for the powerful effects that food, sex, and acquired addictive precursors have on human motivation; all have common molecular genetic antecedents that if impaired lead to aberrant behaviors. In discussing RDS, we refer specifically to an insensitivity and inefficiency in the brain's reward system. RDS also encompasses acquired needs to escape or avoid painful states or negative feelings. Impairment in the mechanisms involved in these processes can lead to multiple impulsive, compulsive, and addictive behaviors.

Applications and clinical relevance are discussed with reference to reward deficiency disorders and substance abuse treatment.

10.1 The Neural Network for Reward–Reinforcement and Role of Dopamine

High-level cognitive and emotional processes important in the learning of reward values and affective properties of stimuli are controlled by an extensive network of cortical–subcortical connections that modulate the behavioral responses (Rushworth et al. 2011; Wood and Grafman 2003). Key cortical and subcortical centers within this network are frontal cortex (dorsolateral prefrontal, orbitofrontal, and anterior cingulate cortices), insular cortex, and limbic regions—hippocampus, amygdala, nucleus accumbens, septal nuclei, and the ventral diencephalon: the hypothalamus, basal forebrain, and sublenticular extended amygdala, as well as a large portion of the ventral tegmentum (Barbas 2007; Barrett et al. 2007; Makris et al. 2008). This network has also been named the *Extended Reward and Oversight System* (EROS) by Makris et al. (2008). The network is outlined in Fig. 10.1.

Within the extended network, the nucleus accumbens (NAc) is a site that plays a prominent role in mediating the reinforcing effects of drugs of abuse, food, sex,

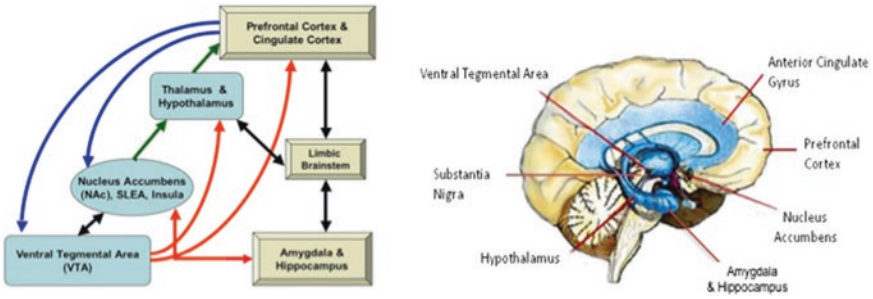

Fig. 10.1 Outline of the extended reward and oversight system (Makris et al. 2008), and medial view of the human brain showing components of mesocorticolimbic circuitry

and other addictions. This structure mandates motivated behaviors such as eating, drinking, and sexual activity, which are elicited by natural rewards and other strong incentive stimuli.

The mesocorticolimbic pathway within the network (see Fig. 10.1) represents the neural circuitry closely linked to positive reinforcement. It is strongly involved in many biobehavioral functions impaired in addicted individuals, and its breakdown and dysfunction are responsible for a variety of abnormalities, e.g., insensitivity to rewards and other feedbacks (Brand et al. 2009; Wrase et al. 2007), impaired maintenance and monitoring of incoming information (Muller et al. 2002), disruption of decision making (Bechara 2003; Bolla et al. 2005; Brand et al. 2005; LeDoux 2000; Pandya and Yeterian 2002; Patterson et al. 2002; Poldrack et al. 1999), impairments in emotional control and behavioral inhibition (Ochsner and Gross 2007), and initiating drug use or relapse after protracted abstinence (Goldstein and Volkow 2011a; Oscar-Berman and Bowirrat 2005).

Goldstein and Volkow (2002) proposed that disrupted function of the mesocorticolimbic system leads to a syndrome of *Impaired Response Inhibition and Salience Attribution* in all addictions. This syndrome is characterized by "attributing excessive salience to the drug and drug-related cues, decreased sensitivity to non-drug reinforcers, and decreased ability to inhibit maladaptive or disadvantageous behaviors" (Goldstein and Volkow 2011a). The authors provided an informative schematic figure, based upon neuroimaging findings, that shows differences in brain activity between addicted and healthy individuals involving functional domains such as attention and memory, decision making and inhibitory control, and emotion and motivation.

10.1.1 Dopamine: The Major Neurotransmitter for Reward and Reinforcement

The brain's major reward neurotransmitter is dopamine (DA) (Goldstein and Volkow 2002; Kirsch et al. 2006). Although other neurotransmitters, e.g.,

glutamate, gamma-aminobutyric acid (GABA) (Dick et al. 2004), serotonin (Goldman et al. 1992), and enkephalins (Comings et al. 1999), may be important in determining some rewarding and stimulating effects of substances such as alcohol, DA may be critical for initiating drug use and for reinstating drug use during protracted abstinence (Comings et al. 1999; Connor et al. 2002; Gordon et al. 2001; Gardner 2005; Goldstein and Volkow 2011b; Rommelspacher et al. 1992).

When released into the synapse, DA stimulates a number of receptors (D_1–D_5), which results in increased feelings of well-being and stress reduction (Koob and Kreek 2007). The D_2 receptor especially has been associated with pleasure. The mesocorticolimbic dopaminergic pathway plays an important role in mediating reinforcement of natural rewards such as food and sex, as well as unnatural rewards such as alcohol and drugs of abuse, and the hedonic feelings derived from gambling and certain other risk-taking behaviors (Bruijnzeel et al. 2004; Joutsa et al. 2012; Olsen 2011). The distinction between natural and unnatural rewards is an important one. Natural rewards include satisfaction of ubiquitous physiological drives (e.g., hunger and reproduction), and unnatural rewards are learned and involve satisfaction of acquired pleasures such as hedonic sensations derived from alcohol and other drugs, as well as from a variety of behaviors considered by some to be addictive (Hodge et al. 1996; Hodge and Cox 1998; Schmitz 2005; Wightman and Robinson 2002). Of note, drugs of abuse have been considered to be stronger positive reinforcers than natural reinforcers such as food and sex (Cooper et al. 1995; Epping-Jordan et al. 1998; Wightman and Robinson 2002).

10.1.2 Dopamine and Pleasure

As noted, the NAc, a site within the mesocorticolimbic system, is best known for its prominent role in mediating the reinforcing effects of drugs of abuse. Indeed, it is generally believed that this structure mandates many natural biologically motivated behaviors, such as feeding, drinking, and sexual behavior, as well as behaviors elicited by unnatural acquired rewards or incentive stimuli that can become addictive. Pleasure produced from drugs of abuse occurs because most of these drugs target the brain's reward system by flooding the circuit with DA (Budygin et al. 2012). When some drugs like cocaine are taken, they can release two to ten times the amount of DA; the resultant effects on the brain's pleasure circuit dwarf those produced by natural rewards such as food and sex. This fact alone strongly motivates people to take drugs again and again. Independent of one's genetic makeup, if one keeps taking drugs, the brain adjusts to the overwhelming surge in DA and other neurotransmitters causing a breakdown in the natural process of brain reward by producing less DA or by reducing the number of DA D_2 receptors. This process causes abnormally low DA function, high cravings, and reduced ability to perceive pleasure (Chen et al. 2011a, b).

Similarly, hedonic *liking* for sensory pleasures is an important aspect of reward, and a disproportionate liking of particular rewards might contribute to

excessive consumption and to disorders involving response deficiency (discussed in Sect. 10.3). Modern neuroscience tools such as drug microinjection for immunoreactivity mapping have further identified hedonic hot spots within the NAc, where opioids are especially tuned to magnify liking of food rewards (see Konkle and Bielajew 2004 review of functional neuroanatomical tools that have played an important role in proposing which structures underlie brain stimulation reward circuitry). Moreover, hedonic hot spots in different brain structures may interact with each other within the larger functional circuitry that interconnects them (Peciña et al. 2006). For example, when Peciña et al. (2012) examined gene effects on the functional connectivity of the prefrontal cortex during reward anticipation, and subcortical stress-induced DA release, they showed significant neurobiological effects of genotype variation in the DA D_2 receptor gene (DRD2) on multiple functional domains, such as emotion, stress, and reward processing. As such, it contributes to normal variation and potentially to vulnerability to psychopathology associated with those functions, such as risk for mood and substance use disorders.

Additionally, sexual activity activates brain mesocorticolimbic reward circuitry (Blum 2011), and an orgasm provides a primary natural blast of DA. Accordingly, Georgiadis et al. (2009) scanned the brains of people having orgasm and noted that they resembled scans of heroin rushes. Orgasms and addictive substances or behaviors have two things in common. They produce an initial pleasurable experience, and both are followed by neurochemical fluctuations that appear to continue for a week or two. Thus, activation of the dopaminergic system induces feelings of reward and pleasure (Eisenberg et al. 2007; Volkow et al. 2002).

10.1.3 The Brain Reward Cascade

Chemical messengers including serotonin, enkephalins, GABA, and DA, work in concert to provide a net release of DA at the NAc, a site within the ventral striatum of the mesocorticolimbic system. The cascade of these neuronal events has been termed “brain reward cascade” (Blum et al. 1990) (see Fig. 10.2). The brain reward cascade starts in the hypothalamus where serotonin acts as the neurotransmitter activating the enkephalins (one type of brain endorphin). Enkephalins released in the hypothalamus stimulate receptors in the substantia nigra, which contains the inhibitory neurotransmitter GABA that stimulates $GABA_B$ receptors projecting to the ventral tegmental area; there, DA neurons are inhibited, allowing just the right amount of DA to be released at the NAc. A breakdown of this cascade ultimately will lead to a dysregulation and dysfunction of DA. Any reduction in function could lead to reward deficiency and resultant aberrant substance seeking behavior and a lack of wellness (Blum et al. 2000).

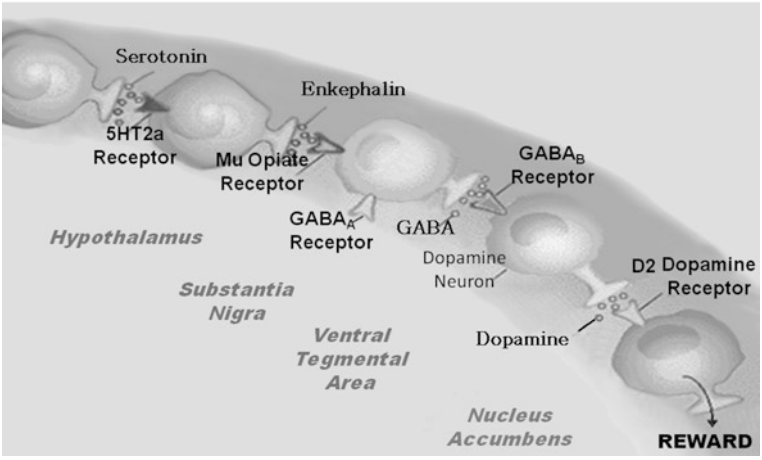

Fig. 10.2 The interaction of various neurotransmitters, including serotonin, enkephalins, GABA, and DA, constitute the *brain reward cascade* (Erickson 2007)

10.1.4 Evolutionary Genetics of Dopamine

The role of DA in brain reward functions has been fraught with controversy, and there are many unanswered questions related to what makes us human and what drives our unique behaviors. While many brain theories have focused on the role of brain size and genetic adaptations, Previc (1999) proposed the provocative concept of a *dopaminergic society* to explain the differences between modern humans and their hominid relatives. Previc proposed that increased levels of DA were part of a general physiological adaptation that evolved with the increased consumption of meat around two million years ago by *homo habilis* and/or *Australopithecus sediba* (Berger et al. 2010) and later (beginning approximately 80,000 years ago) by other dietary changes and environmental and social factors. This hypothesis was supported by recent discoveries about seaside settlements of early man, suggesting that dietary changes, like the inclusion of fish oils—known to increase DA receptors—could have further enhanced DA function (Kuperstein et al. 2005).

Previc further hypothesized that the "high-dopamine" society is characterized by high intelligence, a sense of personal destiny, a religious/cosmic preoccupation, and an obsession with achieving goals and conquests. According to this hypothesis, and because DA increases activity levels, speeds up our internal clocks, and creates a preference for novel over unchanging environments, a dopaminergic society is extremely goal-oriented, fast-paced, and even manic (Previc 1999). Moreover, it was proposed that high levels of DA underlie increased psychological disorders in industrialized societies.

Although behavioral evidence and some indirect anatomical evidence, like the enlargement of the DA-rich striatum in humans revealed by the work of Rapoport

(1990), support a dopaminergic expansion in humans, according to Raghanti and associates (Raghanti et al. 2008), there is still no direct evidence that DA levels are markedly higher in humans relative to apes. However, recent discoveries about the seaside settlements of early man may provide evidence of dietary changes consistent with this hypothesis. There are a number of studies that report the positive relationship between omega-3 fish oil and DA D_2 receptor density. Specifically, decreased tissue levels of n-3 (omega-3) fatty acids, particularly docosahexaenoic acid, are implicated in the etiologies of non-puerperal and postpartum depression.

Davis et al. (2010) examined the effects of a diet-induced loss of brain docosahexaenoic acid content and concurrent reproductive status on dopaminergic parameters in adult female Long–Evans rats. Decreased brain docosahexaenoic acid produced a significant main effect of decreased density of ventral striatal DA D_2-like receptors. Virgin females with decreased docosahexaenoic acid also exhibited higher density of D_1-like receptors in the caudate nucleus than virgin females with normal docosahexaenoic acid. These receptor alterations are similar to those found in several rodent models of depression and are consistent with the proposed hypodopaminergic basis for anhedonia and motivational deficits in depression.

10.1.5 Evolutionary Genetics and the DRD2 Gene

The possibility exists that prehistoric ancestral species over two million years ago carried low DA brain function due to low DA receptors (Blum et al. 2012a). As a neurotransmitter, DA activates the five known types of DA receptors (D_1 through D_5) and their variants. DA from l-tyrosine, abundant in meat, is produced in several areas of the brain, including the brain reward site in the NAc (see Fig. 10.1). Two major variant forms of the human DRD2 gene regulate the synthesis of D_2 receptors; they are the A1 and A2 alleles. As these forms (polymorphisms) exist in pairs, there are at least three variants of the DA D_2 receptors: the A1/A1, the A1/A2, and the A2/A2. DRD2, the most widely studied gene, accounts for major aspects of modern human behavior. The DRD2 A2 allele, which in today's world is considered the "normal" variation, is carried by two-thirds of the US population. Carriers of the DRD2 A1 allele constitute about one-third of our population and have 30–40% lower D_2 receptors; this is a subset of approximately 100 million people (Blum et al. 2011b). However, within this subset, the prevalence varies significantly between Caucasians, African Americans, Hispanics, Asians, and Native Americans (Castiglione et al. 1995). It is prudent to speculate that the older gene allele (DRD2 A1) leading to low DA function may have afforded certain survival benefits. But as *Homo habilis* or *Australopithecus sediba* (Berger et al. 2010) increased their meat consumption, feeding the brain with the needed l-tyrosine to synthesize more DA required to overcome the D_2 receptor deficit (competitive edge), a new society was born—the high DA society carrying the DRD2 A2 allele of this gene (Blum et al. 1996b, c).

Comings (1996) in his popular book *The Gene Bomb* suggested that while it may be true that genetic adaptations are very slow, there could be some exceptions, like the Tibetan altitude gene that allowed for adaptation to high altitudes. Comings also discussed the future of the DRD2 gene: Let us assume that a gene variant called X causes addiction and that individuals with this X gene drop out of school earlier, cohabitate with others carrying the same genotype, and start having children earlier than individuals who do not carry that gene. Let us also assume that the average age at birth of the first child of DRD2 A1 allele carriers is 20 years, while for those not carrying the variation, it is 25 years. As a result, the DRD2 A1 form of the gene will reproduce faster, namely every 20 years, while the normal form (DRD2 A2) of the gene will reproduce every 25 years. The ratio of 25/20 is 1.25. Although this gene DRD2 A1 may seem to not have any selective benefit, one must consider the fact that having low D_2 receptors in our current society may confer certain competitive advantages such as enhanced aggression, novelty seeking, and risk taking, leading to greater survival as it did in the past (Comings 1996).

10.2 Reward Dependence and Deficiency as Phenotypes

10.2.1 Genetic Components of Brain Reward Circuitry

In a *candidate gene approach,* scientists pick and choose genes to examine in people with a specific disease condition based on prior knowledge of physiological processes. Using this approach, Blum et al. (1990) searched for the "reward gene." Initially, they found at least one important variant of the DA D_2 receptor gene (Taq A1) that is associated with severe alcoholism. However, this apparently simple finding turned out to be complicated by the fact that many other genes and their polymorphisms in brain reward pathways work in concert to provide the genetically controlled phenotype. That is, with the advent of microarray analysis of the genome, researchers now can examine a collection of microscopic DNA samples, thereby allowing important discoveries to be made regarding the role of genes and behavior. For example, array work has been accomplished by Li et al. (2008), who integrated 2343 items of evidence from peer-reviewed publications between 1976 and 2006 linking genes and chromosome regions to addiction. Using single-gene strategies, microarray, proteomics, or genetic studies, they identified 1500 human addiction-related genes. They used a computer network system called KARG (Knowledgebase for Addiction-Related Genes; see http://karg.cbi.pku.edu.cn) to explore the association of gene polymorphisms and addiction, and they developed the first molecular database for addiction-related genes, with extensive annotations and a friendly Web interface. Then, they performed a meta-analysis of 396 genes that were supported by two or more independent items of evidence to identify 18 molecular pathways that were enriched, covering both upstream signaling events and downstream effects. Based on this work, Li and colleagues proposed

that the 396 genes work in a network of common pathways to influence the final net release of DA and glutamate in the brain's reward centers, thus effecting drug-seeking behavior. This is further of interest because the discovery made using the candidate gene approach provided a specific therapeutic target for potentially treating the phenotype Reward Deficiency Syndrome (RDS) with a DA D_2 agonist—discussed in Sect. 10.3.

While there is a plethora of genetic variation at the level of mesocorticolimbic activity, certain candidate genes and their polymorphisms predispose individuals to excessive cravings and resultant aberrant behaviors (Blum et al. 1996b). Examples are as follows: DA D_2 receptor (DRD2), DA D_4 receptor (DRD4), DA transporter (DAT1), serotonergic-2A receptor (5-HTT2a), serotonergic transporter (5HTTLPR), catechol-o-methyl-transferase (COMT), and monoamine oxidase (MOA) genes. Of these, the DRD2 gene has been one of the most widely studied for its relationship to neuropsychiatric disorders, in general, and in alcoholism and other addictions in particular (Blum et al. 2000), and the DRD2 A1 allele has been referred to as a reward gene (Blum et al. 1990). In 1996, Blum and his colleagues first described RDS to define the common genetic variant involving DA D_2 receptor gene (DRD2) polymorphisms (Grandy et al. 1989; Smith et al. 1989) as putative predictors of impulsive, compulsive, and addictive behaviors (Blum et al. 1996c). It is an umbrella term to describe the common genetic antecedents of such behaviors.

The DRD2 TaqIA polymorphism is associated with DA D_2 receptor density, which plays an important role in the context of reward. Persons carrying an A1 allele have a lower D_2 receptor density. In turn, a deficiency or absence of the D_2 receptors then predisposes individuals to high risk for multiple addictive, impulsive, and compulsive behaviors (Comings et al. 1995; Comings and Blum 2000; Koob 2003; Koob and Le Moal 2001; Serý et al. 2006).

One study was designed to investigate the influence of the DRD2 TaqIA polymorphism and the selective D_2 receptor agonist bromocriptine on the activation of the reward system by means of functional magnetic resonance imaging (fMRI). In a double-blind crossover study with 24 participants, Kirsch et al. (2006) found an increase of reward system activation from placebo to bromocriptine only in subjects carrying the A1 allele. Furthermore, only A1 carriers showed an increase of performance under bromocriptine. The results were interpreted as reflecting a specific sensitivity for DA agonists in persons carrying an A1 allele and may complement actual data and theories of the development of addiction disorders that postulate a higher genetic risk for substance abuse in carrier of the A1 allele (see Lawford et al. 1995).

Individuals possessing a paucity of serotonergic and/or dopaminergic receptors and an increased rate of synaptic DA catabolism due to high catabolic genotype of the COMT gene, or high MOA activity, are predisposed to self-medicating with any substance or behavior that will activate DA release. These self-imposed treatments include alcohol, opiates, psychostimulants, nicotine, glucose, gambling, sexual promiscuity, and even excessive Internet gaming, as well as others (Comings and Blum 2000). Use of most drugs of abuse, including alcohol, is

associated with the release of DA in the mesocorticolimbic system or brain reward circuitry (Di Chiara 1999, 2002; Reuter et al. 2005). Thus, activation of the dopaminergic system induces feelings of reward and pleasure (Eisenberg et al. 2007; Volkow et al. 2002), decreases negative feelings, and satisfies abnormal cravings (Adler et al. 2000; Blum et al. 1991; Carboni et al. 2000; Di Chiara 1999, 2002; Noble et al. 1994). However, reduced activity of the DA system (hypodopaminergic functioning) can trigger drug-seeking behavior (Dackis et al. 1985; Volkow et al. 2001). Variant alleles can induce hypodopaminergic functioning through reduced DA receptor density, blunted response to DA, or enhanced DA catabolism in the reward circuitry (Hietala et al. 1994). Possibly, cessation of chronic drug use induces a hypodopaminergic state that predisposes individuals to a high risk for multiple addictive, impulsive, and compulsive behaviors (Comings et al. 1995; Comings and Blum 2000; Koob 2003; Koob and Le Moal 2001; Serý et al. 2006) and prompts drug-seeking behavior in an attempt to address the withdrawal-induced state (Melis et al. 2005).

Low DA receptor density due to carrying the DRD2 A1 allelic genotype can lead to excessive cravings and consequential behaviors, whereas normal or high DA receptors typically result in low craving-induced behaviors. In sum, when the mesocorticolimbic DA reward system functions abnormally (potentially caused by certain genetic variants), the end result is RDS (Blum et al. 1996c) and subsequent drug-seeking behavior.

10.2.2 The Dopamine-Addiction Connection

Understanding how low DA function leads to impulsive, compulsive, and addictive behaviors paves the way for defining addiction as a brain disorder involving impairments in reward circuitry (Blum et al. 2000). The definition of addiction has now been adopted by the American Society of Addiction Medicine (ASAM 2011).

This new definition is based in part on our initial conceptualization of the brain reward cascade (see Fig. 10.2) and our discovery in 1990, in collaboration with Earnest Noble, of the genetic association between alcohol addiction and the reward gene DRD2 (Blum et al. 1990, 1996a, b, c). This was the first evidence of the link between addictive behavior genes and neurotransmitters. As indicated, the term RDS was first introduced as an umbrella term to include behaviors associated with genetic antecedents that result in a hypodopaminergic state and a predisposition to obsessive, compulsive, and impulsive traits. All of these behaviors have been linked with low DA function due to an association with the presence of the DRD2 A1 gene form (Blum et al. 1996a, b, c, 2012a). Based on an abundance of literature indicating that low brain DA function confers a high vulnerability to substance use and aberrant behavior seeking, it is not surprising that every known abusable drug, as well as gaming, sex, and even music (Blum et al. 2012b), can cause the neuronal release of DA at brain reward sites. In essence, this helps explain the concept of self-medication. An individual with low DA function will seek out substances

or behaviors known to boost DA function. This can be achieved temporarily with drugs and behaviors that provide a pseudo-sense of well-being, happiness, and a temporary feeling of "normalization" (Blum et al. 2012b).

While having any genetic deficit in reward sites of the brain may predispose an individual to a higher risk for RDS, it is always the combination of our genes and their interaction with environmental elements that predict not only addictive behaviors in general, but also the specificity of the type of drug or behavior of choice. Research by Clark and Grunstein (2000), studying identical twins raised in different families, showed that about half of human behavior (including aggression, sexuality, mental function, eating disorder, alcoholism, and drug abuse or generalized RDS), was influenced by genes (Uhl et al. 1993). In this regard, it has been shown that individuals with RDS features may have a reduced number of DA D_2 receptors (Hietala et al. 1994; Noble et al. 1991) and a high number of DA transporter sites (Tiihonen et al. 1995; Tupala et al. 2001a, b, 2003). Certainly, the finding of hypodominergic function as discovered in pathological gambling (Comings et al. 1996, 2001; Reuter et al. 2005), an example of a RDS behavior, helps us understand the potential driving force of some to induce activation of the DA system (Thut et al. 1997).

However, it should be emphasized that very few behaviors depend upon a single gene. Complexes of genes (polygenic) drive most of our heredity-based actions, suggesting that genetic panels or algorithms organized into genetic indexes, such as a *gene profile,* may be valuable clinically. As Sussman and Sussman (2011) pointed out, RDS is highly impacted by environmental epigenetic factors affecting our RNA, rather than just genetic factors involving our DNA. While people are not doomed to become addicted because of their genes, they are definitely at high risk and, as such, may be helped by this genetic knowledge earlier rather than later in life. Understanding the interaction of these many influences is likely to lead to better treatment. It is important to consider one of the most important new areas in neurobiology and genetics termed "epigenetics" and its role in RDS. Epigenetics refers to heritable alterations in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA.

The role of dopaminergic genes as a predictor of risk concerning personality traits has been positively identified in molecular genetic studies. Earlier work in our laboratory identified the relationship between schizoid avoidance (Blum et al. 1997) and impulsive and compulsive behaviors (Blum et al. 1995). We also reviewed the epigenetics of ADHD and detailed the important interaction of environmental elements and gene expression in that disorder (Archer et al. 2011). The work of Teh et al. (2012) showing significantly higher frequency for the DRD2 TaqIA polymorphism among addicts (69.9%) compared to control subjects (42.6%; $p < 0.05$) also showed that the addicts had higher scores for novelty seeking and harm avoidance personality traits but lower scores for reward dependence.

In a recent study, Blum et al. (2011a) evaluated the potential association of DA D_2 receptor gene (DRD2), DA D_1 receptor gene (DRD1), DA transporter gene (DAT1), and DA beta-hydroxylase gene (DBH) polymorphisms in RDS subjects in order to illustrate the relevance of a generalized RDS behavior set as the

phenotype. Blum and his group genotyped an experimental cohort of 55 subjects derived from up to five generations of two independent multiple-affected families. Data related to RDS behaviors were collected on these subjects plus 13 deceased family members. Among the genotyped family members, 78% carried the DRD2 Taq1 allele, 58% carried the DAT1 10/10 allele, 66% carried the DBHB1 allele, and 35% carried either the DRD1 A1/A1 or A2/A2 genotypes. The experimental positive rate for the DRD2 Taq1 allele was significantly greater ($\chi^2 = 43.6$, $p < 0.001$), with an odds ratio of 103.9 (12.8, 843.2).

Researchers estimate that genetic factors account for between 40 and 60% of a person's vulnerability to addiction (especially alcoholism), including the effects of environment on gene expression and function. It is noteworthy that adolescents and individuals with comorbid mental disorders are at greater risk of drug abuse and addiction than the general population (Pickens et al. 1991).

10.3 Applications and Clinical Relevance

The neural mechanisms of reward dependence and deficiency we have described, including the current knowledge of receptor genes and polymorphisms, have clear relevance in the realm of substance abuse treatment. In terms of preventing substance abuse or excessive cravings, one goal would be to induce a proliferation of DA D_2 receptors in genetically prone individuals (Rothman et al. 2007). Experiments in vitro have shown that constant stimulation of the DA receptor system with a known D_2 agonist in low doses results in significant proliferation of D_2 receptors in spite of genetic antecedents (Boundy et al. 1995). In essence, D_2 receptor stimulation signals negative feedback mechanisms to induce mRNA expression causing proliferation of D_2 receptors. This proliferation of D_2 receptors, in turn, could induce the attenuation of craving behavior. In fact, this has been proven with work showing DNA-directed overexpression (a form of gene therapy) of the DRD2 receptors and significant reduction in both alcohol and cocaine craving-induced behavior in rodents (Thanos et al. 2001, 2008).

Such observations are the basis for the development of a functional hypothesis of drug seeking and drug use: The presence of a hypodopaminergic state, regardless of the source, is a primary cause of drug-seeking behavior. Thus, genetic polymorphisms that induce hypodopaminergic functioning may be the causal mechanism of a genetic predisposition to chronic drug use and relapse (Merlo et al. 2008). Moreover, it is likely that utilizing a long-term dopaminergic activation approach will ultimately lead to a common safe and effective modality to treat RDS behaviors including substance use disorders, attention deficit/hyperactivity disorder (ADHD), and obesity, among other reward deficient aberrant behaviors.

Based on neurochemical and genetic evidence, Blum et al. (2008) suggested that prevention and treatment of multiple addictions, such as dependence to alcohol, nicotine, and glucose, should involve a biphasic approach. Thus, acute treatment should consist of preferential blocking of postsynaptic NAc DA receptors (D_1–D_5), whereas long-term activation of the mesocorticolimbic dopaminergic

system should involve activation and/or release of DA at the NAc site. Failure to do so will result in abnormal mood, behavior, and potential suicide ideation. Individuals possessing a paucity of serotonergic and/or dopaminergic receptors, and an increased rate of synaptic DA catabolism due to high catabolic genotype of the COMT gene, are predisposed to self-medicating any substance or behavior that will activate DA release, including alcohol, opiates, psychostimulants, nicotine, gambling, sex, and even excessive Internet gaming. Acute utilization of these substances and/or stimulatory behaviors induces a feeling of well-being. Unfortunately, sustained and prolonged abuse leads to a toxic pseudo-feeling of well-being resulting in tolerance and disease or discomfort. Thus, a reduced number of DA receptors, due to carrying the DRD2 A1 allelic genotype, result in excessive craving behavior, whereas a normal or sufficient amount of DA receptors results in low craving behavior. In terms of preventing substance abuse, one goal would be to induce a proliferation of DA D_2 receptors in genetically prone individuals. While in vivo experiments using a typical D_2 receptor agonist induce downregulation, experiments in vitro have shown that constant stimulation of the DA receptor system with a known D_2 agonist results in significant proliferation of D_2 receptors in spite of genetic antecedents. In essence, D_2 receptor stimulation signals negative feedback mechanisms in the mesocorticolimbic system to induce mRNA expression causing proliferation of D_2 receptors.

With regard to utilizing this long-term dopaminergic activation approach, treatment providers should address the following areas within the broader, holistic therapeutic approach:

- Genetic testing to determine risk for RDS;
- Drug testing to assist in determining medication adherence and use as outcome measures;
- Tests related to alterations of reward gene expression as a molecular outcome measure; and
- Safe and effective nutrigenomic and neuromodulation solutions to activate dopaminergic pathways in the brain.

The patient who carries certain high-risk genetic deficits, such as low DA function (*dopamine resistance*) in the brain reward site, is at a high risk of relapse. Following treatment—residential or non-residential—where no attempt is made to enhance the function of brain DA, the patients who most likely carry gene variants that cause low DA function in the brain are released back into society and may be doomed to relapse.

We propose that utilizing exercise, pharmacological treatments, and/or natural dopaminergic repletion therapy to promote long-term dopaminergic activation could lead to a common, safe, and *effective modality to treat RDS conditions.* We also share the consensus in the literature drawn from many years of study, suggesting that prevention of drug-seeking relapse can be attenuated by enriched environments. For example, Chauvet et al. (2009) showed in rats the potential "curative" influence of enriched environments in reducing cocaine-induced craving effects, thereby highlighting the importance of positive life conditions in facilitating abstinence and preventing relapse to cocaine addiction.

10.4 Conclusion

Advances in neuroscience, neuroimaging, pharmacology, genetics, and genomics have confirmed and extended early ideas about the critical role of DA and related genes and gene deficits in the etiology and risk for drug dependence. Nonetheless, while DA hypotheses have been reported, corroborated, and strengthened, changes have been slow to move from the bench to the bedside (Dackis and Gold 1985; Goldstein and Volkow 2011a; Giordano and Blum 2010; Dackis and Gold 1985; Giordano and Blum, 2010). The field is poised to embark on large population studies incorporating new ideas, especially as they relate to dopaminergic targeting of mesocorticolimbic pathways. Prevention, diagnosis, treatment, and relapse-deterrence tactics can be augmented considerably by promoting rigorous multiyear outcome research in outpatient and residential inpatient programs. Diagnostic precision and better treatment outcomes are more likely when consideration is given to the neuroanatomy of reward and control circuitry, the common pathway of DA release, and the concept of RDS as an inherited and acquired change in sensitivity and calibration of this circuitry.

Utilizing exercise, pharmacological treatments, and/or natural dopaminergic repletion therapy to promote long-term dopaminergic activation could lead to a common, safe, and effective modality to treat substance use disorders and other RDS conditions. This concept is further supported by the more comprehensive understanding of the role of DA in the NAc as a "wanting" messenger in the mesocorticolimbic DA system. We further recommend that DSM diagnosis should include gene polymorphic testing using candidate gene analysis to assist in individualizing diagnosis, risk assessment, and therapy. By classifying risk severity and striving to remediate these deficits with medication, diet, and exercise, we could improve future clinical trials.

In summary, there may be a common neurogenetic mechanism underlying addictive, impulsive, and compulsive disorders, of which RDS is a basic phenotype. RDS covers many reward behaviors and psychiatric disorders (including spectrum disorders and posttraumatic stress disorder) that could possibly benefit from future inclusion in DSM revisions as an umbrella for diagnostic conditions with strong genetic influences.

References

Adler CM, Elman I, Weisenfeld N et al (2000) Effects of acute metabolic stress on striatal dopamine release in healthy volunteers. Neuropsychopharmacology 22(5):545–550

Archer T, Oscar-Berman M, Blum K (2011) Epigenetics in developmental disorder: ADHD and endophenotypes. J Genet Syndr Gene Ther 2(104)

ASAM (2011) Public policy statement: definition of addiction. American Society of Addiction Medicine. http://www.asam.org/quality-practice/definition-of-addiction

Barbas H (2007) Flow of information for emotions through temporal and orbitofrontal pathways. J Anat 211(2):237–249

Barrett LF, Mesquita B, Ochsner KN et al (2007) The experience of emotion. Annu Rev Psychol 58:373–403

Bechara A (2003) Risky business: emotion, decision-making, and addiction. J Gambl Stud 19(1):23–51

Berger LR, de Ruiter DJ, Churchill SE et al (2010) *Australopithecus sediba*: a new species of homo-like *australopith* from South Africa. Science 328(5975):195–204

Blum K (2011) Neurogenetics and nutrigenomics of reward deficiency syndrome. In: Barh D, Blum D, Madigan K (eds) Omcics biomedical perspectives and application. CRC Press, Boca Raton, pp 535–564

Blum K, Noble EP, Sheridan PJ et al (1990) Allelic association of human dopamine D_2 receptor gene in alcoholism. JAMA 263(15):2055–2060

Blum K, Noble EP, Sheridan PJ et al (1991) Association of the A1 allele of the D_2 dopamine receptor gene with severe alcoholism. Alcohol 8(5):409–416

Blum K, Sheridan PJ, Wood RC et al (1995) Dopamine D_2 receptor gene variants: association and linkage studies in impulsive-addictive-compulsive behaviour. Pharmacogenet 5(3):121–141

Blum K, Braverman ER, Wood RC et al (1996a) Increased prevalence of the Taq I A1 allele of the dopamine receptor gene (DRD2) in obesity with comorbid substance use disorder: a preliminary report. Pharmacogenetics 6(4):297–305

Blum K, Cull JG, Braverman ER et al (1996b) Reward deficiency syndrome. Am Sci 84:132–145

Blum K, Sheridan PJ, Wood RC et al (1996c) The D_2 dopamine receptor gene as a determinant of reward deficiency syndrome. J R Soc Med 89(7):396–400

Blum K, Braverman ER, Wu S et al (1997) Association of polymorphisms of dopamine D_2 receptor (DRD2), and dopamine transporter (DAT1) genes with schizoid/avoidant behaviors (SAB). Mol Psychiatry 2(3):239–246

Blum K, Braverman ER, Holder JM et al (2000) Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. J Psychoactive Drugs 32 Suppl:i–iv, 1–112

Blum K, Chen AL, Chen TJ et al (2008) Activation instead of blocking mesolimbic dopaminergic reward circuitry is a preferred modality in the long term treatment of reward deficiency syndrome (RDS): a commentary. Theor Biol Med Model 5:24

Blum K, Chen AL, Oscar-Berman M et al (2011a) Generational association studies of dopaminergic genes in Reward Deficiency Syndrome (RDS) subjects: selecting appropriate phenotypes for reward dependence behaviors. Int J Environ Res Public Health 8(12):4425–4459

Blum K, Liu Y, Shriner R et al (2011b) Reward circuitry dopaminergic activation regulates food and drug craving behavior. Curr Pharm Des 17(12):1158–1167

Blum K, Chen AL, Giordano J et al (2012a) The addictive brain: all roads lead to dopamine. J Psychoactive Drugs 22(2):134–143

Blum K, Werner T, Carnes S et al (2012b) Sex, drugs, and rock 'n' roll: hypothesizing common mesolimbic activation as a function of reward gene polymorphisms. J Psychoactive Drugs 44(1):38–55

Bolla KI, Eldreth DA, Matochik JA et al (2005) Neural substrates of faulty decision-making in abstinent marijuana users. Neuroimage 26(2):480–492

Boundy VA, Pacheco MA, Guan W et al (1995) Agonists and antagonists differentially regulate the high affinity state of the D2L receptor in human embryonic kidney 293 cells. Mol Pharmacol 48(5):956–964

Brand M, Fujiwara E, Borsutzky S et al (2005) Decision-making deficits of Korsakoff patients in a new gambling task with explicit rules: associations with executive functions. Neuropsychology 19(3):267–277

Brand M, Pawlikowski M, Labudda K et al (2009) Do amnesic patients with Korsakoff's syndrome use feedback when making decisions under risky conditions? An experimental investigation with the game of dice task with and without feedback. Brain Cogn 69(2):279–290

Bruijnzeel AW, Repetto M, Gold MS (2004) Neurobiological mechanisms in addictive and psychiatric disorders. Psychiatr Clin North Am 27(4):661–674

Budygin EA, Park J, Bass CE et al (2012) Aversive stimulus differentially triggers subsecond dopamine release in reward regions. Neuroscience 201:331–337

Carboni E, Silvagni A, Rolando MT et al (2000) Stimulation of in vivo dopamine transmission in the bed nucleus of stria terminalis by reinforcing drugs. J Neurosci 20(20):RC102

Castiglione CM, Deinard AS, Speed WC et al (1995) Evolution of haplotypes at the DRD2 locus. Am J Hum Genet 57(6):1445–1456

Chauvet C, Lardeux V, Goldberg SR et al (2009) Environmental enrichment reduces cocaine seeking and reinstatement induced by cues and stress but not by cocaine. Neuropsychopharmacology 34(13):2767–2778

Chen TJ, Blum K, Chen AL et al (2011a) Neurogenetics and clinical evidence for the putative activation of the brain reward circuitry by a neuroadaptagen: proposing an addiction candidate gene panel map. J Psychoactive Drugs 43(2):108–127

Chen YI, Famous K, Xu H et al (2011b) Cocaine self-administration leads to alterations in temporal responses to cocaine challenge in limbic and motor circuitry. Eur J Neurosci 34(5):800–815

Clark WR, Grunstein M (2000) Are we hardwired? The role of genes in human behavior, 1st edn. Oxford University Press, New York

Comings D (1996) The gene bomb: does higher education and advanced technology accelerate the selection of genes for learning disorders, ADHD, addictive, and disruptive behaviors?. Hope Press, Toronto

Comings DE, Blum K (2000) Reward deficiency syndrome: genetic aspects of behavioral disorders. Prog Brain Res 126:325–341

Comings DE, MacMurray J, Johnson P et al (1995) Dopamine D_2 receptor gene (DRD2) haplotypes and the defense style questionnaire in substance abuse, Tourette syndrome, and controls. Biol Psychiatry 37(11):798–805

Comings DE, Gade R, MacMurray JP et al (1996) Genetic variants of the human obesity (OB) gene: association with body mass index in young women, psychiatric symptoms, and interaction with the dopamine D_2 receptor (DRD2) gene. Mol Psychiatry 1(4):325–335

Comings DE, Dietz G, Johnson JP et al (1999) Association of the enkephalinase gene with low amplitude P300 waves. NeuroReport 10(11):2283–2285

Comings DE, Gade-Andavolu R, Gonzalez N et al (2001) The additive effect of neurotransmitter genes in pathological gambling. Clin Genet 60(2):107–116

Connor JP, Young RM, Lawford BR et al (2002) D(2) dopamine receptor (DRD2) polymorphism is associated with severity of alcohol dependence. Eur Psychiatry 17(1):17–23

Cooper ML, Frone MR, Russell M et al (1995) Drinking to regulate positive and negative emotions: a motivational model of alcohol use. J Pers Soc Psychol 69(5):990–1005

Dackis CA, Gold MS (1985) New concepts in cocaine addiction: the dopamine depletion hypothesis. Neurosci Biobehav Rev 9(3):469–477

Dackis CA, Gold MS, Davies RK et al (1985) Bromocriptine treatment for cocaine abuse: the dopamine depletion hypothesis. Int J Psychiatry Med 15(2):125–135

Davis PF, Ozias MK, Carlson SE et al (2010) Dopamine receptor alterations in female rats with diet-induced decreased brain docosahexaenoic acid (DHA): interactions with reproductive status. Nutr Neurosci 13(4):161–169

Di Chiara G (1999) Drug addiction as dopamine-dependent associative learning disorder. Eur J Pharmacol 375(1–3):13–30

Di Chiara G (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 137(1–2):75–114

Dick DM, Edenberg HJ, Xuei X et al (2004) Association of GABRG3 with alcohol dependence. Alcohol Clin Exp Res 28(1):4–9

Eisenberg DT, Campbell B, Mackillop J et al (2007) Season of birth and dopamine receptor gene associations with impulsivity, sensation seeking and reproductive behaviors. PLoS ONE 2(11):e1216

Epping-Jordan MP, Markou A, Koob GF (1998) The dopamine D-1 receptor antagonist SCH 23390 injected into the dorsolateral bed nucleus of the stria terminalis decreased cocaine reinforcement in the rat. Brain Res 784(1–2):105–115

Erickson C (2007) The science of addiction: from neurobiology to treatment. WW Norton & Company, New York

Gardner EL (2005) Brain-reward mechanisms. In: Lowinson J, Ruiz P, Millman R et al (eds) Substance abuse: a comprehensive textbook, 4th edn. Lippincott Williams & Wilkins, Philadelphia

Georgiadis JR, Reinders AA, Paans AM et al (2009) Men versus women on sexual brain function: prominent differences during tactile genital stimulation, but not during orgasm. Hum Brain Mapp 30(10):3089–3101

Giordano J, Blum K (2010) Probing the mysteries of recovery through nutrigenomics and holistic medicine: science meets recovery. The Counselor 17(52)

Goldman D, Dean M, Brown GL et al (1992) D_2 dopamine receptor genotype and cerebrospinal fluid homovanillic acid, 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxyphenylglycol in alcoholics in Finland and the United States. Acta Psychiatr Scand 86(5):351–357

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159(10):1642–1652

Goldstein RZ, Volkow ND (2011a) Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. Nat Rev Neurosci 12(11):652–669

Goldstein RZ, Volkow ND (2011b) Oral methylphenidate normalizes cingulate activity and decreases impulsivity in cocaine addiction during an emotionally salient cognitive task. Neuropsychopharmacology 36(1):366–367

Gordon AS, Yao L, Jiang Z et al (2001) Ethanol acts synergistically with a D_2 dopamine agonist to cause translocation of protein kinase C. Mol Pharmacol 59(1):153–160

Grandy DK, Litt M, Allen L et al (1989) The human dopamine D_2 receptor gene is located on chromosome 11 at q22-q23 and identifies a TaqI RFLP. Am J Hum Genet 45(5):778–785

Hietala J, West C, Syvalahti E et al (1994) Striatal D_2 dopamine receptor binding characteristics in vivo in patients with alcohol dependence. Psychopharmacology 116(3):285–290

Hodge CW, Cox AA (1998) The discriminative stimulus effects of ethanol are mediated by NMDA and GABA(A) receptors in specific limbic brain regions. Psychopharmacology 139(1–2):95–107

Hodge CW, Chappelle AM, Samson HH (1996) Dopamine receptors in the medial prefrontal cortex influence ethanol and sucrose-reinforced responding. Alcohol Clin Exp Res 20(9):1631–1638

Joutsa J, Johansson J, Niemela S et al (2012) Mesolimbic dopamine release is linked to symptom severity in pathological gambling. Neuroimage 60(4):1992–1999

Kirsch P, Reuter M, Mier D et al (2006) Imaging gene-substance interactions: the effect of the DRD2 TaqIA polymorphism and the dopamine agonist bromocriptine on the brain activation during the anticipation of reward. Neurosci Lett 405(3):196–201

Konkle AT, Bielajew C (2004) Tracing the neuroanatomical profiles of reward pathways with markers of neuronal activation. Rev Neurosci 15(6):383–414

Koob GF (2003) Alcoholism: allostasis and beyond. Alcohol Clin Exp Res 27(2):232–243

Koob G, Kreek MJ (2007) Stress, dysregulation of drug reward pathways, and the transition to drug dependence. Am J Psychiatry 164(8):1149–1159

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24(2):97–129

Kuperstein F, Yakubov E, Dinerman P et al (2005) Overexpression of dopamine receptor genes and their products in the postnatal rat brain following maternal n-3 fatty acid dietary deficiency. J Neurochem 95(6):1550–1562

Lawford BR, Young RM, Rowell JA et al (1995) Bromocriptine in the treatment of alcoholics with the D_2 dopamine receptor A1 allele. Nat Med 1(4):337–341

LeDoux JE (2000) Emotion circuits in the brain. Annu Rev Neurosci 23:155–184
Li CY, Mao X, Wei L (2008) Genes and (common) pathways underlying drug addiction. PLoS Comput Biol 4(1):e2
Makris N, Oscar-Berman M, Jaffin SK et al (2008) Decreased volume of the brain reward system in alcoholism. Biol Psychiatry 64(3):192–202
Melis M, Spiga S, Diana M (2005) The dopamine hypothesis of drug addiction: hypodopaminergic state. Int Rev Neurobiol 63:101–154
Merlo LJ, Gold MS (2008) Prescription opioid abuse and dependence among physicians: hypotheses and treatment. Harv Rev Psychiatry 16(3):181–194
Muller NG, Machado L, Knight RT (2002) Contributions of subregions of the prefrontal cortex to working memory: evidence from brain lesions in humans. J Cogn Neurosci 14(5):673–686
Noble EP, Blum K, Ritchie T et al (1991) Allelic association of the D_2 dopamine receptor gene with receptor-binding characteristics in alcoholism. Arch Gen Psychiatry 48(7):648–654
Noble EP, Syndulko K, Fitch RJ et al (1994) D_2 dopamine receptor TaqI A alleles in medically ill alcoholic and nonalcoholic patients. Alcohol Alcohol 29(6):729–744
Ochsner KN, Gross JJ (2007) The neural architecture of emotional regulation. Handbook of emotion regulation. Guilford Press, New York, pp 87–109
Olsen CM (2011) Natural rewards, neuroplasticity, and non-drug addictions. Neuropharmacology 61(7):1109–1122
Oscar-Berman M, Bowirrat A (2005) Genetic influences in emotional dysfunction and alcoholism-related brain damage. Neuropsychiatr Dis Treat 1(3):211–229
Pandya D, Yeterian E (2002) The anatomical substrates of emotional behavior: the role of the cerebral cortex. In: Grafman IJ, Boller F (eds) The Frontal Lobes, 2nd edn. Elsevier, New York
Patterson JC, Ungerleider LG, Bandettini PA (2002) Task-independent functional brain activity correlation with skin conductance changes: an fMRI study. Neuroimage 17(4):1797–1806
Peciña S, Smith KS, Berridge KC (2006) Hedonic hot spots in the brain. Neuroscientist 12(6):500–511
Peciña M, Mickey BJ, Love T et al (2012) DRD2 polymorphisms modulate reward and emotion processing, dopamine neurotransmission and openness to experience. Cortex 49(3):877–890
Pickens RW, Svikis DS, McGue M et al (1991) Heterogeneity in the inheritance of alcoholism. A study of male and female twins. Arch Gen Psychiatry 48(1):19–28
Poldrack RA, Wagner AD, Prull MW et al (1999) Functional specialization for semantic and phonological processing in the left inferior prefrontal cortex. Neuroimage 10(1):15–35
Previc FH (1999) Dopamine and the origins of human intelligence. Brain Cogn 41(3):299–350
Raghanti MA, Stimpson CD, Marcinkiewicz JL et al (2008) Cortical dopaminergic innervation among humans, chimpanzees, and macaque monkeys: a comparative study. Neuroscience 155(1):203–220
Rapoport SI (1990) Integrated phylogeny of the primate brain, with special reference to humans and their diseases. Brain Res Brain Res Rev 15(3):267–294
Reuter J, Raedler T, Rose M et al (2005) Pathological gambling is linked to reduced activation of the mesolimbic reward system. Nat Neurosci 8(2):147–148
Rommelspacher H, Raeder C, Kaulen P et al (1992) Adaptive changes of dopamine-D_2 receptors in rat brain following ethanol withdrawal: a quantitative autoradiographic investigation. Alcohol 9(5):355–362
Rothman RB, Blough BE, Baumann MH (2007) Dual dopamine/serotonin releasers as potential medications for stimulant and alcohol addictions. AAPS J 9(1):E1–E10
Rushworth MF, Noonan MP, Boorman ED et al (2011) Frontal cortex and reward-guided learning and decision-making. Neuron 70(6):1054–1069
Schmitz JM (2005) The interface between impulse-control disorders and addictions: are pleasure pathway responses shared neurobiological substrates? Sex Addict Compulsivity 12(2–3):149–168

Serý O, Drtilkova I, Theiner P et al (2006) Polymorphism of DRD2 gene and ADHD. Neuro Endocrinol Lett 27(1–2):236–240

Smith M, Wasmuth J, McPherson JD (1989) Cosegregation of an 11q22.3-9p22 translocation with affective disorder: proximity of the dopamine D_2 receptor gene relative to the translocation breakpoint. Am J Hum Genet 45(A220)

Sussman S, Sussman AN (2011) Considering the definition of addiction. Int J Environ Res Public Health 8(10):4025–4038

Teh LK, Izuddin AF, M HF et al (2012) Tridimensional personalities and polymorphism of dopamine D_2 receptor among heroin addicts. Biol Res Nurs 14(2):188–196

Thanos PK, Volkow ND, Freimuth P et al (2001) Overexpression of dopamine D_2 receptors reduces alcohol self-administration. J Neurochem 78(5):1094–1103

Thanos PK, Michaelides M, Umegaki H et al (2008) D2R DNA transfer into the nucleus accumbens attenuates cocaine self-administration in rats. Synapse 62(7):481–486

Thut G, Schultz W, Roelcke U et al (1997) Activation of the human brain by monetary reward. NeuroReport 8(5):1225–1228

Tiihonen J, Kuikka J, Bergstrom K et al (1995) Altered striatal dopamine re-uptake site densities in habitually violent and non-violent alcoholics. Nat Med 1(7):654–657

Tupala E, Hall H, Bergstrom K et al (2001a) Dopamine D(2)/D(3)-receptor and transporter densities in nucleus accumbens and amygdala of type 1 and 2 alcoholics. Mol Psychiatry 6(3):261–267

Tupala E, Kuikka JT, Hall H et al (2001b) Measurement of the striatal dopamine transporter density and heterogeneity in type 1 alcoholics using human whole hemisphere autoradiography. Neuroimage 14(1 Pt 1):87–94

Tupala E, Hall H, Bergstrom K et al (2003) Dopamine D_2 receptors and transporters in type 1 and 2 alcoholics measured with human whole hemisphere autoradiography. Hum Brain Mapp 20(2):91–102

Uhl G, Blum K, Noble E et al (1993) Substance abuse vulnerability and D_2 receptor genes. Trends Neurosci 16(3):83–88

Volkow ND, Chang L, Wang GJ et al (2001) Low level of brain dopamine D_2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am J Psychiatry 158(12):2015–2021

Volkow ND, Fowler JS, Wang GJ (2002) Role of dopamine in drug reinforcement and addiction in humans: results from imaging studies. Behav Pharmacol 13(5–6):355–366

Wightman RM, Robinson DL (2002) Transient changes in mesolimbic dopamine and their association with 'reward'. J Neurochem 82(4):721–735

Wood JN, Grafman J (2003) Human prefrontal cortex: processing and representational perspectives. Nat Rev Neurosci 4(2):139–147

Wrase J, Schlagenhauf F, Kienast T et al (2007) Dysfunction of reward processing correlates with alcohol craving in detoxified alcoholics. Neuroimage 35(2):787–794

Chapter 11
Face Perception

Jennifer Richler and Isabel Gauthier

11.1 Definition and Overview

Face perception is a critical skill for survival: Among other information gathered from the face, specifying identity is necessary for deciding whether an individual is a known ally or enemy. Consequently, the cognitive demands of face perception differ from most instances of object recognition: Unlike objects, which are typically identified at the category level (e.g., "chair"; Rosch et al. 1976), recognizing faces as individuals (e.g., "Bob") is essential in day-to-day interactions. But, all faces consist of the same kinds of features (eyes, nose, and mouth) in the same configuration (eyes above nose, nose above mouth). Thus, one challenge of face recognition is to successfully individuate a large number of visually similar objects, while at the same time generalizing across perceptual features that are not critical to identity such as differences in illumination or emotional expression. Ultimately we master this task, but we continue to improve over many years of experience, with recent work suggesting that face recognition abilities do not peak until after 30 years of age (Germine et al. 2011).

The sociobiological necessity of individuating and differentiating group members combined with the differences in cognitive demands for faces compared with other object categories has led to specialization for face processing. For example, despite the limitations of the newborn visual system in terms of visual acuity, contrast sensitivity, and spatial frequency range (Nelson 2001), infants are

J. Richler (✉) · I. Gauthier
Department of Psychology, Vanderbilt University, Nashville, USA
e-mail: jennifer.j.richler@vanderbilt.edu

I. Gauthier
e-mail: isabel.gauthier@vanderbilt.edu

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_11

able to discriminate their mother's face from the face of strangers on the basis of visual information alone (e.g., Bushnell et al. 1989; Field et al. 1984). Infants also exhibit a more general visual preference for faces and spend more time looking at or tracking faces—including schematic faces—compared with other highly salient visual stimuli (e.g., Goren et al. 1975; Johnson et al. 1991; Maurer and Young 1983; Morton and Johnson 1991; Valenza et al. 1996; but see Easterbrook et al. 1999). These findings are sometimes taken as evidence that there is an innate face module; infants are born with a subcortical mechanism that contains structural information about faces and is responsible for orienting responses to objects that match this structure (Slater and Kirby 1998; but see Simion et al. 2007 for an alternative account proposing that infant preferences reflect general properties of the developing visual system).

Specialization for face perception is also supported by the discovery of neurons in the inferotemporal cortex (IT), particularly the superior temporal sulcus (STS), of non-human primates that respond preferentially to faces (Bruce et al. 1981; Gross et al. 1972; Perrett et al. 1982). These "face cells" are systematically organized in visual areas (e.g., cells within the same cortical columns in the 6-layer cortical lamina respond to similar head views), and they generally do not respond to most other visual or arousing stimuli, and their responses to faces are unaffected by image transformations (e.g., gray scale, size). In general, as long as a face is easy to perceive, face cells respond with little modulation; if faces are difficult or impossible to see, face cell activity is greatly reduced or eliminated. One notable exception is profile views, which reduce or eliminate face cell responses despite being easily recognized as faces. Individual face parts also elicit neural responses from face cells, although most face cells exhibit a larger response to stimuli containing multiple face features compared to stimuli containing a single face feature (Perrett et al. 1982). Indeed, cell tuning to individual features is enhanced when features are presented within a whole face context that includes other face features (Freiwald et al. 2009). Thus, there is an underlying organization of face cells in the primate brain that selectively respond to face perception.

Evidence for cortical areas specialized for face perception is also provided by adults suffering from prosopagnosia, an impairment in face recognition. Cases of prosopagnosia differ greatly in etiology: Prosopagnosia can be present from childhood (developmental or congenital prosopagnosia; McConachie 1976), or it can be the result of brain injury, stroke, or degenerative disease that affects occipitotemporal brain regions, specifically the fusiform gyrus, after normal face recognition skills have been acquired (Farah 1990). Critically, prosopagnosia is characterized by impaired face recognition while object recognition abilities are relatively spared and elementary visual processing remains intact. The existence of an impairment that disproportionately affects face recognition and at least one documented case of the opposite impairment (spared face recognition in the presence of object recognition deficits; Moscovitch et al. 1997) suggests that there is an anatomically segregated system dedicated to face recognition; it is possible to incur damage to this specific region without damaging brain areas necessary for general object recognition, and vice versa.

In summary, there is strong neurophysiological and neuropsychological evidence for specialization of parts of the visual system for face processing. Next, we turn to behavioral and cognitive neuroscience methods that afford more flexibility and experimental control and which have been used to study the nature of the mechanisms underlying such specialization.

11.2 Cognitive and Neural Mechanisms of Face Perception

Consistent with the location of brain lesions in prosopagnosic patients and face-selective cells identified by single-cell recordings in non-human primates, brain imaging studies in healthy adults have revealed several distinct regions in the brain that show more activity in response to faces relative to other objects, including scrambled faces: the STS, regions in the occipital fusiform area (OFA), the lateral fusiform gyrus (Puce et al. 1995, 1998; Sergent et al. 1992; Kanwisher et al. 1997), and the anterior temporal lobe (aIT) (Gauthier et al. 1999b; Sergent et al. 1992). The lateral fusiform gyrus has received the most attention, and the selectivity in this region is so robust that it has been named the fusiform face area (FFA; see Weiner and Grill-Spector 2011, for a review). Note that these regions are defined functionally, most often using a comparison of activation in response to images of faces versus a baseline of other objects or scenes. The label FFA, for instance, often maps onto two separate areas of activity, about 15 mm apart along the posterior–anterior axis of the fusiform gyrus (Pinsk et al. 2009; see Fig. 11.1). Recent advances in the spatial resolution of fMRI, from cubic voxels that are 3 mm to 1.5 or even 1 mm on each side, recently led to the proposal of a topography of relatively face-selective and body part-selective areas in high-level visual cortex that could be helpful in standardizing the labeling of functional areas across studies (Weiner and Grill-Spector 2011). The applicability of this scheme remains difficult to evaluate because most studies do not localize body parts-responsive areas. This is not the place to discuss all of the evidence relevant to the functional role of these different regions (see for instance Haxby et al. 2000 for an influential model), but it is generally suggested that the FFA represents an intermediate stage of processing in a ventral temporal cortex route for face perception that is critical to the representation of individual faces (Gauthier et al. 2000), between that of the OFA which seems to represent facial features (Pitcher et al. 2011) and the aIT, where individual faces elicit even more distinct response patterns (Kriegeskorte et al. 2007). However, new evidence based on dynamic causal modeling of fMRI data suggests that inputs may reach OFA and FFA in parallel, with the two regions reciprocally connected (Ewbank et al. 2012). The understanding of the anatomical and functional organization of high-level visual areas will no doubt continue to evolve in the next decades.

Moving on to human studies using methods that have even better temporal resolution, there is also evidence that an ERP component measured at occipitotemporal electrodes that emerges 170 ms after stimulus presentation is face-specific.

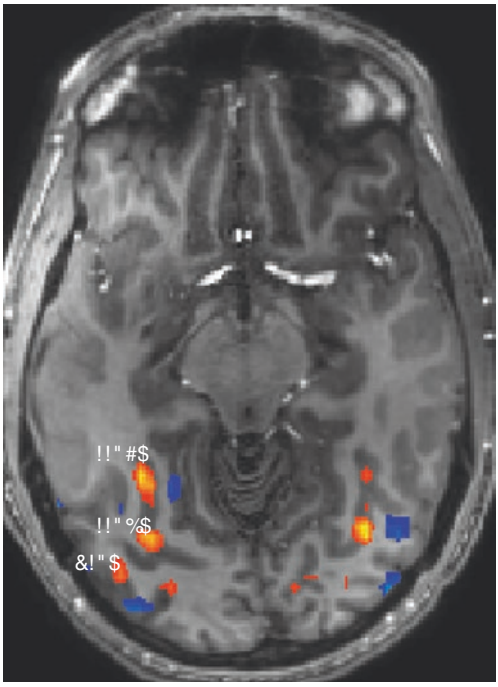

Fig. 11.1 Example of functional localization of face-selective areas in an individual subject, using a comparison of faces to various objects, in a task where subjects detect 1-back repetitions of identical images presented foveally, one per second. Other face-selective areas in the superior temporal sulcus and anterior temporal pole are also found and are not visible in this slice. Image courtesy of Rankin McGugin

This component, called the N170, is found for faces but not other categories of objects, such as cars or butterflies (Bentin et al. 1996; Eimer 1998), and is significantly reduced in prosopagnosic individuals (Eimer and McCarthy 1999). Similar to the pattern of single-cell recordings in non-human primates, the N170 is sensitive to the presentation of an intact face, showing a smaller amplitude and longer latency when face parts are presented in isolation (Bentin et al. 1996).

Neural activity in the FFA and the N170 ERP component are associated with two key behavioral signatures of face perception, holistic processing, and the inversion effect. Holistic processing refers to the fact that faces are processed as unified wholes rather than as a collection of features. The strongest evidence that faces are processed holistically comes from studies based on the composite illusion (see Fig. 11.2) where participants exhibit an inability to selectively attend to a single face half and ignore information in the rest of a face, even when instructed to do so, and even when a failure to do so is disadvantageous for performance (Young et al. 1987; Farah et al. 1998)—participants cannot ignore irrelevant information in a face because faces are processed as wholes. Holistic processing facilitates the extraction of information about spatial relations that goes beyond the shape of individual parts or their coarse configuration, enabling more rapid identification of visually similar objects, consistent with the unique goals of face perception. Indeed, holistic processing is observed for faces but not for non-face objects (Farah et al. 1998; Richler et al. 2011). Supporting the role of holistic

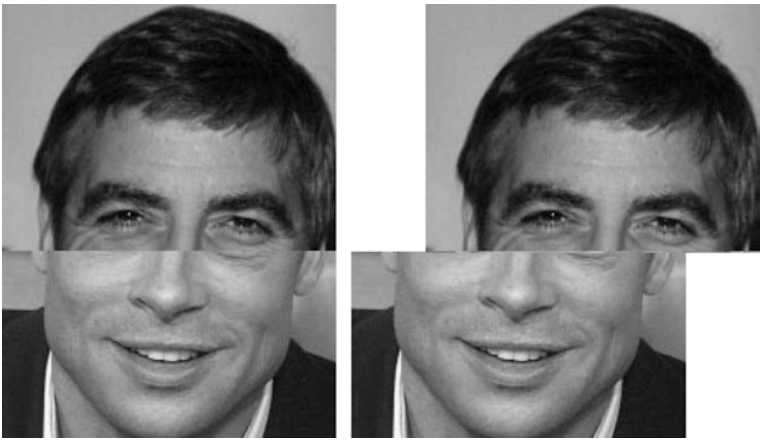

Fig. 11.2 Composite illusion. Participants are slower to name the top face half ("George Clooney") when it is aligned with a bottom face half belonging to a different individual (e.g., Brad Pitt) compared to when the parts are misaligned

processing in successful face recognition, recent work has shown that people who process faces more holistically are better at recognizing faces (Richler et al. 2011).

The inversion effect refers to the finding that inversion disrupts memory for faces more so than it does for other objects that have a clear canonical orientation (e.g., houses; Yin 1969; Carey and Diamond 1977; Valentine and Bruce 1986). In other words, although all mono-oriented objects show a processing advantage when upright, the difference in performance between upright and inverted is more pronounced for faces. One explanation for this phenomenon is that inversion disrupts the perception of metric distances between features (e.g., interocular distance) more so than the perception of individual local features (Leder and Bruce 2000; Searcy and Bartlett 1996; Rhodes et al. 1993). Because information about precise spatial relations between features is especially critical to face perception, inversion is particularly disruptive to performance.

An inversion effect is also observed in the FFA: Although the FFA responds preferentially to both upright and inverted faces (Kanwisher et al. 1998), FFA activity is reduced for inverted versus upright faces (Gauthier et al. 1999b; Yovel and Kanwisher 2005). These results are consistent with behavioral work showing that both upright and inverted faces are processed holistically, but overall performance is reduced for inverted faces (Richler et al. 2011; Sekuler et al. 2004). Moreover, longer presentation times are required to obtain holistic effects (Richler et al. 2011) and to achieve above-chance identification performance (Curby and Gauthier 2009) for inverted versus upright faces, findings that map on remarkably well to the delay in the N170 response when faces are inverted (Bentin et al. 1996; Rossion et al. 2000). Taken together, these results suggest that upright and inverted faces are processed in a qualitatively similar manner, but our more extensive

experience with upright faces leads to an advantage in processing efficiency over inverted faces, promoting better performance.

In summary, behavioral and neural evidence link the mechanisms specialized for face perception to holistic processing and reveal that this mechanism operates most efficiently for upright faces, although its action also generalizes to inverted faces, for which it is less effective. While such work attempts to capture what differs between faces and non-face objects, the results on inversion illustrate that the domain of operation of this mechanism is not all or none. Next, we review efforts to understand when non-face objects can be processed using the same mechanism as faces, and what it suggests about the nature of the phenotype.

11.3 Face Perception as a Behavioral Phenotype

Decades of research have established that face perception is supported by specialized cognitive and neural mechanisms. More recent work has shown that performance on face processing tasks is more strongly correlated with monozygotic versus dizygotic twins (Wilmer et al. 2010; Zhu et al. 2010). Together with growing evidence that developmental prosopagnosia is hereditary (de Haan 1999; Grueter et al. 2008), these results suggest that face perception is a heritable cognitive ability. But what exactly is being inherited?

On the one hand, individual differences in face perception have been shown to be dissociable from object perception, suggesting that face perception is a domain-specific heritable skill. In twin studies, performance on face processing tasks is unrelated to performance on tasks with other visual objects (Wilmer et al. 2010) or more general cognitive abilities (Zhu et al. 2010). Variability in performance on face versus object processing tasks is sometimes found to be independent (Furl et al. 2011; Garrido et al. 2009), and variability associated with performance with faces, but not objects, predicts overall activity (Furl et al. 2011) and gray matter volume (Garrido et al. 2009) in the FFA.

But, such evidence does not exhaust all the possible ways that face perception could be related to more domain-general skills. Indeed, while "faces" can approximately be considered one domain, "objects" cannot: Performance with one object category (e.g., cars) can be relatively independent from that with another object category (e.g., birds; Gauthier et al. 2000; Bukach et al. 2010). Indeed, the fact that recent twin studies (Wilmer et al. 2010; Zhu et al. 2010) base their conclusion that face recognition is a domain-specific heritable skill on a comparison with a single category of non-face objects is problematic; an approach that compares faces to a single object category does not reveal potential differences between non-face categories themselves. As an example, the Thatcher illusion, where it is difficult to detect that local features (e.g., eyes, mouth) have been inverted when the entire face is presented upside down (see Fig. 11.3) was believed to be face-specific because the illusion was larger for faces than for a single non-face category (Thompson 1980). However, the illusion for faces is not exceptionally large

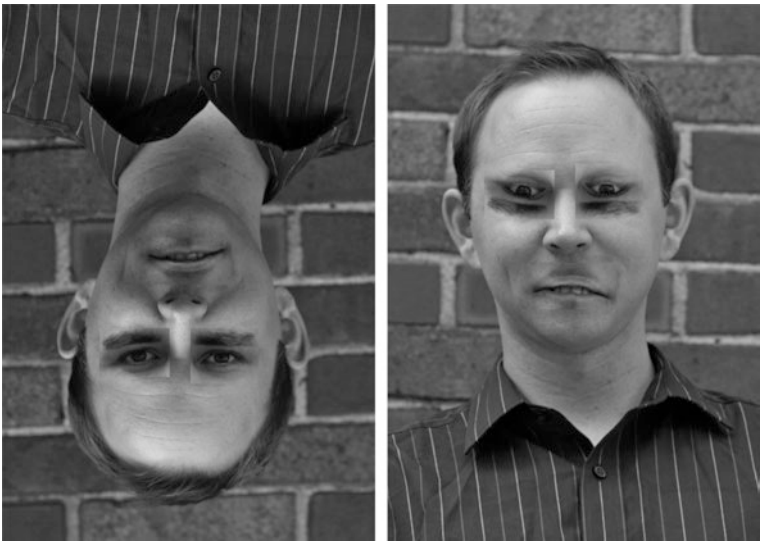

Fig. 11.3 Thatcher illusion. It is difficult to detect that local features (e.g., eyes, mouth) have been inverted when the entire face is presented upside down compared to when the face is presented upright

compared to the distribution obtained when many non-face categories are used (Wong et al. 2010). Therefore, it is not sufficient to claim that a face-specific phenotype has been found based solely on the evidence that performance with faces differs from that with a single non-face category because such a contrast does not capture the regular variability that exists between different non-face object categories. Thus, a unique challenge that arises when attempting to measure a potential domain-specific phenotype is to properly characterize domain specificity itself.

Of particular relevance to determining the domain specificity of face recognition is the distinction between *objects* and *objects of expertise*. In contrast to faces that tend to be processed at the individual level, objects are typically categorized at the basic level (Rosch et al. 1976). However, this is not always, nor does it have to be, the case. For example, an avid birder's goal is not simply to spot a bird, but rather to identify its species. Accordingly, bird experts can be as fast to categorize birds at the subordinate level (e.g., robin) as the basic level (e.g., bird; Tanaka and Taylor 1991). Moreover, individuals with extensive real-world experience individuating non-face objects within a visual homogenous category (e.g., cars or birds) process them more like faces: Objects of expertise are processed holistically (Bukach et al. 2012), and quantitative measures of expertise predict the magnitude of several neural and behavioral signatures of face perception, such as the inversion effect (Curby and Gauthier 2009), activity in the FFA (Gauthier et al. 2000; Xu 2005; Engel et al. 2009; McGugin et al., submitted A), and the magnitude of the N170 ERP component (Gauthier et al. 2003). Therefore, while individual differences in face recognition may dissociate from individual differences in object recognition, with the latter

being less heritable (Furl et al. 2011; Garrido et al. 2009; Wilmer et al. 2010; Zhu et al. 2010), these results may not hold when using objects of expertise.

Of course, these similarities do not necessarily mean that the perception of faces and objects of expertise are related abilities. However, recent research suggests that the perception of faces and objects of expertise do not merely occupy brain real estate in roughly the same neighborhood, but that they are in fact not functionally independent. For example, when face targets are interspersed among task-irrelevant cars, interference from the car distractors is observed as a function of car expertise, with car experts showing more interference than car novices (McKeeff et al. 2010; McGugin et al. 2011a; see also Gauthier et al. 2003; Rossion et al. 2004). Put more simply, processing non-face objects of expertise disrupts face perception. Such evidence suggests that performance with both faces and objects of expertise reflect a common mechanism that supports holistic processing.

Research suggests that holistic processing may develop in response to the individuation demands that are similar in these domains. That is, provided sufficient practice at the task of individuating visually similar objects, holistic processing mechanisms, to the extent that they are available to a given individual, appear to be recruited. Indeed, in addition to face recognition deficits, individuals with prosopagnosia often exhibit difficultly discriminating between items within visually homogenous non-face categories, such as cars or birds (Bornstein 1963; Damasio et al. 1982; see also Gauthier et al. 1999a). The idea that face perception is closely related to individuation is supported by training studies, where, unlike with real-world experts, the precise kind and amount of experience can be carefully controlled. These studies demonstrate that behavioral and neural signatures of face perception are obtained for novel objects following individuation training (Gauthier et al. 1998; Wong et al. 2009a, b), while other kinds of training regimens that teach categorization based on simple dimensions or local features but do not require individuation do not produce face-like outcomes (McGugin et al. 2011b; Wong et al. 2009a). These results suggest that one important property of high-level visual areas, including putative face-selective regions, is that they demonstrate functional flexibility and can be tuned by experience.

Thus, the alternative to a face-specific phenotype is that the observed genetic differences in face perception are the result of a more general aptitude for a particular kind of visual learning that happens to be critical in face perception. In fact, this phenotype may be most fully realized in face perception: Faces are a category where sufficient exposure coupled with motivating factors may lead most individuals to realize their full potential. For this very reason while face recognition ability may be one good measure of this phenotype, it is not sufficient for interpreting what the phenotype is about; it is difficult to find a non-face domain where the motivation and opportunity to develop expertise is universally high, and any relationship between faces and objects of expertise will break down if there is significant variability in subjects' experience. For example, a birder's individuation ability for birds will reflect a combination of the individual's aptitude for learning

subtle visual distinctions, their motivation to do so, and the intensity and duration of their efforts. Indeed, face recognition deficits in autism have been attributed to a breakdown in the normal acquisition of face expertise due to a lack of social motivation to attend to faces (Schultz 2005). One case study of a boy with autism revealed that while his FFA was not responsive to faces, FFA activity was elicited in response to Digimon characters, a category of objects with which the boy showed intense interest (Grelotti et al. 2005). These results support the notion that although individuals with autism show impaired face recognition, the underlying cognitive phenotype—individuation learning—remains intact. Therefore, training studies present an optimal approach to studying whether there is a domain-general behavioral phenotype related to individuation learning.

Having identified a potential domain-general ability that may underlie the heritability of face recognition, we must now turn to the issue of how we actually measure this ability. To properly characterize a behavioral phenotype, a measure that successfully taps into the construct of interest and that does so reliably within an individual is essential. Unfortunately, important concepts in face recognition that have since been applied to objects of expertise have been poorly measured in the past, in ways that do not capture the underlying trait of interest. For example, the composite task is the most popular measure of holistic processing. Yet, one often-used version of this task (*partial design*) has been shown to track response biases that are not stable and that can be influenced by task factors independently of the construct of interest, perceptual interference due to holistic processing (e.g., Cheung et al. 2008; Richler et al. 2011b, c). Although an alternative composite task measure (*complete design*) does not suffer from issues related to response biases and has been successfully used in studies of individual differences (McGugin et al., submitted B; Richler et al. 2011a), it too is not ideal for an individual differences approach: Holistic processing is operationalized as a difference of differences, and difference scores can often be less reliable than their component parts (Thorndike et al. 1991; see Zhu et al. 2010, supplemental information for an example). Thus, measuring the trait of interest is difficult even with faces, a category with which people have many years of experience (e.g., Germine et al. 2011). These issues will become even more important in research on a more domain-general aptitude where the goal is to find meaningful variance in individual performance that can be captured in brief learning studies.

Additionally, recent work has found striking sex differences in object recognition, with females showing superior performance with some categories, and males showing superior performance with others. Critically, the relationship between the individual differences in face recognition and object recognition is mediated by an interaction with sex (McGugin et al., submitted B): In other words, performance in face recognition is only correlated with performance for objects with sex-congruent categories (e.g., cars in men, birds in women). Although the cause of these sex differences are unknown (and, intriguingly, they are unrelated to self-reported experience and interest, see also Dennett et al. 2011), these results demonstrate that using a single category of control non-face objects to draw conclusions about

the domain specificity of what is heritable in face recognition is unlikely to be sufficient.

11.4 Conclusion

Face recognition is a task for which there is strong evidence of specialization in the primate brain. A great deal of cognitive psychology and cognitive neuroscience work has linked face processing to a particular perceptual strategy, that of holistic processing, which reflects observers' inability to selectively attend to parts of a face while ignoring the other parts. There is evidence suggesting that the system that supports holistic processing is not unique to faces, as objects in a non-face category can be processed holistically and engage face-selective responses in the brain, when individuals have had extensive experience individuating objects from this category. At least some of the abilities supporting face recognition appear to be heritable, but existing data does not allow us yet to conclude whether the ability is specific to faces or more general, reflecting the ability to learn holistically. The case of face recognition illustrates special difficulties inherent in establishing evidence for a domain-specific phenotype, such as the need to compare the putative domain to more than one control object and the importance of considering large differences in experience when comparing performance across domains.

References

Bentin S, Allison T, Puce A et al (1996) Electrophysiological studies of face perception in humans. J Cogn Neurosci 8(6):551–565

Bornstein B (1963) Prosopagnosia. In: Halpern L (ed) Problems of dynamic neurology. Hebrew University Hadassah Medical School, Jerusalem, pp 283–318

Bruce C, Desimone R, Gross CG (1981) Visual properties of neurons in a polysensory area in superior temporal sulcus of the macaque. J Neurophysiol 46:369–384

Bukach CM, Philips WS, Gauthier I (2010) Limits of generalization between categories and implications for theories of category specificity. Atten Percept Psychophys 72:1865–1874

Bukach CM, Vickery T, Kinka D, Gauthier I (2012) Training experts: individuation without naming is worth it. J Exp Psychol Hum Percept Perform 38:14–17

Bushnell IW, Sai F, Mullin JT (1989) Neonatal recognition of the mother's face. Br J Dev Psychol 7:3–15

Carey S, Diamond R (1977) From piecemeal to configurational representation of faces. Science 195:312–314

Cheung OS, Richler JJ, Palmeri TJ, Gauthier I (2008) Revisiting the role of spatial frequencies in the holistic processing of faces. J Exp Psychol Hum Percept Perform 34:1327–1336

Curby KM, Gauthier I (2009) The temporal advantage for individuating objects of expertise: perceptual expertise is an early riser. J Vis 9:1–13

Damasio AR, Damasio H, Van Hoesen GW (1982) Prosopagnosia: anatomical basis and behavioral mechanisms. Neurology 32:331–341

De Haan EHF (1999) A familial factor in the development of face recognition deficits. J Clin Exp Neuropsychol 21:312–315

Dennett HW, McKone E, Tavashami R et al (2011) The Cambridge car memory test: a task matched in format to the Cambridge Face Memory Test, with norms, reliability, sex differences, dissociations from face memory, and expertise effects. Behav Res Methods 44(2):587–605
Easterbrook MA, Kisilevsky BS, Hains SMJ, Muir DW (1999) Faceness or complexity: evidence from newborn visual tracking of facelike stimuli. Infant Behav Dev 22(1):17–35
Eimer M (1998) Does the face-specific N170 component reflect the activity of a specialized eye processor? NeuroReport 9:2945–2948
Eimer M, McCarthy RA (1999) Prosopagnosia and structural encoding of faces: evidence from event-related potentials. NeuroReport 10:255–259
Engel SA, Harley EM, Pope WB et al (2009) Activity in the fusiform face area supports expert perception in radiologists and does not depend upon holistic processing of images. pp 72630M-72630M-72638
Ewbank MP, Henson RN, Rowe JB et al (2012) Different neural mechanisms within occipitotemporal cortex underlie repetition suppression across same and different-size faces. Cereb Cortex. Epub ahead of print
Farah MJ (1990) Visual Agnosia: disorders of object recognition and what they tell us about normal vision. MIT Press, Cambridge
Farah MJ, Wilson KD, Drain M, Tanaka JN (1998) What is "special" about face perception? Psychol Rev 105:482–498
Field TM, Cohen D, Garcia R, Greenberg R (1984) Mother-stranger face discrimination by the newborn. Infant Behav Dev 7:19–25
Freiwald WA, Tsao DY, Livingstone MS (2009) A face feature space in the macaque temporal lobe. Nat Neurosci 12:1187–1196
Furl N, Garrido L, Dolan RJ et al (2011) Fusiform gyrus face selectivity relates to individual differences in face recognition ability. J Cogn Neurosci 23:1723–1740
Garrido L, Furl N, Draganski B et al (2009) Voxel-based morphometry reveals reduced grey matter volume in the temporal cortex of developmental prosopagnosics. Brain 132:3443–3455
Gauthier I, Behrmann M, Tarr MJ (1999a) Can face recognition really be dissociated from object recognition? J Cogn Neurosci 11:349–370
Gauthier I, Tarr MJ, Anderson AW et al (1999b) Activation of the middle fusiform "face area" increases with expertise in recognizing novel objects. Nat Neurosci 2:568–573
Gauthier I, Curran T, Curby KM, Collins D (2003) Perceptual interference supports a non-modular account of face processing. Nat Neurosci 6:428–432
Gauthier I, Tarr MJ, Moylan J et al (2000) The fusiform "face area" is part of a network that processes faces at the individual level. J Cogn Neurosci 12:495–504
Gauthier I, Williams P, Tarr MJ, Tanaka J (1998) Training "greeble" experts: a framework for studying expert object recognition processes. Vis Res 38:2401–2428
Germine LT, Duchaine B, Nakayama K (2011) Where cognitive development and aging meet: face learning ability peaks after age 30. Cognition 118:201–210
Goren CC, Sarty M, Wu PY (1975) Visual following and pattern discrimination of face-like stimuli by newborn infants. Pediatrics 56:544–549
Grelotti DJ, Klin AJ, Gauthier I, Skudlarski P, Cohen DJ, Gore JC, Volkmar FR, Schultz RT (2005) FMRI activation of the fusiform gyrus and amygdala to cartoon characters but not to faces in a boy with autism. Neuropsychologia 43(3):373–385
Gross CG, Rocha-Miranda CE, Bender DB (1972) Visual properties of neurons in inferotemporal cortex of the Macaque. J Neurophysiol 35:96–111
Grueter T, Grueter M, Carbon CC (2008) Neural and genetic foundations of face recognition and prosopagnosia. J Neuropsychol 2:79–97
Haxby JV, Hoffman EA, Gobbini MI (2000) The distributed human neural system for face perception. Trends Cogn Sci 4:223–233
Johnson MH, Dziurawiec S, Ellis H, Morton J (1991) Newborns' preferential tracking of facelike stimuli and its subsequent decline. Cognition 40:1–19

Kanwisher N, McDermott J, Chun MM (1997) The fusiform face area: a module in human extrastriate cortex specialized for face perception. J Neurosci 17:4302–4311
Kanwisher N, Tong F, Nakayama K (1998) The effect of face inversion on the human fusiform face area. Cognition 68:B1–B11
Kriegskorte N, Formisano E, Sorger B, Goebel R (2007) Individual faces elicit distinct response patterns in human anterior temporal cortex. Proc Natl Acad Sci U S A 104:20600–20605
Leder H, Bruce V (2000) When inverted faces are recognized: The role of configural information in face recognition. Q J Exp Psychol 53:513–536
Maurer D, Young RE (1983) Newborn's following of natural and distorted arrangements of facial features. Infant Behav Dev 6:127–131
McConachie HR (1976) Developmental prosopagnosia: a single case report. Cortex 12:76–82
McGugin RW, Gatenby C, Gore JC, Gauthier I (Submitted A) High-resolution imaging of expertise reveals reliable object selectivity in the FFA related to perceptual performance
McGugin RW, Richler JJ, Herzmann G et al (Submitted B) The Vanderbilt Expertise Test and the contribution of general object recognition abilities to face recognition
McGugin RW, McKeeff TJ, Tong F, Gauthier I (2011a) Irrelevant objects of expertise compete with faces during visual search. Atten Percept Psychophys 73:309–317
McGugin RW, Tanaka JW, Lebrecht S et al (2011b) Race-specific perceptual discrimination training improvement following short individuation training with faces. Cogn Sci 35:330–347
McKeeff TJ, McGugin RW, Tong F, Gauthier I (2010) Expertise increases the functional overlap between face and object perception. Cognition 117:355–360
Morton J, Johnson MH (1991) CONSPEC and CONLERN: a two-process theory of infant face recognition. Psychol Rev 98:164–181
Moscovitch M, Winocur G, Behrmann M (1997) What is special about face recognition? Nineteen experiments on a person with visual object agnosia and dyslexia but normal face recognition. J Cogn Neurosci 9:555–604
Nelson CA (2001) The development and neural bases of face recognition. Infant Child Dev 10:3–18
Perrett DI, Rolls ET, Caan W (1982) Visual neurones responsive to faces in the monkey temporal cortex. Exp Brain Res 47:329–342
Pinsk MA, Arcaro M, Weiner KS et al (2009) Neural representations of faces and body parts in Macaque and human cortex: a comparative fMRI study. J Neurophysiol 101:2581–2600
Pitcher D, Duchaine B, Walsh V et al (2011) The role of lateral occipital face and object areas in the face inversion effect. Neuropsychologia 49:3448–3453
Puce A, Allison T, Bentin S (1998) Temporal cortex activation in humans viewing eye and mouth movements. J Neurosci 18:2188–2199
Puce A, Allison T, Gore JC, McCarthy G (1995) Face-sensitive regions in human extrastriate cortex studied by functional MRI. J Neurophysiol 74:1192–1199
Rhodes G, Brake S, Atkinson AP (1993) What's lost in inverted faces? Cognition 47:25–57
Richler JJ, Cheung OS, Gauthier I (2011a) Holistic processing predicts face recognition. Psychol Sci 22:464–471
Richler JJ, Cheung OS, Gauthier I (2011b) Beliefs alter holistic face processing… if response bias is not taken into account. J Vis 11:1–13
Richler JJ, Mack ML, Palmeri TJ, Gauthier I (2011c) Inverted faces are (eventually) processed holistically. Vis Res 51:333–342
Rosch E, Mervis CB, Gray WD et al (1976) Basic objects in natural categories. Cogn Psychol 8:382–439
Rossion B, Gauthier I, Tarr MJ et al (2000) The N170 occipito-temporal component is enhanced and delayed to inverted faces but not to inverted objects: an electrophysiological account of facespecific processes in the human brain. NeuroReport 11:69–74
Rossion B, Kung CC, Tarr MJ (2004) Visual expertise with nonface objects leads to competition with the early perceptual processing of faces in the human occipitotemporal cortex. Proc Natl Acad Sci U S A 101:14521–14526

Schultz RT (2005) Developmental deficits in social perception in Autism: the role of the amygdala and fusiform face area. Int J Dev Neurosci 23:125–141
Searcy JH, Bartlett JC (1996) Inversion and processing of component and spatial-relational information in faces. J Exp Psychol Hum Percept Perform 22:904–915
Sekuler AB, Gaspar CM, Gold JM, Bennett PJ (2004) Inversion leads to quantitative, not qualitative, changes in face processing. Curr Biol 14:391–396
Sergent J, Ohta S, MacDonald B (1992) Functional neuroanatomy of face and object processing. A positron emission tomography study. Brain 115:15–36
Simion F, Leo I, Turati C et al (2007) How face specialization emerges in the first months of life. Prog Brain Res 164:169–185
Slater A, Kirby R (1998) Innate and learned perceptual abilities in the newborn infant. Exp Brain Res 123:90–94
Tanaka JW, Taylor M (1991) Object categories and expertise: is the basic level in the eye of the beholder? Cogn Psychol 23:457–482
Thompson P (1980) Margaret Thatcher: a new illusion. Perception 9:483–484
Thorndike RM, Cunningham GK, Thorndike RL, Hagen EP (1991) Measurement and evaluation. In: Psychology and education, 5th edn. Macmillan, New York
Valentine T, Bruce V (1986) The effect of race, inversion and encoding activity upon face recognition. Acta Psychol 61:259–273
Valenza E, Simion F, Cassia VM, Umiltà C (1996) Face preference at birth. J Exp Psychol Hum Percept Perform 22:892–903
Weiner KS, Grill-Spector K (2011) Neural representations of faces and limbs neighbor in human high-level visual cortex: evidence for a new organization principle. Psychol Res 77(1):74–97
Wilmer JB, Germine L, Chabris CF et al (2010) Human face recognition ability is specific and highly heritable. Proc Natl Acad Sci U S A 107:5238–5241
Wong YK, Twedt E, Sheinberg D, Gauthier I (2010) Does Thompson's Thatcher effect reflect a face-specific mechanism? Perception 1125–1141
Wong ACN, Palmeri TJ, Gauthier I (2009a) Conditions for facelike expertise with objects: becoming a Ziggerin expert—but which type? Psychol Sci 20:1108–1117
Wong ACN, Palmeri TJ, Rogers BT et al (2009b) Beyond shape: how you learn about objects affects how they are represented in visual cortex. PLoS ONE 4:e8405
Xu Y (2005) Revisiting the role of the fusiform face area in visual expertise. Cerebr Cortex 15:1234–1242
Yin RK (1969) Looking at upside-down faces. J Exp Psychol 81:141–145
Young AW, Hellawell D, Hay DC (1987) Configurational information in face perception. Perception 16:747–759
Yovel G, Kanwisher N (2005) The neural basis of the behavioral face-inversion effect. Curr Biol 15:2256–2262
Zhu Q, Song Y, Hu S et al (2010) Heritability of the specific cognitive ability of face perception. Curr Biol 20:1–6

Chapter 12
Language Phenotypes

Mabel Rice and Helen Tager-Flusberg

12.1 Introduction

Language is a complex, uniquely human capacity. At birth, infants are prepared to acquire language, which develops over time with most of the essential elements in place by the time a child enters school. The components of language that children typically acquire in an effortless way with little or no direct instruction include phonology (the basic speech sounds), lexicon (vocabulary or morphemes—the minimal unit of meaning), semantics (meaning in words and sentences), grammar (rules for combining words and grammatical elements such as tense or number), and discourse (use of language in different social settings). The rapid acquisition of this hierarchically organized communication system is an excellent example of a biologically based cognitive system that develops over the course of time as the child interacts with rich social and nonsocial environments. While the foundations of language are acquired by age five or so, language continues to develop beyond this time, particularly the acquisition of complex grammatical constructions, discourse, and vocabulary, often within the context of more formal school settings as the child learns to read (see Gleason 2013).

M. Rice (✉)
Child Language Doctoral Program, University of Kansas, Lawrence, USA
e-mail: mabel@ku.edu

H. Tager-Flusberg
Department of Psychology, Boston University, Boston, USA
e-mail: htagerf@bu.edu

H. Tager-Flusberg
Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, USA

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_12

But for a minority of children, despite the available and appropriate environmental contexts, speech and language development do not proceed as expected, especially in the early years. For example, most children with specific neurodevelopmental disorders such as Down syndrome or Williams syndrome are significantly delayed in language and may never reach the same end point as their peers (Luyster et al. 2011). In these disorders, language deficits travel fairly closely with delays in cognitive development that result in intellectual disability. The known genes (duplicated or deleted, depending on the syndrome) presumably have a fairly global impact on brain development, though there are clearly syndrome-specific neurocognitive and linguistic profiles that reflect the differential effects of specific genes on the growth of neural systems (Flint 1998). There are also examples of complex neurodevelopmental disorders, in which impairments in language and communication are among the *primary* diagnostic symptoms. These include specific language impairment (SLI), autism spectrum disorder (ASD), and speech sound disorder (SSD), which are all highly heritable (De Rubeis and Buxbaum 2015; Graham and Fisher 2013). However, to date, relatively few of the estimated thousand or more risk genes have been identified. Finding the genes associated with these language disorders is an important research agenda for which the use of valid phenotypes is clearly needed (Graham et al. 2015).

In this chapter, we focus our attention on language phenotypes that have shown significant promise in current research on developmental language disorders and that are grounded in what is known about typical language acquisition. This line of research not only is important for advancing our understanding about the underlying etiology of these disorders, which can have lasting impact on the lives of the children, but also holds the potential for discovering the genetic bases of language, the most remarkable achievement of human evolution. Early work on the search for language genes associated with language disorder focused on the investigations of the famous KE family in which about half of the family members suffered severe language, but not cognitive, impairments (Fisher et al. 2003). But this research was hampered by researchers' failure to cast a broad net in their characterization of the full phenotype of the affected individuals (e.g., Gopnik and Crago 1991). Thus, when mutations in the FOXP2 gene were discovered as the basis of the KE family syndrome, initial reactions were that this was the "grammar gene." We now know that the phenotype of the FOXP2 syndrome includes not only grammar, but also motor and phonological features of language (Vargha-Khadem et al. 1995; Watkins et al. 2002). The lessons learned from this work are that studies of language phenotypes need to encompass measures that tap multiple language domains and that are developmentally sensitive.

12.2 Overview of Language Phenotyping for Genetics Inquiry

An investigator faced with the choice of domains and particular instruments to serve as measurements for language phenotypes encounters a number of factors to be considered. First, as noted, language acquisition is a robust and dynamic age-dependent process. The manifestations of children's language abilities change over time, from infancy to late adolescence, and the appropriate forms of assessment vary by the developmental level of the children of interest. Second, the preferred phenotype method is direct assessment of children's language abilities, which can be time-consuming and requires training on the part of examiners. If the scope of the investigation rules out direct assessment, then indirect assessments such as questionnaires for caregivers can be used although there is the potential for informant error. Third, language is comprised of different dimensions that unfold in age-dependent progression. Speech, vocabulary, grammar, and discourse have different phenotypes as children mature, differences that may tap into distinct as well as shared underlying neurocognitive structures. Fourth, conventional assessment methods include standardized tests that are designed for a given age range. Some standardized tests evaluate all dimensions of language in an "omnibus" approach (e.g., Test of Language Development; Preschool Language Scale; and Clinical Evaluation of Language Fundamentals), whereas others assess a particular domain, such as vocabulary (e.g., Peabody Picture Vocabulary Test and Expressive Vocabulary Test). The outcome typically is a standardized score (usually with a mean of 100 and a standard deviation of 15 points) adjusted for age expectations. In effect, this yields an estimate of a particular child's performance relative to other children in the same age-group, which may or may not be predictive of a child's respective level of performance at previous or subsequent age levels. These tests provide static snapshots at a given level of development but do not reveal growth trajectories of change over time. Fifth, language phenotypes are associated with other phenotypes, leading to the possibility of cognitive phenotypes that are measurable indicators of hypothesized shared neurocognitive infrastructure. Some candidate phenotypes, such as nonword repetition, can be measured over a wide age range and require little time or training of examiners, thereby providing obvious advantages. Sixth, the phenotypes must align with the design and methods of the genetics inquiry, as well as the available resources. Some methods require large numbers of participants, such as genome-wide association studies that can require thousands of participants, whereas others, such as family-based studies, can be informative with modest sample sizes. Thus, direct assessment may be possible for smaller scale studies but prohibitive for the resources available in large-scale studies. Seventh, for obvious reasons, there are no animal models of human language acquisition and no animal models of potential endophenotypes. Some features of "speech" production are detectable in some mouse or avian models, with the possibility that such models may reveal neurological pathways similar to the pathways that could lead to speech impairments in humans, although this

remains to be determined (Konopka and Roberts 2016). At the level of language abilities, such as vocabulary and grammar development, there are no animal models of the sort that have been helpful in corroborating other human disease models. The complex higher processes involved in human language acquisition have yet to be modeled in animals. Thus, the genetics of language acquisition requires the study of people, and the etiology of ontological differences requires the study of young children, an expensive and time-consuming process that cannot be avoided.

In light of the many challenges summarized here, it is not surprising that genetic investigations of developmental language phenotypes are in early stages, with initial discoveries based on opportunities to study clinically ascertained families or samples of children with language impairments drawn from the records of school or clinical treatment facilities (Evans et al. 2015). More recently, programmatic investigations are beginning to appear, with the aim of identifying developmental phenotypes for language impairments of children and their family members in family-based linkage and association studies (Fisher and Vernes 2015). On the horizon are language growth phenotypes and phenotyping methods that would be applicable to large-scale investigation.

As genetic investigations move forward, there is an increasing recognition of the advantages of employing common phenotypic measures. Comparisons across studies will be facilitated by common measures, in order to assess replication at target gene locations and validity of outcomes for clinical groups. Further, the opportunity to aggregate participants across studies will be enhanced for large-scale investigations of many participants and attendant increases in statistical power to detect significant associations. Here, we focus on the first effort of this type including speech and language measures sponsored by the National Institutes of Health.

12.3 PhenX Project

The PhenX project, consensus measures for **Phen**otypes and e**X**posures, was developed to help researchers consistently measure and report human traits and environmental exposures in genetic studies. It is funded by the National Institutes of Health, National Human Genome Research Institute. The goal of the project was to select 15 high-priority, low-burden measures for each of the 21 medical research domains that are recommended for use in genome-wide association studies (GWAS) and other large-scale genomic studies. The measures were developed using a consensus-based process and are available in a free online "PhenX Toolkit" (www.phenxtoolkit.org), which provides information about each measure including references and links to resources. The comprehensive process involved more than 200 scientists from diverse scientific and health disciplines. The toolkit is intended to bolster efforts to compare data from multiple studies, accelerating efforts to understand the complex genetic and environmental factors that

contribute to diseases, and to usher in a new era of collaborative research that will have a positive impact on biomedical science and the human condition.

One of the 21 research domains included in the toolkit is speech and hearing, which includes language measures.[1] The goal of the workgroup was to select a set of standardized measures that researchers can use in GWAS and other large-scale genomic studies. Fifteen measures were selected for inclusion. Out of the fifteen, nine are in the areas of speech and language: family history, onset and early childhood speech and language assessment, grammatical impairments in preschool and school-age children, vocabulary assessments, phonemic inventories of speech development, stuttering, reading words and reading comprehension (as possible pleiotropic genetic effects) and a verbal memory measure as a possible endophenotype. The other six measures are in the areas of hearing and balance. Each of the measures is summarized in a standardized format in the toolkit. The PhenX protocol includes questions to record the child's age, recorded as the birth date and the age in years and months, as well as the date of the evaluation, in order to avoid possible ambiguities of interpretation of the phenotypes. The PhenX protocols are classified according to the age level for children's assessments.

An important consideration in the selection of direct assessments for speech and language in the toolkit was to avoid tests that provide only age-equivalent scores. Such scores appear frequently in some sectors of the literature and pose challenges to interpretation and replication across studies (Maloney and Larrivee 2007). Age equivalence is benchmarked to the average score of a group of children at a particular age level. For example, a 5-year-old child could have a raw score of 50 on a given test which is the same value as the mean score for 3-year-old children on the same test. The age-equivalent score could be used to characterize the child as two years behind in development. The problem is that such scores are not on an interval scale and cannot be interpreted in terms of calendar months of difference. For example, the differences between the mean scores for 36 months versus 42 months might be greater or less than the differences between mean scores for 54 months versus 60 months. For this reason, age-equivalent scores should not be used in arithmetic calculations of associations with genetic variants. Overall, age-equivalent scores can be misleading for clinical interpretation and are unsuited to the technical analyses of genetic investigations. Standardized scores have the advantage of providing comparable measurements across age levels on an interval scale. Depending on the design of the study, raw scores can be used for some scales or tests in some technical analyses.

[1]The Speech and Hearing Workgroup was chaired by Cynthia Morton (Brigham and Women's Hospital, Boston, MA) and Mabel Rice (University of Kansas). Committee researcher members were as follows: Ellen Cohn (University of Pittsburgh, Pittsburgh, PA), Dennis Drayna (National Institute on Deafness and Other Communication Disorders, Rockville, MD), Kenneth Grundfast (Boston University Medical Center, Boston, MA), and Bruce Tomblin (University of Iowa). Mary Marazita (University of Pittsburg, Pittsburgh, PA) was a liaison researcher with the Steering Committee, and Wayne Huggins, Noëlle Richa Siegfried, and Carol M. Hamilton were staff of RTI International, RTP, NC.

12.3.1 Summaries of Key Speech and Language Measures in PhenX

Family History Questionnaire: Children with speech and/or language impairments are likely to have a member of their nuclear family with a history of childhood speech and/or language impairments (Tomblin 1989). One of the best predictors of late language emergence (LLE) in young children is a positive history of familial speech and/or language impairments (Zubrick et al. 2007). Beginning in the late 1980s, a number of studies established that children with language or speech impairments without other developmental disabilities, as well as children who stutter, had positive family histories for these impairments (Lewis et al. 2007; Poulos and Webster 1991; Rice et al. 2009a, b; Tomblin 1989). Various forms of questions and questionnaires were used to elicit the family history data from informants, usually the child's mother. Rice et al. (1998) reported results from a short list of questions that were designed to elicit general information, such as "Has anyone in your child's family been slow in learning to talk?" as well as indications of particular symptoms. Because family members often are not attuned to symptoms of language impairment, it is necessary to utilize wording that is meaningful for describing possible language phenotypes. Examples are as follows: "…less talkative" or "…awkward sentence structures." The Family History Questionnaire for speech and language impairment in the PhenX Toolkit is a 20-item instrument that yields possible phenotypes for LLE and speech, language, reading, and spelling impairments, based on the Rice et al. (1998) study. Many of the items have been used in epidemiological longitudinal investigations of children in Norway and Australia and are in the current version of the National Health and Nutrition Examination Survey (NHANES—http://www.cdc.gov/nchs/nhanes.htm).

Onset and Early Childhood Speech and Language: Phenotypes of early language milestones are emerging as important benchmarks in the literature. For example, the *CNTNAP2* gene was reported to be an autism susceptibility gene in a study of children with autism using the phenotype of age at which a child spoke the first word (Alarcon et al. 2008). Studies of children with SLI (but not autism) identified an association of *CNTNAP2* variants with lower levels of language ability (Vernes et al. 2008). Further, *CNTNAP2* variants were associated with language acquisition at 24 months of age, in a general population sample (Whitehouse et al. 2011) in which the phenotype was based on the parental report, from the Communication Subscale of the Infant Monitoring Questionnaire (Bricker and Squires 1989), an early version of the *Ages and Stages Questionnaire* (Squires et al. 1999). The PhenX Toolkit includes the *Communication Subscale* of the *Ages and Stages Questionnaire*, a developmental screening tool, recommended for children of 24–30 months. The scale is comprised of 6 questions for a parent or caretaker to answer, designed to screen children for language impairments. A child's scores are compared to cutoff scores to determine a need for further assessment. The cutoff points are defined as 2 standard deviations below the mean, and the manual provides means and standard deviations for the Communication Subscale

in the target age range for the calculation of more lenient classification of impairment if desired for phenotyping.

The early preschool-age levels are challenging for direct assessment of speech and language skills. The methods require trained examiners, often involve more than 30 min of time, and may have questionable validity and reliability. Large-scale studies usually do not have the resources for direct assessment of children and therefore require a questionnaire form of measurement from a parent or caretaker. Although clinical interview forms are frequently used in this manner, there is little evidence that parental reports are valid, when compared to clinical judgments (Bishop and McDonald 2009). The *Speech and Language Assessment Scale* (*SLAS*) was developed for use with preschool children, as a supplement to formal clinical assessment and as an instrument to serve as a focal point for working with parents to enhance their awareness of their child's language and communicative abilities (Hadley and Rice 1993). The scale elicits parental judgments of a child's speech and language ability, benchmarked to a 7-point Likert scale that compares the target child to other children of the same age. When parental responses were compared to judgments made by certified speech language pathologists, they were found to have high validity. The scale includes 19 questions that yield an overall composite score and 5 subscales comprised of 2–3 items each: *Assertiveness and Responsiveness* measure the use of language in social contexts; *Semantics* measures vocabulary; *Syntax* measures grammar; and *Articulation* measures speech sound production and intelligibility. The format is a series of items for the respondent to judge using the same response scale across all items: 1 = very low for age; 4 = normal for age; and 7 = very high for age. Examples of the items are as follows: "My child's ability to use his/her words correctly is…"; "My child's ability to get what he/she wants by talking is…"; and "My child's ability to understand directions spoken to him/her is…"

Grammatical Impairments in Preschool- and School-Age Children: The domain of grammar shows strong promise for language phenotyping. Tense-marking, such as past tense—ed endings and third person singular present tense—s endings on verbs, has been identified as a strong clinical marker of SLI in English-speaking children (Tager-Flusberg and Cooper 1999). Tense-marking also shows significant heritability in twin samples (Bishop et al. 2006). Falcaro et al. (2008) reported significant linkage of tense-marking to a candidate gene area on chromosome 19q in a sample of children with SLI (Falcaro et al. 2008). Tense-marking is an area of linguistic weakness in children with ASD (Roberts et al. 2004), fragile X syndrome (Sterling et al. 2012), and Down Syndrome (Finestack 2013).

Three assessments of grammar are recommended in PhenX for preschool- and school-age children. The first is a sentence imitation task. A similar test was sensitive to the condition of SLI in an epidemiologically ascertained sample of 5-year-old children (Conti-Ramsden et al. 2001; Tomblin et al. 1996). The PhenX Toolkit includes the *Recalling Sentences* subtest of the *Clinical Evaluation of Language Fundamentals* (*CELF*), which requires a child to imitate sentences spoken by the examiner (Semel et al. 2013). As the test progresses, the sentences become longer

and more complicated. The child's responses are scored for the number of errors made in each sentence.

A second option included in PhenX is a set of subtests of the *Rice/Wexler Test of Early Grammatical Impairment* (*TEGI*): the *Past Tense Probe* and the *Third Person Singular Probe.* The subtests from *TEGI* are relatively brief (about 10 min) and are available free (https://cldp.ku.edu/rice-wexler-tegi), along with the standardization norms developed by the original test publisher. The tasks used in these subtests involve an interviewer showing pictures that elicit statements from the respondent with target morphemes that mark tense. The subtests have high sensitivity and selectivity for the identification of children with language impairments in both SLI (Rice and Wexler 2001) and ASD (Roberts et al. 2004) and are reported to have high and discrete heritability in twin studies (Bishop et al. 2006). There is suggestive evidence of gene linkage in studies reported by Falcaro et al. (2008) and Rice et al. (2009a, b). One limitation of the two probes is that the peak sensitivity and specificity are in a relatively narrow age range (3–8 years) which can limit power to detect genetic effects in a family-based study with a wide age range (cf. Rice et al. 2009a, b).

A third option in PhenX addresses the age issue in a test of tense-marking judgments, in a test adapted from an experimental task that is a clinical marker of SLI with longitudinal validity (Rice et al. 2009a, b). The *Rice Grammaticality Judgment Task-WH Questions* tests tense-marking in sentence contexts sensitive to language impairments in a broad age range, in children aged 4–16 years and beyond. The judgment task consists of the interviewer presenting recorded sentences that the respondent says are either good (grammatical) or not good (ungrammatical).

Vocabulary: One of the earliest indicators of language impairments in children is a limited vocabulary, relative to age norms. Vocabulary development is strongly associated with reading abilities in family samples, and reading ability is associated with language impairments (Rice et al. 2009a, b). Although vocabulary is not always below age expectations in children with ASD (Tager-Flusberg et al. 2005), it is often impaired in children with SLI (Rice and Hoffman 2015) and other forms of language impairment, such as children with Down Syndrome (Zampini and D'Odorico 2013) or fragile X syndrome (Roberts et al. 2007). A psychometrically robust method of assessing vocabulary is a straightforward picture identification task. PhenX recommends the *Peabody Picture Vocabulary Test*, fourth edition (*PPVT-4*), suitable for a wide age range from 30 months into adulthood (Dunn and Dunn 2007). Rice et al. (2009a, b) report a significant association with one SNP location on *KIAA0319*, as a weak signal but of interest because this is a candidate gene for reading impairments and there is a likely effect of language on reading comprehension (Bishop and Snowling 2004).

Phonemic Inventory: Speech sound disorder (SSD) is characterized by deficits in articulation, in phonological processing, and in the cognitive representation of sounds in words and morphemes. In the general population of 5-year-olds, speech and language disorders show little overlap (Shriberg et al. 1999). In clinical samples, there is a greater overlap, and in twin studies, there is evidence that

heritability estimates for SLI are increased when probands are ascertained through clinical referral for speech problems (Bishop and Hayiou-Thomas 2008). SSD is also associated with reading impairments. In studies of children ascertained for SSD, findings link this condition to reading-related loci on chromosomes 3, 6, and 15 (Miscimarra et al. 2007; Rice et al. 2009a, b; Smith et al. 2005; Stein et al. 2004, 2006). The PhenX Toolkit includes one measure of SSD: the *Sounds in Words* section of the *Goldman-Fristoe Test of Articulation*, a test of speech sound production (Goldman and Fristoe 2015). The examiner provides verbal cues to elicit single-word answers from the respondent that demonstrate common speech sounds. Two levels of scoring are provided depending on the qualifications of the examiner: at the first level, correct or incorrect production of speech sound, and at the second level, the types of errors produced in incorrect responses.

Stuttering: Stuttering is a speech disorder distinct from speech sound production (SSD) and language abilities (SLI). Stuttering is characterized by dysfluencies in speech production, which can occur as a single disorder or in conjunction with speech and/or language impairments (Yairi et al. 2001). Inherited forms of stuttering and genetic etiologies have been identified in family case studies of large pedigrees (Drayna and Kang 2011). The Toolkit includes the *Stuttering Severity Instrument*—fourth edition (*SSI-4*; Riley 2009). This is an interview-administered test that records the respondent's speech as he or she describes their job or school and reads a short passage. Computerized scoring software calculates the frequency and duration of stuttering dysfluencies and yields an estimate of speech naturalness. The scores are ranked according to the age-specific population norms.

Reading Words and Reading Comprehension: Converging evidence from molecular studies indicates the likelihood of pleiotropic effects of candidate genes that influence reading and language acquisition (Paracchini 2011; Pennington and Bishop 2009; Smith 2011). This suggests that genes that influence reading could also influence language, perhaps in part because early language acquisition is a strong predictor of subsequent reading abilities, especially reading comprehension (Catts et al. 2006). One implication is that a possible entry point into genetic investigations of language impairments is via genes linked or associated with reading impairment (Paracchini 2011; Smith 2011; Rice et al. 2009a, b). For example, a candidate gene on chromosome 6, *KIAA0319*, produced statistically significant signals in association analyses for speech, vocabulary, reading comprehension, and omnibus language abilities in a sample in which the same children received all tests (Rice et al. 2009a, b). PhenX includes the two subtests of word reading accuracy taken from the *Test of Word Reading Efficiency* (*TOWRE*; Torgesen et al. 2012). The *Sight Word Efficiency* (*SWE*) subtest records the number of real printed words accurately read by a respondent in 45 s. The *Phonetic Decoding Efficiency* (*PDE*) subtest records the number of pronounceable printed nonwords that are accurately decoded in 45 s. PhenX also includes a test of reading comprehension: the *Passage Comprehension Subtest* of the *Woodcock-Johnson Tests of Achievement.* The respondent is asked to read a short passage and identify a missing key word that makes sense in the context.

Verbal Memory/Nonword Repetition: Nonword repetition (NWR) tasks are measures of phonological short-term memory that have been suggested as "core deficits" in SLI (Newbury et al. 2005) or as a "key contributory trait of SLI" (Conti-Ramsden et al. 2001). Bishop et al. (2006) concluded that the evidence that nonword repetition deficits are causally related to syntactic deficits in SLI (such as the grammatical tense marker) is quite limited, leading to the current view that both nonword repetition and grammatical tense tasks measure distinct components of language phenotype. A possible role for nonword repetition ability as an endophenotype of language impairments is of great interest, in part because it is readily assessed in children aged 4 and older and in part because there is lively debate about the possible role of verbal memory in the causal pathways of language impairment (Gathercole 2006). The evidence varies across studies (Pawlowska 2014). Thus, in a candidate gene linkage and association study that yielded statistically significant signals for speech, language, and reading measures, there was little evidence of significance for a NWR phenotype (Rice et al. 2009a, b); however, Falcaro et al. (2008) reported significant linkage for NWR on chromosome 16. The PhenX Toolkit includes one assessment, the *Non-Word Repetition* (*NWR*) *Test* taken from the *Comprehensive Test of Phonological Processing* (CTOPP). The respondent listens to a series of nonsense words varying in syllable length that are played on an audiotape and repeats them back to the interviewer. Accuracy is scored as the number of consonants and vowels repeated correctly.

12.4 Other Language Phenotypes

12.4.1 Discourse Phenotypes

The hallmark feature of language impairment in ASD involves deficits in conversational discourse and other uses of language in social contexts (Tager-Flusberg et al. 2005). Even in children and adults who have no other language or reading deficit problems with social uses of language including conversational discourse and narrative, skills are evident in ASD and are often subtly impaired in other language disorders. PhenX includes a very brief assessment of conversational skills with a few questions included on the *Speech and Language Assessment Scale*, which is suitable for children at the preschool level.

Methods for assessing these aspects of language in older children and adults usually involve parent report questionnaires or expert ratings of conversational discourse. For children aged 4–16 years, the Children's Communication Checklist (CCC-2) was developed by Bishop and her colleagues to tap a range of language skills, including several scales of pragmatic abilities such as initiation of conversation, ability to interpret language in context, use of scripted language, and nonverbal communication (Bishop 2006). The CCC-2 is a questionnaire that is completed by a parent or teacher and scored by a clinician. For adults, the Pragmatic Rating Scale (PRS), an interviewer-based instrument, was developed by Landa et al.

(1992) to assess language in first-degree relatives of probands with ASD. This scale was modified (PRS-M) by Ruser et al. (2007) to include additional measures of nonverbal communication and administered to parents of children with ASD or SLI. In both versions of the PRS, the interviewer engages the adult in a conversation for about 20 min, with the interviewer beginning with open-ended questions about the participant's occupation, hobbies, and so forth. The interview is videotaped, and later, the key items (15 or 19, depending on the version used) are coded on a 3-point scale (0 = typical; 1 = somewhat abnormal; and 2 = frequently abnormal). The PRS-M items loaded on three subscales tapping pragmatic behavior: emotional expressiveness and awareness of the other; communicative performance; and overtalkativeness. Interestingly, Ruser et al. (2007) found that about 15% of both the ASD and SLI parents (many of whom had a history of SLI themselves) were scored as significantly impaired, with fathers showing overall greater pragmatic impairment than mothers. To date, no genetic studies have incorporated measures of discourse phenotypes in their analyses.

12.4.2 Brain Phenotypes

A considerable amount is known about the neural systems underlying language, dating back to the earliest autopsy studies carried out by Paul Broca and Heinz Wernicke (Poeppel et al. 2012). Patient studies and neuroimaging research on healthy right-handed people demonstrate that for most people, the left cortical hemisphere is more specialized for language perception and production than the right and that there are both structural and functional differences in the inferior frontal gyrus region and in the posterior superior temporal gyrus region favoring the left hemisphere (Foundas 1995). Anatomically, these language areas are relatively larger in the left hemisphere, and there is some evidence that this asymmetry may be present at birth (Galaburda et al. 1978). In functional imaging studies (primarily using fMRI or MEG), there is greater activation in the primary language regions of the left hemisphere for most language tasks. While these patterns are evident early in life, developmental studies using MRI have demonstrated that cortical areas associated with language, particularly those in prefrontal regions, continue to grow throughout childhood and into adolescence (Giedd et al. 2009; Thompson et al. 2005).

Several studies have investigated the brain bases of language impairment in both SLI and ASD. The findings demonstrate that individuals with SLI (e.g., Leonard et al. 2006; Plante et al. 1991) and ASD (Herbert et al. 2002; Mody and Belliveau 2013; Tager-Flusberg et al. 2008) show atypical asymmetry patterns for language structures and functional organization. Specifically, there is reduced (or reversed) left hemisphere asymmetry in inferior frontal regions (particularly *pars opercularis*) and exaggerated left asymmetry in *planum temporale*. The volume of frontal regions is generally reduced in both disorders (Gauger et al. 1997; Joseph et al. 2014; Knaus et al. 2010). Functional imaging studies of language using

fMRI also found reduced activation in left frontal regions in SLI (e.g., Weismer et al. 2005) and ASD (e.g., Harris et al. 2006; Just Cherkassy et al. 2004; Knaus et al. 2008). Importantly, it appears that these atypical patterns of language asymmetry not only are found in individuals with SLI or ASD, but are also seen in unaffected infant siblings of children with SLI (Benasich et al. 2006) and ASD (Seery et al. 2013). These findings suggest that atypical asymmetry in neural structure and organization of language may be an endophenotype that could potentially be incorporated in future genetic studies.

12.5 Toward Dynamic Growth Phenotypes

The static age-specific assessments currently available as phenotypes are providing informative glimpses of the genetics of children's language acquisition and impairments. However, growth phenotypes of individual children's acquisition of particular dimensions of language have the potential of being far more powerful and sensitive to genetic influences. Although more effortful and costly to obtain, it is important to keep in mind that language and especially language disorders are *developmental* phenomena, and therefore, genetic research will be significantly accelerated by incorporating longitudinal developmental phenotypes into their designs.

Growth data show that children with SLI are, on average, likely to be delayed in start-up of language acquisition, but when the acquisition process is underway, the patterns of growth are surprisingly similar to younger children (Rice 2012). In effect, some elements of language acquisition are vulnerable, but growth, per se, shows strengths, even in the areas of grammar acquisition that are the weakest among the dimensions of language to be acquired. Examination of growth trajectories also documents that if the grammar is not well established by middle childhood, it is quite likely that the grammar will remain at levels below expected accuracy as children with SLI move into adulthood. Rice (2012) notes the interpretive challenges of such growth evidence and how the evidence implicates underlying genetic mechanisms of growth and change, in the form of a hypothesized growth signaling dysfunction. The implication for language phenotypes, within a growth context, is to focus on the early start-up period of language acquisition as well as on the development of growth behavioral phenotypes estimating rate and path of change over time per linguistic dimension.

In ASD, there are several different developmental trajectories that have been identified and carefully described (Anderson et al. 2007; Lord et al. 2004). A small number of children show no delays, and their acquisition of language may be within the norms for typically developing children. At the other end of the spectrum, there are children who, despite years of intervention, fail to acquire spoken language at all (Tager-Flusberg and Kasari 2013). Other children are significantly delayed in the onset of language milestones (anywhere between two and four years delay), but once they begin, their rate of acquisition is extremely rapid

beyond what is seen in typical children, and they catch up with their peers by the time they reach school (Szatmari et al. 2000). A third pattern more closely resembles the trajectories seen in SLI, but with most children in this group remaining below expectations, particularly in grammar (Kjelgaard and Tager-Flusberg 2001). The final pattern is one that appears to be unique to ASD and is often referred to as "regression": children who begin speaking on time, but who lose words and even phrases in the second or third year of life along with the onset of other ASD symptoms (Pickles et al. 2009). We do not fully understand the significance of these different developmental trajectories in the language acquisition of children with ASD; however, several ongoing genetic studies have attempted to incorporate even the limited information available in phenotypic datasets about these patterns into their analyses (Lord et al. 2015; Miles 2011).

On the molecular level, in addition to candidate gene investigations, there is a need to evaluate regulatory mechanisms in the form of gene x gene interactions and epigenetic influences that contribute to strengths and weaknesses in the development of the neurocognitive infrastructures needed for the complex human linguistic abilities (Graham and Fisher 2013). Ultimately, language impairments are about deficiencies in the growth and development of the necessary neurocognitive mechanisms. Selection of appropriate phenotypes that can be common across studies will be the vital components of scientific progress on this important endeavor.

Acknowledgements The preparation of this chapter was supported by the following grants: MR: NIH P30DC005803, R01DC001803, R01DC005226, and P30HD002528; HTF: NIH RO1DC10290; P50DC13027 and RO1 DC11339 and support from the Simons Foundation.

References

Alarcon M, Abrahams BS, Stone JL et al (2008) Linkage association and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. Am J Hum Genet 82(1):150–159

Anderson DK, Lord C, Risi S et al (2007) Patterns of growth in verbal abilities among children with autism spectrum disorder. J Consult Clin Psychol 75(4):594–604

Benasich AA, Choudhury N, Friedman JT et al (2006) The infant as a prelinguistic model for language learning impairments: predicting from event-related potentials to behavior. Neuropsychologia 44(3):396–411

Bishop DVM, Hayiou-Thomas ME (2008) Heritability of specific language impairment depends on diagnostic criteria. Genes Brain Behav 7:365–372

Bishop D, McDonald D (2009) Identifying language impairment in children: combining language test scores with parental report. Int J Lang Commun Disord 44:600–615

Bishop DVM, Snowling MJ (2004) Developmental dyslexia and specific language impairment: same or different? Psychol Bull 130:858–886

Bishop DVM, Adams CV, Norbury CF (2006) Distinct genetic influences on grammar and phonological short-term memory deficits: evidence from 6-year-old twins. Genes Brain Behav 5(2):158–169

Bricker D, Squires J (1989) The effectiveness of parental screening of at risk infants: the infant monitoring questionnaires. Topics Early Child Spec Educ 9(3):67–85

Catts HW, Adlof SM, Ellis WS (2006) Language deficits in poor comprehenders: a case for the simple view of reading. J Speech Lang Hear Res 49(2):278–293
Conti-Ramsden G, Botting N, Faragher B (2001) Psycholinguistic markers for specific language impairment (SLI). J Child Psychol Psychiatry 42(6):741–748
De Rubeis S, Buxbaum J (2015) Recent advances in the genetics of autism spectrum disorder. Curr Neurol Neurosci Rep 15(6):553
Drayna D, Kang C (2011) Genetic approaches to understanding the causes of stuttering. J Neurobehav Disord 3(4):374–380
Dunn LM, Dunn DM (2007) PPVT-4: peabody picture vocabulary test [assessment instrument]. Pearson Assessments, Minneapolis
Evans P, Mueller K, Gamazon E et al (2015) A genome-wide sib-pair scan for quantitative language traits reveals linkage to chromosomes 10 and 13 Genes. Brain Behav 14(5):387–397
Falcaro M, Pickles A, Newbury DF et al (2008) Genetic and phenotypic effects of phonological short-term memory and grammatical morphology in specific language impairment. Genes Brain Behav 7(4):393–402
Finestack LH, Sterling AM, Abbeduto L (2013) Discriminating Down syndrome and Fragile X syndrome based on language ability. J Child Lang 40(1):244–265
Fisher S, Vernes SC (2015) Genetics and the language science. Ann Rev Linguist 1:289–310
Fisher S, Lai C, Monaco A (2003) Deciphering the genetic basis of speech and language disorders. Ann Rev Neurosci 26:57–80
Flint J (1998) Behavioral phenotypes: conceptual and methodological issues. Am J Med Genet 81:235–240
Foundas AL (1995) Language dominance and MRI asymmetries. Neurology 45:1635–1636
Galaburda AM, LeMay M, Kemper TL, Geschwind N (1978) Right-left asymmetrics in the brain. Science 199(4331):852–856
Gathercole SE (2006) Nonword repetition and word learning: the nature of the relationship. Appl Psycholinguist 27(4):513–543
Gauger LM, Lombardino LJ, Leonard CM (1997) Brain morphology in children with specific language impairment. J Speech Lang Hear Res 40(6):1272–1284
Giedd JN, Lalonde FM, Celano MJ et al (2009) Anatomical brain magnetic resonance imaging of typically developing children and adolescents. J Am Acad Child Adolesc Psychiatry 48(5):465–470
Gleason JB (2013) The development of language, 8th edn. Allyn and Bacon, Boston
Goldman R, Fristoe M (2015) GFTA-3: Goldman-Fristoe test of articulation 3 [assessment instrument]. Pearson Assessments, Minneapolis
Gopnik M, Crago M (1991) Family aggregation of a developmental language disorder. Cognition 39:1–50
Graham S, Fisher S (2013) Decoding the genetics of speech and language. Curr Opin Neurobiol 23:43–51
Graham S, Deriziotis P, Fisher S (2015) Insights into the genetic foundations of human communication. Neuropsychol Rev 25:3–26
Hadley PA, Rice ML (1993) Parental judgments of preschoolers' speech and language development: a resource for assessment and IEP planning. Semin Speech Lang 14(4):278–288
Harris GJ, Chabris CF, Clark J et al (2006) Brain activation during semantic processing in autism spectrum disorders via functional magnetic resonance imaging. Brain Cogn 61(1):5468
Herbert MR, Harris GJ, Adrien KT et al (2002) Abnormal asymmetry in language association cortex in autism. Ann Neurol 52(5):588–596
Joseph RM, Fricker Z, Fenoglio A et al (2014) Structural asymmetries of language-related gray and white matter and their relationship to language function in young children with ASD. Brain Imag Behav 8:60–72
Just MA, Cherkassky VL, Keller TA, Minshew NJ (2004) Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain 127:1811–1821

Kjelgaard M, Tager-Flusberg H (2001) An investigation of language impairment in autism: implications for genetic subgroups. Lang Cogn Process 16:287–308
Knaus TA, Silver AM, Lindgren KA et al (2008) fMRI activation during a language task in adolescents with ASD. J Int Neuropsychol Soc 14:967–979
Knaus TA, Silver AM, Kennedy M et al (2010) Language laterality in autism spectrum disorder and typical controls: a functional volumetric and diffusion tensor MRI study. Brain Lang 112(2):113–120
Konopka G, Roberts T (2016) Animal models of speech and vocal communication deficits associated with psychiatric disorders. Biol Psychiatry 79:53–61
Landa R, Piven J, Wzorek M et al (1992) Social language use in parents of autistic individuals. Psychol Med 22(1):245–254
Leonard C, Eckert M, Given B et al (2006) Individual differences in anatomy predict reading and oral language impairments in children. Brain 129:3329–3342
Lewis B, Freebairn L, Hansen A et al (2007) Speech and language skills of parents of children with speech sound disorders. Am J Speech Lang Pathol 16:108–118
Lord C, Risi S, Pickles A (2004) Trajectory of language development in autistic spectrum disorders. In: Rice ML, Warren SF (eds) Developmental language disorders: from phenotypes to etiologies. Lawrence Erlbaum Associates Publisher, Mahwah, pp 7–29
Lord C, Bishop S, Anderson D (2015) Developmental trajectories as autism phenotypes. Am J Med Genet C Semin Med Genet 169(2):198–208
Luyster R, Seery AM, Thompson MR, Tager-Flusberg H (2011) Identifying early risk markers and developmental trajectories for language impairment in neurodevelopmental disorders. Dev Disabil Res Rev 17:151–159
Maloney E, Larrivee L (2007) Limitations of age-equivalent scores in reporting the results of norm-referenced tests. Contemp Issues Commun Sci Disord 34:86–93
Miles JH (2011) Autism spectrum disorders—a genetics review. Genet Med 13:278–294
Miscimarra L, Stein C, Millard C et al (2007) Further evidence of pleiotropy influencing speech and language: analysis of the DYX8 region. Hum Hered 63(1):47–58
Mody M, Belliveau JW (2013) Speech and language impairments in autism: insights from behavior and neuroimaging. N Am J Med Sci 5(3):157–161
Newbury DF, Bishop DVM, Monaco AP (2005) Genetic influences on language impairment and phonological short-term memory. Trends Cogn Sci 9:528–534
Paracchini S (2011) Dissection of genetic associations with language-related traits in population-based cohorts. J Neurodev Disord 3(4):365–373
Pawłowska M (2014) Evaluation of three proposed markers for language impairment in English: a meta-analysis of diagnostic accuracy studies. J Speech Lang Hear Res 57(6):2261–2273
Pennington B, Bishop DVM (2009) Relations among speech language and reading disorders. Ann Rev Psychology 60:283–306
Pickles A, Simonoff E, Conti-Ramsden G et al (2009) Loss of language in early development of autism and specific language impairment. J Child Psychol Psychiatry 50(7):843–852
Plante E, Swisher L, Vance R, Rapcsak S (1991) MRI findings in boys with specific language impairment. Brain Lang 41:52–66
Poeppel D, Emmorey K, Hickok G, Pylkkanen L (2012) Towards a new neurobiology of language. J Neurosci 32(14):14125–14131
Poulos MG, Webster WG (1991) Family history as a basis of subgrouping people who stutter. J Speech Lang Hear Res 34(1):5–10
Rice ML (2012) Toward epigenetic and gene regulation models of specific language impairment: looking for links among growth genes and impairments. J Neurodev Disord 4(27):2–14
Rice ML, Hoffman L (2015) Predicting vocabulary growth in children with and without specific language impairment: a longitudinal study from 2; 6 to 21 years of age. J Speech Lang Hear Res 58(2):345–359
Rice ML, Wexler K (2001) Rice/Wexler test of early grammatical impairment [assessment instrument]. Psychological Corporation, San Antonio

Rice M, Haney K, Wexler K (1998) Family histories of children with SLI who show extended optional infinitives. J Speech Lang Hear Res 41(2):419–432
Rice M, Hoffman L, Wexler K (2009a) Judgments of omitted BE and DO in questions as extended finiteness clinical markers of specific language impairment (SLI) to 15 years: a study of growth and asymptote. J Speech Lang Hear Res 52:1417–1433
Rice M, Smith S, Gayan J (2009b) Convergent genetic linkage and association to language speech and reading measures in families of probands with specific language impairment. J Neurodev Disord 1:264–282
Riley G (2009) The stuttering severity instrument for adults and children (SSI-4), 4th edn. Pro-Ed, Austin
Roberts J, Rice M, Tager-Flusberg H (2004) Tense marking in children with autism. Appl Psycholinguist 25:429–448
Roberts J, Price J, Barnes E et al (2007) Receptive vocabulary expressive vocabulary and speech production of boys with Fragile X syndrome in comparison to boys with Down syndrome. Am J Ment Retard 112(3):177–193
Ruser T, Arin D, Dowd M et al (2007) Communicative competence in parents of children with autism and parents of children with specific language impairment. J Autism Dev Disord 37:1323–1336
Seery AM, Vogel-Farley V, Tager-Flusberg H, Nelson CA (2013) Atypical lateralization of ERP response to native and non-native speech in infants at risk for autism spectrum disorder. Dev Cogn Neurosci 5:10–24
Semel E, Wiig EH, Secord WA (2013) CELF-5: clinical evaluation of language fundamentals fifth edition [assessment instrument]. Pearson Assessments, Minneapolis
Shriberg LD, Tomblin JB, McSweeny JL (1999) Prevalence of speech delay in 6-year-old children and comorbidity with language impairment. J Speech Lang Hear Res 42(6):1461–1481
Smith SD (2011) Approach to epigenetic analysis in language disorders. J Neurodev Disord 3(4):356–364
Smith SD, Pennington BF, Boada R, Shriberg LD (2005) Linkage of speech sound disorder to reading disability loci. J Child Psychol Psychiatry 46(10):1057–1066
Squires JK, Potter L, Bricker DD (1999) The ASQ user's guide, 2nd edn. Paul H. Brookes Publishing Co., Baltimore
Stein CM, Schick JH, Gerry TH et al (2004) Pleiotropic effects of a chromosome 3 locus on speech-sound disorder and reading. Am J Hum Genet 74(2):283–297
Stein CM, Millard C, Kluge A et al (2006) Speech sound disorder influenced by a locus in 15q14 region. Behav Genet 36(6):858–868
Sterling AM, Rice ML, Warren SF (2012) Finiteness marking in boys with Fragile X syndrome. J Speech Lang Hear Res 55(6):1704–1705
Szatmari P, Bryson SE, Streiner DL et al (2000) Two-year outcome of preschool children with autism or Asperger's syndrome. Am J Psychiatry 157(12):1980–1987
Tager-Flusberg H, Cooper J (1999) Present and future possibilities for defining a phenotype for specific language impairment. J Speech Lang Hear Res 42:1275–1278
Tager-Flusberg H, Kasari C (2013) Minimally verbal school-aged children with autism spectrum disorder: the neglected end of the spectrum. Autism Res 6:468–478
Tager-Flusberg H, Paul R, Lord CE (2005) Language and communication in autism. In: Volkmar F, Paul R, Klin A, Cohen DJ (eds) Handbook of autism and pervasive developmental disorder, 3rd edn. Wiley, New York, pp 335–364
Tager-Flusberg H, Lindgren K, Mody M (2008) Structural and functional imaging research on language disorders: specific language impairment and autism spectrum disorders. In: Wolf L, Schreiber H, Wasserstein J (eds) Adult learning disorders: contemporary issues. Psychology Press, New York, pp 127–157
Thompson PM, Sowell ER, Gogtay N et al (2005) Structural MRI and brain development. Int Rev Neurobiol 67:285–323
Tomblin JB (1989) Familial concentration of developmental language impairment. J Speech Hear Disord 54:287–295

Tomblin JB, Records NL, Zhang X (1996) A system for the diagnosis of specific language impairment in kindergarten children. J Speech Lang Hear Res 39(6):1284–1294

Torgesen JK, Wagner RK, Rashotte CA (2012) TOWRE: test of word reading efficiency-2 [assessment instrument]. Pro-Ed, Austin

Vargha-Khadem F, Watkins K, Alcock K et al (1995) Praxic and nonverbal cognitive deficits in a large family with a genetically transmitted speech and language disorder. Proc Natl Acad Sci U S A 92:930–933

Vernes SC, Newbury DF, Abrahams BS et al (2008) A functional genetic link between distinct developmental language disorders. N Engl J Med 359(22):2337–2345

Watkins K, Dronkers N, Varga-Khadem F (2002) Behavioral analysis of an inherited speech and language disorder: comparison with acquired aphasia. Brain 125:452–464

Weismer ES, Plante E, Tomblin JB (2005) A functional magnetic resonance imaging investigation of verbal working memory in adolescents with specific language impairment. J Speech Lang Hear Res 48(2):405–425

Whitehouse AJO, Bishop DVM, Ang QW et al (2011) CNTNAP2 variants affect early language development in the general population. Genes Brain Behav 10(4):451–456

Yairi E, Ambrose N, Watkins R, Paden E (2001) What is stuttering. J Speech Lang Hear Res 44:585–592

Zampini L, D'Odorico L (2013) Vocabulary development in children with Down syndrome: longitudinal and cross-sectional data. J Intellect Dev Disabil 38(4):310–317

Zubrick S, Taylor S, Rice M, Slegers D (2007) Late language emergence at 24 months: an epidemiological study of prevalence predictors and covariates. J Speech Lang Hear Res 50:1562–1592

Chapter 13
Phenotypic Markers in Event-Related Potentials

Mei Hua-Hall

13.1 Introduction to the Event-Related Potential

Event-related brain potentials (ERPs) are a noninvasive method of measuring brain activity during cognitive processing. ERPs can be recorded from all of the primary sensory modalities (visual, auditory, somatosensory, and gustatory) and from motor events (e.g., a button press). ERPs are voltage fluctuations in the electroencephalogram (EEG) that are time-locked to sensory, motor, or cognitive events (e.g., the presentation of an image or sound) and reflect coordinated neural network activity in the brain. ERPs provide a neurophysiological reflection of the processing of events and can provide an insight into very early stages of information processing. Since ERPs have excellent temporal resolution, to the level of milliseconds, they are a "real-time" representation of the processing of an event.

ERPs are recorded from electrodes placed at multiple locations on the scalp. ERPs recorded from the scalp are relatively small compared with the ongoing background EEG activity. ERP responses therefore need to be extracted from the EEG by means of digital filtering and signal averaging. Filtering involves off-line application of mathematical procedures to the EEG recording to selectively attenuate frequencies that are not relevant to the measurements of interest. For example, most of the relevant portion of the ERP waveform in a typical cognitive neuroscience experiment consists of frequencies between 0.01 and 40 Hz and majority of muscle activity (electromyogram, EMG) consists of frequencies above 100 Hz or

M. Hua-Hall (✉)
Schizophrenia and Bipolar Disorder Program, McLean Hospital, Belmont, USA
e-mail: mhall@mclean.harvard.edu

M. Hua-Hall
Department of Psychiatry, Harvard Medical School, Boston, USA

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_13

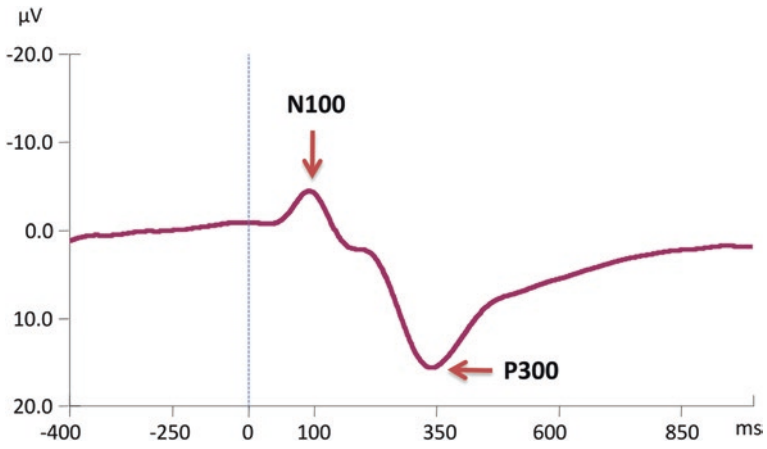

Fig. 13.1 Example of N100 and P300 ERP waveforms

below 0.01 Hz. The EMG activity can be eliminated by applying a band-pass filter, which attenuates both high and low frequencies, passing only an intermediate range of frequencies (Luck 2005). Signal averaging is used to enhance the signal-to-noise ratio (SNR). When a sufficient number of ERP trials are averaged, the background noise (i.e., the EEG) will approximate zero and the signal (i.e., the ERP) waveform can be extracted. The number of trials needed to achieve acceptable SNR increases as the size of the signal relative to background EEG decreases (Luck 2005). In addition to background EEG noise, non-brain activity, for example, from eye movements (the electrooculogram, EOG) and EMG also influence the scalp recording. Recording procedures may be adapted to minimize the occurrence of EOG and EMG activity, and off-line analyses usually exclude trials contaminated with high EOG/EMG activity prior to averaging (Luck 2005).

The resulting averaged ERP waveforms consist of a sequence of positive and negative voltage deflections that are called peaks, waves, or components. In Fig. 13.1, the peaks are labeled N100 and P300. P and N are traditionally used to indicate positive and negative peaks, respectively; the number indicates the latency of a peak (most commonly measured from stimulus onset). For example, P300 denotes a positive peak (in relation to baseline) at 300 ms. Each ERP component has a distinctive scalp distribution. When a large number of electrodes are used to record ERP activity (up to 256 electrodes), source localization approaches can be applied to evaluate the electrical sources of scalp-derived activity within brain regions. However, it remains challenging to determine the precise location of the neural generator source simply by examining the distribution of voltage over the scalp (Sehatpour et al. 2006).

In general, the ERP components that occur prior to 100 ms, e.g., auditory brain stem ERP, are thought to reflect information processing in early sensory pathways. The middle latency components, occurring between 100 and 200 ms, e.g., N170,

are thought to reflect late sensory and early perceptual processes arising from the thalamus and possibly the cortical areas. Later latency components, e.g., P300, after 250 ms or so, are thought to reflect higher level cognitive processes (e.g., memory and language) (Coles and Rugg 1995).

13.2 Cognitive Phenotype of ERPs

Because of their high temporal resolution, noninvasiveness, relatively low cost, and ability to provide a continuous measure of the brain's information processing, ERPs have been widely adopted by cognitive neuroscientists, psychologists, psycholinguists, and neurologists to study normal cognitive processing and functions at different developmental stages, as well as to illuminate how and why these cognitive processes are altered in neurological and psychiatric disorders. Over the years, a number of ERPs have been identified as intermediate markers or endophenotypes for psychiatric illnesses. Endophenotypes are heritable, disease-associated neurophysiological, cognitive, or neurobiological traits that are believed to be in the etiological pathway (i.e., intermediate) between risk genotype and the clinical syndrome but to be more proximally related to the genetic substrate than the higher order construct of a "disorder" (Ritsner 2009; Freedman et al. 1999; Gottesman and Shields 1973; Wickham and Murray 1997). Since psychiatric disorders are genetically and phenotypically complex, researchers have turned to ERP endophenotypes, using them in patient and non-patient populations to help uncover the genetic architecture of disease risk, to characterize functional brain patterns of affected individuals, and to understand the neurobiology of a disorder (Braff et al. 2008; Hall and Smoller 2009; Rangaswamy and Porjesz 2008; Turetsky et al. 2007). Endophenotypes may lead to the development of new drug targets or alternative treatment strategies and may help to identify individuals at higher risk for developing a disorder. As the ERP literature is very large, this chapter selectively describes six ERP phenotypes that are commonly studied in neuropsychiatric disorders. Each ERP phenotype may be useful in diagnosis prediction, identifying genetic susceptibility, and understanding cognitive behavior and brain function.

13.2.1 The Mismatch Negativity (MMN)

The mismatch negativity (MMN) is a frontal negativity occurring at around 100–200 ms. It is generated by an automatic cortical change detection process whereby the brain detects a difference between the current auditory input and the regularity of the immediately preceding auditory input (Naatanen 1992). For example, when a sequence of repetitive (standard) sounds is interrupted periodically by a clearly different stimulus, such as a change in the frequency or duration

of simple tones, or by complex sounds such as phonemes, MMN is elicited. Importantly, MMN can be elicited even in the absence of attention (Naatanen 1992). The MMN is commonly derived by subtracting the ERP response to the standard stimulus from that of the deviant stimulus. This difference of ERP activity is thought to reflect the mismatch between a trace in sensory memory (of the standard stimulus) and the representation of the current stimulus to which the trace is compared. MMN is considered to be an index of the pre-attentive stage of auditory information processing. Because it reflects the automatic detection of deviant auditory stimuli in the brain independent of attention, it is particularly suitable for studies of populations such as young infants, children with developmental disorders, or coma patients.

MMN has been used to study phonological and auditory dysfunctions in dyslexia. Diminished MMN has been reported in children with dyslexia, especially for detecting differences between speech sounds (e.g.,/ba/vs./da/), suggesting that the central auditory system of dyslexic individuals is more vulnerable to subtle phonemic difference (Kujala et al. 2001; Naatanen 2003). This difference in central auditory processing can be detected as early as at birth. Leppanen and colleagues investigated whether dyslexic children with familial risk showed atypical auditory/speech processing, as measured by MMN, at birth, at six months, and at school age. They found that children later diagnosed with dyslexia at age 9 had diminished MMN at birth compared with control children. These investigators also found that 6-month-old infants at high genetic risk for dyslexia exhibited reduced MMN compared with control infants (Leppanen et al. 2012). These results suggest that reduced MMN is a risk factor, or possibly an endophenotype, for dyslexia. Recently, a genome-wide association (GWA) analysis on MMN in 200 dyslexic children identified a risk variant located on chromosome 4q32.1. This risk variant was found to be associated not only with the late MMN component, but also with mRNA expression levels of the *SLC2A3* gene on chromosome 12 (Roeske et al. 2011). The *SLC2A3* gene belongs to a brain-expressed member of the facilitative glucose transporter family and its product has been shown to provide energy for synaptic transmission in the brain. The authors hypothesized that there might be a possible trans-regulation effect on the *SLC2A3* gene leading to risk for dyslexic and to attenuated MMN (Roeske et al. 2011).

In patients with a diagnosis of schizophrenia, the MMN deficit emerges after illness onset. Several studies have shown that individuals in their first episode have normal MMN amplitude, but an MMN deficit emerges over the first two years of the illness and is present in chronically ill patients (Michie 2001; Salisbury et al. 2002, 2007; Umbricht et al. 2006). Progressive reduction of MMN was correlated with cortical gray matter volume loss (Salisbury et al. 2002). The severity of MMN deficits in chronically ill schizophrenia patients correlated with severity of negative symptoms (Javitt et al. 1995) and with impaired global outcome (Light and Braff 2005). Taken together, these results suggest that the brain mechanisms relevant to auditory perception and sensory memory storage are affected by progressive pathologic processes in schizophrenia. MMN deficits also provide an index of structural alteration in the brain. Thus, measuring MMN in schizophrenia

patients at the early stage of the illness can be used to predict functional outcome and may be relevant to different therapeutic intervention strategies.

Another line of MMN research has had a substantial clinical impact. MMN has been used, among other clinical measures, to predict recovery of consciousness in coma patients (Fischer et al. 2004; Wijnen et al. 2007). The presence, or return of, an MMN is significantly correlated with recovery of consciousness and negatively associated with moving to a permanent vegetative state. Also, increased amplitude of the MMN has been observed in patients in vegetative states during the period preceding the recovery of consciousness (Wijnen et al. 2007). In the absence of behavioral responses in vegetative states in minimally conscious patients, increased MMN suggests increased neural activity and a sign of residual cerebral function. The clearly enhanced MMN found in these patients might therefore be indicative of the consolidation of neural networks underlying interactive communication (Wijnen et al. 2007).

13.2.2 The Face-Sensitive N170 Component

Electrophysiological studies using ERPs have suggested the existence of face-selective cortical processing mechanisms. The N170 is an early face-sensitive ERP component (Bentin et al. 1996; Botzel et al. 1995). N170 amplitude is typically larger, and latency is shorter, when evoked by images of face stimuli than by non-face stimuli (e.g., butterflies, cars), and is especially prominent over the right hemisphere in occipital–temporal regions of the scalp (Bentin et al. 1996). The N170 has been linked with the structural encoding of faces (Rossion and Jacques 2008). Thus, many studies have used the N170 component to gain insights into the time course and functional properties of different aspects of face processing in the human brain (see Eimer 2011; Rossion and Jacques 2011 for reviews). When viewing inverted faces, the N170 latency was delayed (Bentin et al. 1996) and its amplitude was enhanced (Rossion et al. 1999). This effect, called the face inversion effect, is, in part, thought to be due to the fact that inverted faces are more difficult to process than upright faces, as it requires additional attentional processing (George et al. 1996). The N170 is not affected by the familiarity of faces, and it is therefore interpreted as reflecting the earliest stages of face processing, the "structural encoding of facial features," that precedes recognition processes (Eimer 2000).

Multiple behavioral studies have shown that children and adults with autism exhibit abnormal face discrimination, recognition, and emotion perception (Carver and Dawson 2002). Autistic children tend not to attend to faces (Osterling and Dawson 1994; Jeste and Nelson 2009). McPartland and colleagues found that, compared to age and IQ-matched individuals, individuals with autism exhibited significantly longer N170 latencies to faces. Also, in contrast to the robust face inversion effect shown in non-autistic individuals, autistic subjects showed minimal differences in N170 latencies to upright versus inverted faces (McPartland

et al. 2004). These abnormalities have been reported in high-functioning adults of autism spectrum disorder as well. O'Connor et al. (2007) found that, compared to controls, adults with Asperger's syndrome exhibited slower N170 latencies to both faces and facial parts, but the groups did not differ in response to objects (O'Connor et al. 2007). Poor face discrimination and recognition abilities are hypothesized to reflect abnormal information processing strategies and may be a phenotypic marker for deficits in social cognition. Dawson and colleagues found that impaired face processing is present early in autism, as young as 3 years of age (Dawson et al. 2005a). Children with typical development and children with developmental delays showed differential ERP activity to familiar versus unfamiliar faces and objects. In contrast, children with autism showed differential brain activity only to objects. The latency of the P400 ERP (a developmental precursor of the N170) to faces was correlated with severity of joint attention, the ability to shift attention between another person and an object or event. Joint attention is an early emerging and fundamental impairment in children with autism. This result suggests that impairments in joint attention may be related to a failure to adequately process information regarding faces. In another study, Dawson et al. (2005a, b) found that non-autistic parents of children with autism failed to show shorter N170 to faces compared to non-face stimuli and failed to show the expected right lateralized pattern. This pattern of neural responses to faces was similar to that found in individuals with autism (Dawson et al. 2005b). These data suggest that impaired face recognition may be a potential neural trait marker for susceptibility to autism.

An abnormal N170 component has also been found in patients with schizophrenia (Herrmann et al. 2004; Onitsuka et al. 2006). Tsundoda and colleagues reported that patients with schizophrenia exhibited significantly smaller N170 amplitudes to both upright faces and inverted faces compared with normal controls. The N170 amplitudes to upright faces were significantly correlated with the severity of social dysfunction (Tsunoda et al. 2012). Obayashi and colleagues reported significant correlations between reduced global functional scores and reduced N170 to faces in male patients with schizophrenia. Thus, the abnormal face-specific configuration processing observed in patients may underlie some of the social dysfunctions in schizophrenia (Obayashi et al. 2009). Moreover, individuals at risk for schizophrenia exhibited reduction in N170 amplitude, suggesting dysfunctions in visual processing of facial structures may be a trait marker. As face recognition is an important aspect of social cognition that underlies competent social behavior, impaired face recognition may lead to difficulties in facial affect recognition, which may be related to other symptoms, such as social withdrawal.

13.2.3 Prepulse Inhibition of Startle (PPI)

PPI occurs when a relatively weak sensory event (the prepulse) presented 30–500 ms before a strong startle-inducing stimulus reduces the magnitude of the

startle response (Graham 1975). PPI stimuli are usually acoustic. Acoustic stimuli are typically short bursts (20 ms) of white noise; the inhibitory effect is most intense for an interstimulus interval (ISI) range of 50–150 ms (Braff et al. 2001). In humans, the startle response is usually assessed by measuring the EMG activity of the orbicularis oculi muscles. PPI is typically calculated as either a percent or absolute reduction in startle magnitude on trials that include a prepulse compared with startle trials that do not include a prepulse. PPI is commonly viewed as a measure of a process called "sensorimotor gating," by which excess or trivial stimuli are screened or "gated out" of awareness, so that an individual can focus attention on the most salient aspects of the stimulus-laden environment (Braff and Geyer 1990). In the experimental setting, the presentation of prepulse stimuli inhibits ("gates") the motor response to a startling stimulus.

Disruptions of PPI have been studied in humans and many other species. The most studied are deficits of PPI in schizophrenia (Braff et al. 2001). Schizophrenia patients typically exhibit less attenuation of the acoustic startle response following the prepulse, despite having relatively normal responses to startling stimuli. These PPI deficits indicate that in the immediate aftermath of a stimulus, the central nervous system in schizophrenia is overly responsive to a second stimulus, resulting in poorly inhibited motor responses to excessive stimuli. Sensorimotor gating abnormalities have been correlated with thought disturbance in schizophrenia patients (Perry et al. 1999). These correlative studies have led to the hypothesis that sensorimotor gating abnormalities may underlie thought disturbance (Perry et al. 1999). PPI is heritable (Anokhin et al. 2003). Reduced PPI has also been reported in schizophrenia spectrum patients and in their clinically unaffected relatives (Cadenhead et al. 2000) as well as in individuals in the prodromal stages of schizophrenia (Quednow et al. 2008). These findings support the use of PPI as an endophenotype in genetic studies. Recently, Quednow and colleagues found that PPI was significantly associated with a schizophrenia risk gene, the transcription factor 4 (*TCF4*) gene located on chromosome 18q21.2. In two independent samples, carriers of the schizophrenia risk allele C of *TCF4* display decreased PPI levels (Quednow et al. 2011). This finding accords with an animal study showing that transgenic mice overexpressing the *TCF4* gene in the brain display decreased sensorimotor gating (Brzózka et al. 2010, 2016). The role of *TCF4* in the brain is not yet fully understood, but *TCF4* seems to play an important role in the development of the mammalian nervous system (Flora et al. 2007; Hill et al. 2016). Research further characterizing the functional impact of *TCF4* in the brain and its role in the risk of schizophrenia is in progress.

13.2.4 P50 Sensory Gating

P50 sensory gating, also referred to as P50 ERP suppression, is recorded using an auditory conditioning–testing paradigm in which pairs of identical clicks (condition and test stimulus, respectively) are presented 500 ms apart (with an interpair

interval of 10 s). The paradigm elicits conditioning and testing evoked potentials, which occur approximately 50 ms after stimulus onset and are most prominent in the frontal–central scalp sites. Typically, there is a strong evoked potential after the first stimulus, and a much reduced or "gated" response to the second stimulus. This is because the conditioning stimulus not only elicits excitatory neural responses (i.e., a large conditioning amplitude), but also activates the brain's inhibitory pathways, so that the responses to the test stimulus are normally suppressed (i.e., of smaller amplitude than responses to the conditioning stimulus) (Freedman et al. 1994, 1996). The magnitude of the suppression is thought to index inhibitory processes in the brain and is conventionally measured as the ratio of the P50 amplitude of the test response to that of the conditioning response (i.e., test/conditioning ratio or T/C ratio). A ratio of 0 represents complete suppression of the second response and is considered optimal performance of inhibitory circuits; most healthy adults have ratios below 0.5 (Bramon et al. 2004; Patterson et al. 2008).

Sensory gating reflects an individual's ability to screen out (filter) trivial or redundant stimuli from environmental stimuli to prevent an overload of irrelevant information in the brain (Freedman et al. 1996). Abnormalities in P50 sensory gating are linked to deficits in attentional process in schizophrenia (Erwin et al. 1998; Potter et al. 2006). Although both PPI and sensory gating paradigms are thought to measure gating of incoming information, each is based on a different physiological phenomenon: P50 suppression is measured by means of EEG, whereas PPI is measured by means of EMG. The available evidence indicates that PPI and P50 suppression are only weakly related (Braff et al. 2007; Hong et al. 2007; Oranje et al. 1999).

A sensory gating deficit is a robust finding in schizophrenia and in bipolar disorder with psychotic features (Bramon et al. 2004; de Wilde et al. 2007; Olincy and Martin 2005). Unaffected relatives of patients with schizophrenia or bipolar disorder also exhibit sensory gating deficits, suggesting a genetic contribution to this impairment (Adler et al. 1992; de Wilde et al. 2007; Schulze et al. 2007). Hall and colleagues applied a twin methodology to quantify genetic and environmental contributions to P50 sensory gating. They found that sensory gating is a heritable trait with heritability estimates around 60% (Hall et al. 2006). Identical twins with a diagnosis of schizophrenia and their unaffected co-twins exhibited sensory gating deficits. Genetic risk factors to sensory gating deficits overlap with genetic risk for schizophrenia (Hall et al. 2007b). These results support the idea that a sensory gating deficit is a schizophrenia endophenotype. In another study, Hall and colleagues examined genetic and environmental contributions of sensory gating deficits in individuals with psychotic bipolar disorder. Results showed that bipolar patients and their unaffected family members exhibited sensory gating deficits and that genetic risk factors for sensory gating deficits overlapped with genetic risk factors for bipolar illness (Hall et al. 2007a, 2008). Taken together, these results suggest that the P50 sensory gating deficit is an endophenotype for both schizophrenia and psychotic bipolar disorder. The authors hypothesized that the observed impaired P50 sensory gating in both disorders may reflect the impact of shared psychosis susceptibility genes. This possibility is consistent with evidence from

clinical, epidemiological, and genetic findings suggesting shared risk factors between bipolar disorder and schizophrenia (Lichtenstein et al. 2009). Recently, GWA analyses have identified several genetic risk variants that confer risks to both disorders (Ripke et al. 2011).

P50 sensory gating deficits have been linked to the alpha-7 nicotinic acetylcholine receptor gene *CHRNA7* on chromosome 15q13-14 in schizophrenia families (Freedman et al. 1997; Leonard and Freedman 2006). Neurobiological investigations in both humans and animals have further confirmed that decreased function of the α7-nicotinic cholinergic receptor underlies the sensory gating deficit in schizophrenia (Leonard et al. 2002). Failure to activate α7 receptors is thus a putative neurobiological mechanism of attentional dysfunction in schizophrenia. Nicotine is a low-potency agonist at the α7 receptor. High dose of nicotine significantly improves abnormal sensory gating in both human and animal studies (Adler et al. 1993; Ross et al. 2010). In addition, nicotine has positive neurocognitive effects in schizophrenia, particularly on attention (Myers et al. 2004, 2008). These studies implicate a role for the α7 nicotinic receptor in the pathophysiology of sensory gating dysfunction and suggest a potential therapeutic target in schizophrenia. The drug 3-[(2,4-dimethoxy) benzylidene]anabaseine (DMXB-A) is a partial agonist at human α7 nicotinic receptors. In an initial phase-2 clinical trial, DMXB-A significantly improved neurocognitive function and sensory gating in patients with schizophrenia compared with placebo (Olincy et al. 2006). These results suggest that nicotinic cholinergic agonists are a potentially viable novel treatment for neurocognitive impairments in schizophrenia.

13.2.5 P300 ERP

P300 is the most widely studied ERP component. Experimental task manipulations have elucidated at least two functionally discrete P300 subcomponents, the P3a component and the P3b component (Squires et al. 1975). The P3a is a frontocentrally maximal positive ERP wave elicited by novel or unexpected events, and it is considered as an electrophysiological marker of attentional switching, or the attentional orienting response (Squires et al. 1975). When ERP researchers refer to the P3 component or the P300 component, they almost always mean the P3b component. The P3b component is usually elicited in an "oddball" paradigm, in which a subject is instructed to detect a randomly presented infrequent deviant or "target" stimulus within a series of frequent nontarget or "standard" stimuli. Auditory, visual, and somatosensory versions of the oddball task have been studied, but the auditory oddball task is the most commonly studied. P3b occurs later (300–600 ms after target stimulus presentation) than the P3a, has a parietal scalp maximum, and reflects cognitive processes associated with stimulus evaluation and response formation (Polich 2007).

Unlike the recording of earlier ERP components (e.g., MMN), the oddball paradigm entails activation of higher order cognitive processing, involving attention,

memory, and decision-making processes. Although there is still some debate about the underlying mental processes reflected by the P300 wave, P300 amplitude is interpreted as an index of context updating in working memory whereas P300 latency is thought to reflect the timing of mental processes and attentional resource allocation (Polich 2007). P300 latency is negatively correlated with mental functions in normal subjects, such that shorter latencies are associated with superior cognitive performance (Polich and Kok 1995).

Changes in the latency, amplitude, and topography of the P300 correlate with clinical findings in a wide range of psychiatric disorders and brain injuries. For example, reduced visual P300 amplitude is an endophenotype for alcoholism (Begleiter et al. 1998; Dick et al. 2006). In the Collaborative Study on the Genetics of Alcoholism, reduced P300 amplitude was found in alcoholics and their unaffected relatives compared with non-alcoholics and relatives of controls (Dick et al. 2006). Both P300 amplitude and latency are highly heritable (70–80%) (Hall et al. 2006; van Beijsterveldt and van Baal 2002). A quantitative trait locus analysis of alcoholism families found that several chromosomal regions contain genetic loci related to the generation of the visual P300 ERP (Edenberg et al. 2004; Porjesz et al. 2002). Subsequent analyses of the delta and the theta oscillations derived from the P300 ERP pointed to the *CHRM2* gene on chromosome 7 (Edenberg et al. 2004). The *CHRM2* gene is associated not only with brain delta and theta oscillations, but also with clinical diagnoses of alcohol dependence and depression (Rangaswamy et al. 2007). These results demonstrate the utility of using P300 amplitude as an endophenotype for the identification of risk genes for alcohol dependence and related disorders.

Within the auditory modality, patients with schizophrenia and their unaffected relatives show smaller P300 amplitude and prolonged P300 latency, suggesting that P300 deficits are endophenotypic markers for schizophrenia (Bramon et al. 2005; Hall et al. 2007b). Molecular genetic studies have reported associations of P300 abnormalities with risk variations in *COMT*, *Neuregulin 1,* and *DISC* genes (Bramon et al. 2008; Gallinat 2003; Shaikh et al. 2011).

Across different tasks and modalities, P300 has been identified as an objective and sensitive tool for assessing cognitive impairment in Alzheimer's Disease (AD) (Polich and Corey-Bloom 2005). Patients with AD have increased P300 latency and decreased P300 amplitude compared with elderly controls subjects. P300 abnormality is present during early stages of AD. Similar P300 alterations have also been demonstrated in mild cognitive impairment (MCI) (Golob et al. 2002). In a five-year follow-up study, Golob and colleagues found that patients diagnosed with MCI at baseline but later converted to AD had significantly longer P300 latency compared to MCI patients who did not convert to AD (Golob et al. 2007). In addition, one study has documented that P300 amplitude or latency abnormalities were present in individuals at relatively high risk for AD (Ally et al. 2006). These findings imply that simple P300 measures may be useful in evaluating the cognitive effects of dementia in AD or MCI, and that the combination of neuropsychological tests and P300 measurements appear to be useful in improving reliability and increasing sensitivity to early detection and diagnosis of AD (Lai et al. 2010).

13.2.6 Error-Related Negativity (ERN)

ERN is a frontocentrally maximal response-locked negative deflection in the ERP that peaks approximately 50 ms following error detection. ERN can be observed across various choice tasks that employ a variety of stimulus and response modalities and levels of difficulty (see Olvet and Hajcak 2008 for a review). Evidence suggests that the anterior cingulate cortex (ACC) is the generator of the ERN (Taylor et al. 2007). The consistent activation of ACC by response conflict, negative feedback, pain, and errors has led some to suggest that the ERN may reflect the integration of cognitive and affective processing of errors (Luu et al. 2000; Olvet and Hajcak 2008). Consistent with this possibility, there is growing evidence that the ERN reflects the motivational significance of errors: The magnitude of the ERN is larger when errors are more costly or significant, and when accuracy is emphasized over speed (Chiu and Deldin 2007; Hajcak et al. 2005).

Increased ERN is a consistent finding in child and adult with anxiety disorders (see Olvet and Hajcak 2008 for a review). Increased error-related ACC and ERN activity has been observed in obsessive–compulsive disorder (OCD), children with both generalized anxiety disorder (GAD) and OCD, and in adults with GAD. In addition, pathological worry, a trait marker of GAD, and increased self reported anxious distress are associated with increased ERN amplitude (Hajcak et al. 2003). Hajcak and colleagues reported that ERN amplitude does not change after successful treatment in OCD, suggesting that the ERN is not affected by state-related changes in anxious symptoms (Hajcak et al. 2008).

Depressed individuals also exhibit abnormal error-related activity. For example, while performing a Stroop task, depressed subjects had greater ERN amplitude compared with controls (Holmes and Pizzagalli 2008). Additionally, while performing a Flanker task, in which subjects were given either neutral, reward, or punishment feedback on their responses on a simple choice task, depressed subjects had larger ERN amplitudes in neutral and punishment conditions compared with controls, but no difference in ERN amplitude during a reward condition, supporting the notion that depressed individuals are especially sensitive to punishment (Chiu and Deldin 2007). An fMRI study found that depressed patients had increased rostral ACC activity during error trials compared with controls (Steele et al. 2004). Hajcak and colleagues hypothesized that abnormal ERN amplitude may not be specific to pathological conditions of depression or anxiety, but rather reflect negative affect, a characteristic that is central to both these disorders.

Although increased ERN amplitude occurs in anxiety and depression, smaller ERN amplitude is found in individuals with schizophrenia (Bates et al. 2004; Kopp and Rist 1999; Morris et al. 2006). Imaging studies confirm that individuals with schizophrenia have decreased error-related brain activation. During errors of commission, schizophrenic patients had a decreased hemodynamic response in the ACC (Carter et al. 2001) and, more specifically, in the rostral ACC (Laurens et al. 2003). Reduced ERN and ACC activity is thought to represent a general inability of individuals with schizophrenia to self-monitor (Malenka et al. 1982). Reduced

ERN is not specific to schizophrenia, however. Across psychotic disorders, impaired error processing was associated with unemployment and impairment in community functioning, as indicated by hospitalizations during the first 4 years of illness (Foti et al. 2012). Furthermore, Simmonite and colleagues reported reductions of ERN amplitudes in both schizophrenia patients and their unaffected siblings, suggesting that the abnormality may be a trait marker for susceptibility to schizophrenia, rather than a result of the illness (Simmonite et al. 2012).

13.3 Conclusion

For decades, ERPs have been used to shed light on how the human brain normally processes information and how this processing may go awry in neurological or psychiatric disorders. Many of the ERP measures can be recorded in animals, therefore allowing for cross-species translational studies. The availability of ERP measures in both human and animals makes them well suited for pharmacological and genetic studies, as well as permits their use as biomarkers during early drug development. One example is the studies of P50 sensory gating abnormalities described earlier in this chapter. Thus, ERP research will continue to hold promise as an exciting measure that can be used to understand the pathophysiology of brain diseases, improve diagnosis prediction, as endophenotypes to reveal susceptibility genes associated with diseases, and as biomarkers for drug discovery.

References

Adler LE, Hoffer LJ, Griffith J et al (1992) Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. Biol Psychiatry 32(7):607–616

Adler LE, Hoffer LD, Wiser A et al (1993) Normalization of auditory physiology by cigarette smoking in schizophrenic patients. Am J Psychiatry 150(12):1856–1861

Ally BA, Jones GE, Cole JA et al (2006) The P300 component in patients with Alzheimer's disease and their biological children. Biol Psychol 72(2):180–187

Anokhin AP, Heath AC, Myers E et al (2003) Genetic influences on prepulse inhibition of startle reflex in humans. Neurosci Lett 353(1):45–48

Bates AT, Liddle PF, Kiehl KA et al (2004) State dependent changes in error monitoring in schizophrenia. J Psychiatr Res 38(3):347–356

Begleiter H, Porjesz B, Reich T et al (1998) Quantitative trait loci analysis of human event-related brain potentials: P3 voltage. Electromyogr Clin Neurophysiol 108(3):244–250

Bentin S, Allison T, Puce A et al (1996) Electrophysiological studies of face perception in humans. J Cogn Neurosci 8(6):551–565

Botzel K, Schulze S, Stodieck SR (1995) Scalp topography and analysis of intracranial sources of face-evoked potentials. Exp Brain Res 104(1):135–143

Braff DL, Geyer MA (1990) Sensorimotor gating and schizophrenia. Human and animal model studies. Arch Gen Psychiatry 47(2):181–188

Braff DL, Geyer MA, Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology 156(2–3):234–258

Braff DL, Light GA, Swerdlow NR (2007) Prepulse inhibition and P50 suppression are both deficient but not correlated in schizophrenia patients. Biol Psychiatry 61(10):1204–1207
Braff DL, Greenwood TA, Swerdlow NR et al (2008) Advances in endophenotyping schizophrenia. World Psychiatry 7(1):11–18
Bramon E, Rabe-Hesketh S, Sham P et al (2004) Meta-analysis of the P300 and P50 waveforms in schizophrenia. Schizophr Res 70(2–3):315–329
Bramon E, McDonald C, Croft RJ et al (2005) Is the P300 wave an endophenotype for schizophrenia? a meta-analysis and a family study. Neuroimage 27(4):960–968
Bramon E, Dempster E, Frangou S et al (2008) Neuregulin-1 and the P300 waveform–a preliminary association study using a psychosis endophenotype. Schizophr Res 103(1–3):178–185
Brzózka MM, Radyushkin K, Wichert SP, Ehrenreich H, Rossner MJ (2010) Cognitive and sensorimotor gating impairments in transgenic mice overexpressing the schizophrenia susceptibility gene Tcf4 in the brain. Biol Psychiatry 68(1): 33–40
Brzózka MM, Rossner MJ, de Hoz L (2016) Tcf4 transgenic female mice display delayed adaptation in an auditory latent inhibition paradigm. Eur Arch Psychiatry Clin Neurosci 266(6): 505–512
Cadenhead KS, Swerdlow NR, Shafer KM et al (2000) Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. Am J Psychiatry 157(10):1660–1668
Carter CS, MacDonald AW, Ross LL et al (2001) Anterior cingulate cortex activity and impaired self-monitoring of performance in patients with schizophrenia: an event-related fMRI study. Am J Psychiatry 158(9):1423–1428
Carver LJ, Dawson G (2002) Development and neural bases of face recognition in autism. Mol Psychiatry 7(Suppl 2):S18–S20
Chiu PH, Deldin PJ (2007) Neural evidence for enhanced error detection in major depressive disorder. Am J Psychiatry 164(4):608–616
Coles MGH, Rugg MD (1995) Event-related potentials: an introduction. In: Rugg MD, Coles MGH (eds) Electrophysiology of mind. Oxford University Press, New York
Dawson G, Webb SJ, McPartland J (2005a) Understanding the nature of face processing impairment in autism: insights from behavioral and electrophysiological studies. Dev Neuropsychol 27(3):403–424
Dawson G, Webb SJ, Wijsman E et al (2005b) Neurocognitive and electrophysiological evidence of altered face processing in parents of children with autism: implications for a model of abnormal development of social brain circuitry in autism. Dev Psychopathol 17(3):679–697
de Wilde OM, Bour LJ, Dingemans PM et al (2007) A meta-analysis of P50 studies in patients with schizophrenia and relatives: differences in methodology between research groups. Schizophr Res 97(1–3):137–151
Dick DM, Jones K, Saccone N et al (2006) Endophenotypes successfully lead to gene identification: results from the collaborative study on the genetics of alcoholism. Behav Genet 36(1):112–126
Edenberg HJ, Dick DM, Xuei X et al (2004) Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. Am J Hum Genet 74(4):705–714
Eimer M (2000) Effects of face inversion on the structural encoding and recognition of faces. Evidence from event-related brain potentials. Brain Res Cogn Brain Res 10(1–2):145–158
Eimer M (2011) The face-sensitive N170 component of the event-related brain potential. In: Calder AJ, Rhodes G, Johnson M et al (eds) The oxford handbook of face perception. Oxford University Press, Oxford, pp 329–344
Erwin RJ, Turetsky BI, Moberg P et al (1998) P50 abnormalities in schizophrenia: relationship to clinical and neuropsychological indices of attention. Schizophr Res 33(3):157–167
Fischer C, Luaute J, Adeleine P et al (2004) Predictive value of sensory and cognitive evoked potentials for awakening from coma. Neurology 63(4):669–673
Flora A, Garcia JJ, Thaller C, Zoghbi HY (2007) The E-protein Tcf4 interacts with Math1 to regulate differentiation of a specific subset of neuronal progenitors. Proc Natl Acad Sci USA 104(39):15382–15387

Foti D, Kotov, R, Bromet E, Hajcak (2012) Beyond the broken error-related negativity: Functional and diagnostic correlates of error processing in psychosis. Biol Psychiatry 71(10): 864–872

Freedman R, Adler LE, Bickford P et al (1994) Schizophrenia and nicotinic receptors. Harv Rev Psychiatry 2(4):179–192

Freedman R, Adler LE, Myles-Worsley M et al (1996) Inhibitory gating of an evoked response to repeated auditory stimuli in schizophrenic and normal subjects. Human recordings, computer simulation, and an animal model. Arch Gen Psychiatry 53(12):1114–1121

Freedman R, Coon H, Myles-Worsley M et al (1997) Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. Proc Natl Acad Sci USA 94(2):587–592

Freedman R, Adler LE, Leonard S (1999) Alternative phenotypes for the complex genetics of schizophrenia. Biol Psychiatry 45(5):551–558

Gallinat J, Bajbouj M, Sander T et al (2003) Association of the G1947A COMT (Val(108/158) Met) gene polymorphism with prefrontal P300 during information processing. Biol Psychiatry 50:40–48

George N, Evans J, Fiori N et al (1996) Brain events related to normal and moderately scrambled faces. Brain Res Cogn Brain Res 4(2):65–76

Golob EJ, Johnson JK, Starr A (2002) Auditory event-related potentials during target detection are abnormal in mild cognitive impairment. Clin Neurophysiol 113(1):151–161

Golob EJ, Irimajiri R, Starr A (2007) Auditory cortical activity in amnestic mild cognitive impairment: relationship to subtype and conversion to dementia. Brain 130(Pt 3):740–752

Gottesman II, Shields J (1973) Genetic theorizing and schizophrenia. Br J Psychiatry 122(566):15–30

Graham FK (1975) The more or less startling effects of weak prestimulation. Psychophysiology 12(3):238–248 (Presidential Address, 1974)

Hajcak G, McDonald N, Simons RF (2003) Anxiety and error-related brain activity. Biol Psychol 64(1–2):77–90

Hajcak G, Holroyd CB, Moser JS et al (2005) Brain potentials associated with expected and unexpected good and bad outcomes. Psychophysiology 42(2):161–170

Hajcak G, Franklin ME, Foa EB et al (2008) Increased error-related brain activity in pediatric obsessive-compulsive disorder before and after treatment. Am J Psychiatry 165(1):116–123

Hall MH, Smoller JW (2009) A new role for endophenotypes in the GWAS era: functional characterization of risk variants. Harv Rev Psychiatry 18(1):67–74

Hall MH, Schulze K, Rijsdijk F et al (2006) Heritability and reliability of P300, P50 and duration mismatch negativity. Behav Genet 36(6):845–857

Hall MH, Rijsdijk FV, Kalidindi S et al (2007a) Genetic overlap between bipolar illness and event-related potentials. Psychol Med 37(5):667–678

Hall MH, Rijsdijk FV, Picchioni M et al (2007b) Substantial shared genetic influences on schizophrenia and event-related potentials. Am J Psychiatry 164:804–812

Hall MH, Schulze K, Sham P et al (2008) Further evidence for shared genetic effects between psychotic bipolar disorder and P50 suppression: a combined twin and family study. Am J Med Genet B Neuropsychiatric Genet 147B(5):619–627

Herrmann MJ, Ellgring H, Fallgatter AJ (2004) Early-stage face processing dysfunction in patients with schizophrenia. Am J Psychiatry 161(5):915–917

Hill MJ, Killick R, Navarrete K, Maruszak A, McLaughlin GM, Williams BP et al (2016) Knockdown of the schizophrenia susceptibility gene TCF4 alters gene expression and proliferation of progenitor cells from the developing human neocortex. J Psychiatry Neurosci: JPN 41(6): 160073

Holmes AJ, Pizzagalli DA (2008) Spatiotemporal dynamics of error processing dysfunctions in major depressive disorder. Arch Gen Psychiatry 65(2):179–188

Hong LE, Summerfelt A, Wonodi I et al (2007) Independent domains of inhibitory gating in schizophrenia and the effect of stimulus interval. Am J Psychiatry 164(1):61–65

Javitt DC, Doneshka P, Grochowski S et al (1995) Impaired mismatch negativity generation reflects widespread dysfunction of working memory in schizophrenia. Arch Gen Psychiatry 52(7):550–558

Jeste SS, Nelson CA (2009) Event related potentials in the understanding of autism spectrum disorders: an analytical review. J Autism Dev Disord 39(3):495–510
Kopp B, Rist F (1999) An event-related brain potential substrate of disturbed response monitoring in paranoid schizophrenic patients. J Abnorm Psychol 108(2):337–346
Kujala T, Kallio J, Tervaniemi M et al (2001) The mismatch negativity as an index of temporal processing in audition. Clin Neurophysiol 112(9):1712–1719
Lai CL, Lin RT, Liou LM et al (2010) The role of event-related potentials in cognitive decline in Alzheimer's disease. Clin Neurophysiol 121(2):194–199
Laurens KR, Ngan ET, Bates AT et al (2003) Rostral anterior cingulate cortex dysfunction during error processing in schizophrenia. Brain 126(Pt 3):610–622
Leonard S, Freedman R (2006) Genetics of chromosome 15q13-q14 in schizophrenia. Biol Psychiatry 60(2):115–122
Leonard S, Gault J, Hopkins J et al (2002) Association of promoter variants in the alpha7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. Arch Gen Psychiatry 59(12):1085–1096
Leppanen PH, Hamalainen JA, Guttorm TK et al (2012) Infant brain responses associated with reading-related skills before school and at school age. Clin Neurophysiol 42(1–2):35–41
Lichtenstein P, Yip BH, Bjork C et al (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet 373(9659):234–239
Light GA, Braff DL (2005) Mismatch negativity deficits are associated with poor functioning in schizophrenia patients. Arch Gen Psychiatry 62(2):127–136
Luck SJ (2005) An introduction to the event-related potential technique. The MIT Press, Cambridge
Luu P, Collins P, Tucker DM (2000) Mood, personality, and self-monitoring: negative affect and emotionality in relation to frontal lobe mechanisms of error monitoring. J Exp Psychol Gen 129(1):43–60
Malenka RC, Angel RW, Hampton B et al (1982) Impaired central error-correcting behavior in schizophrenia. Arch Gen Psychiatry 39(1):101–107
McPartland J, Dawson G, Webb SJ et al (2004) Event-related brain potentials reveal anomalies in temporal processing of faces in autism spectrum disorder. J Child Psychol Psychiatry 45(7):1235–1245
Michie PT (2001) What has MMN revealed about the auditory system in schizophrenia? Int J Psychophysiol 42(2):177–194
Morris SE, Yee CM, Nuechterlein KH (2006) Electrophysiological analysis of error monitoring in schizophrenia. J Abnorm Psychol 115(2):239–250
Myers CS, Robles O, Kakoyannis AN et al (2004) Nicotine improves delayed recognition in schizophrenic patients. Psychopharmacology 174(3):334–340
Myers CS, Taylor RC, Moolchan ET et al (2008) Dose-related enhancement of mood and cognition in smokers administered nicotine nasal spray. Neuropsychopharmacology 33(3):588–598
Naatanen R (1992) Attention and brain function. Erlbaum, Hillsdale
Naatanen R (2003) Mismatch negativity: clinical research and possible applications. Int J Psychophysiol 48(2):179–188
Obayashi C, Nakashima T, Onitsuka T et al (2009) Decreased spatial frequency sensitivities for processing faces in male patients with chronic schizophrenia. Clin Neurophysiol 120(8):1525–1533
O'Connor K, Hamm JP, Kirk IJ (2007) Neurophysiological responses to face, facial regions and objects in adults with Asperger's syndrome: an ERP investigation. Int J Psychophysiol 63(3):283–293
Olincy A, Martin L (2005) Diminished suppression of the P50 auditory evoked potential in bipolar disorder subjects with a history of psychosis. Am J Psychiatry 162(1):43–49
Olincy A, Harris JG, Johnson LL et al (2006) Proof-of-concept trial of an alpha7 nicotinic agonist in schizophrenia. Arch Gen Psychiatry 63(6):630–638

Olvet DM, Hajcak G (2008) The error-related negativity (ERN) and psychopathology: toward an endophenotype. Clin Psychol Rev 28(8):1343–1354
Onitsuka T, Niznikiewicz MA, Spencer KM et al (2006) Functional and structural deficits in brain regions subserving face perception in schizophrenia. Am J Psychiatry 163(3):455–462
Oranje B, van Berckel BN, Kemner C et al (1999) P50 suppression and prepulse inhibition of the startle reflex in humans: a correlational study. Biol Psychiatry 45(7):883–890
Osterling J, Dawson G (1994) Early recognition of children with autism: a study of first birthday home videotapes. J Autism Dev Disord 24(3):247–257
Patterson JV, Hetrick WP, Boutros NN et al (2008) P50 sensory gating ratios in schizophrenics and controls: a review and data analysis. Psychiatry Res 158(2):226–247
Perry W, Geyer MA, Braff DL (1999) Sensorimotor gating and thought disturbance measured in close temporal proximity in schizophrenic patients. Arch Gen Psychiatry 56(3):277–281
Polich J (2007) Updating P300: an integrative theory of P3a and P3b. Clin Neurophysiol 118(10):2128–2148
Polich J, Kok A (1995) Cognitive and biological determinants of P300: an integrative review. Biol Psychol 41(2):103–146
Polich J, Corey-Bloom J (2005) Alzheimer's disease and P300: review and evaluation of task and modality. Curr Alzheimer Res 2(5):515–525
Porjesz B, Almasy L, Edenberg HJ et al (2002) Linkage disequilibrium between the beta frequency of the human EEG and a GABAA receptor gene locus. Proc Natl Acad Sci USA 99(6):3729–3733
Potter D, Summerfelt A, Gold J et al (2006) Review of clinical correlates of P50 sensory gating abnormalities in patients with schizophrenia. Schizophr Bull 32(4):692–700
Quednow BB, Frommann I, Berning J et al (2008) Impaired sensorimotor gating of the acoustic startle response in the prodrome of schizophrenia. Biol Psychiatry 64(9):766–773
Quednow BB, Ettinger U, Mossner R et al (2011) The schizophrenia risk allele C of the TCF4 rs9960767 polymorphism disrupts sensorimotor gating in schizophrenia spectrum and healthy volunteers. J Neurosci 31(18):6684–6691
Rangaswamy M, Porjesz B (2008) Uncovering genes for cognitive (dys)function and predisposition for alcoholism spectrum disorders: a review of human brain oscillations as effective endophenotypes. Brain Res 1235:153–171
Rangaswamy M, Jones KA, Porjesz B et al (2007) Delta and theta oscillations as risk markers in adolescent offspring of alcoholics. Int J Psychophysiol 63(1):3–15
Ripke S, Sanders AR, Kendler KS et al (2011) Genome-wide association study identifies five new schizophrenia loci. Nat Genet 43(10):969–976
Ritsner M (ed) (2009) The handbook of neuropsychiatric biomarkers, endophenotypes and genes. Springer, Medford
Roeske D, Ludwig KU, Neuhoff N et al (2011) First genome-wide association scan on neurophysiological endophenotypes points to trans-regulation effects on SLC2A3 in dyslexic children. Mol Psychiatry 16(1):97–107
Ross RG, Stevens KE, Proctor WR et al (2010) Research review: cholinergic mechanisms, early brain development, and risk for schizophrenia. J Child Psychol Psychiatry 51(5):535–549
Rossion B, Jacques C (2008) Does physical interstimulus variance account for early electrophysiological face sensitive responses in the human brain? ten lessons on the N170. NeuroImage 39(4):1959–1979
Rossion B, Jacques C (2011) The N170: understanding the time-course of face perception in the human brain. In: Luck S, Kappenman E (eds) The oxford handbook of ERP components. University Press, Oxford, pp 115–142
Rossion B, Delvenne JF, Debatisse D et al (1999) Spatio-temporal localization of the face inversion effect: an event-related potentials study. Biol Psychol 50(3):173–189
Salisbury DF, Shenton ME, Griggs CB et al (2002) Mismatch negativity in chronic schizophrenia and first-episode schizophrenia. Arch Gen Psychiatry 59(8):686–694
Salisbury DF, Kuroki N, Kasai K et al (2007) Progressive and interrelated functional and structural evidence of post-onset brain reduction in schizophrenia. Arch Gen Psychiatry 64(5):521–529

Schulze KK, Hall M-H, McDonald C et al (2007) P50 auditory evoked potential suppression in bipolar disorder patients with psychotic features and their unaffected relatives. Biol Psychiatry 62(2):121–128

Sehatpour P, Molholm S, Javitt DC et al (2006) Spatiotemporal dynamics of human object recognition processing: an integrated high-density electrical mapping and functional imaging study of "closure" processes. Neuroimage 29(2):605–618

Shaikh M, Hall MH, Schulze K et al (2011) Effect of DISC1 on the P300 waveform in psychosis. Schizophr Bull 39(1):161–167

Simmonite M, Bates AT, Groom MJ et al (2012) Error processing-associated event-related potentials in schizophrenia and unaffected siblings. Int J Psychophysiol 84(1):74–79

Squires NK, Squires KC, Hillyard SA (1975) Two varieties of long-latency positive waves evoked by unpredictable auditory stimuli in man. Electroencephalogr Clin Neurophysiol 38(4):387–401

Steele JD, Meyer M, Ebmeier KP (2004) Neural predictive error signal correlates with depressive illness severity in a game paradigm. Neuroimage 23(1):269–280

Taylor SF, Stern ER, Gehring WJ (2007) Neural systems for error monitoring: recent findings and theoretical perspectives. Neuroscientist 13(2):160–172

Tsunoda T, Kanba S, Ueno T et al (2012) Altered face inversion effect and association between face N170 reduction and social dysfunction in patients with schizophrenia. Clin Neurophysiol 123(9):1762–1768

Turetsky BI, Calkins ME, Light GA et al (2007) Neurophysiological endophenotypes of schizophrenia: the viability of selected candidate measures. Schizophr Bull 33(1):69–94

Umbricht DSG, Bates JA, Lieberman JA et al (2006) Electrophysiological indices of automatic and controlled auditory information processing in first-episode, recent-onset and chronic schizophrenia. Biol Psychiatry 59(8):762–772

van Beijsterveldt CE, van Baal GC (2002) Twin and family studies of the human electroencephalogram: a review and a meta-analysis. Biol Psychol 61(1–2):111–138

Wickham H, Murray R (1997) Can biological markers identify endophenotypes predisposing to schizophrenia? Int Rev Psychiatry 9:355–364

Wijnen VJ, van Boxtel GJ, Eilander HJ et al (2007) Mismatch negativity predicts recovery from the vegetative state. Clin Neurophysiol 118(3):597–605

Chapter 14
EEG-Derived Neurophenotypes

William Bosl

14.1 Introduction

The National Institute of Mental Health's Strategic Plan calls for the development of new ways of classifying psychopathology based on observable behavior and neurobiological measures (NIMH 2008). The goal of this strategy is to discover the fundamental "units of behavior"—cognitive phenotypes—that can be reliably measured and used not only for classification of psychopathological disease, but also for planning therapeutic approaches that target the fundamental neurobiological pathology (see Chap. 2 in this volume). In essence, this strategy explicitly states that all neuropsychiatric disorders are brain disorders and lays out a plan for discovering the brain basis for psychological disorders as characterized by cognitive and behavioral phenotypes.

Two goals are actually stated implicitly in the NIMH strategic plan. One is scientific: to discover the brain basis of behavior. The other is clinical and pragmatic: to discover neurobiological measures that are useful for diagnosis and planning therapeutic strategies. Achieving the clinical goal can potentially be done without achieving the scientific goal. That is, discovery of clinical biomarkers of developing mental and cognitive disorders does not require that a complete understanding of why certain measures are highly correlated with specific pathological outcomes to be useful. Medical practice is full of examples where a procedure or test "works," even though the physiological basis is not fully understood. For example, vagal nerve stimulation was accidently discovered to treat intractable epilepsy and treatment-resistant depression, though little is known about how this works (Rong et al. 2012).

W. Bosl (✉)
Department of Healthcare Informatics, Benioff Children's Hospital Oakland Research Institute, University of San Francisco, San Francisco, USA
e-mail: wjbosl@usfca.edu

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_14

In this chapter, a single neurophysiological measure is considered: scalp electrophysiology using electroencephalography or EEG. EEG has long been used clinically for confirming the existence of epilepsy, but may contain far more clinically useful information for monitoring brain function over the life span than has been realized. At the most fundamental level, brain function is electrical. The neural network that comprises the brain and peripheral nervous system, along with all the specialized cellular structures for propagating electrical impulses, is designed to support exquisitely fine control over the electrical patterns that determine all thought and behavior. It would not be an exaggeration to say that the mind is an ever-changing pattern of electrical fields: brain electrical activity is directly related to every thought and behavior. Measurements of brain electrical activity may thus in principle contain information about cognitive phenotypes, if recurring patterns can be found that correlate with them.

14.2 Complex Dynamical Systems

The brain is a complex dynamical system. It may appear so, but this is not an analogy or a model: The brain *is* a complex dynamical system by definition. To be more complete, the brain is an open complex dynamical system, embedded in a body with sensory input from the environment and motor output that enables the brain to sense, respond to, and act upon its environment. This has important implications for understanding the relationship between observed behaviors or cognitive phenotypes and the brain, which is the locus of every behavior, cognitive phenotype, and psychopathology that can be observed. The brain is also an adaptive evolving complex system, implying that its dynamical properties can change over long time periods in response to learning or development.

14.2.1 Cognitive Phenotypes are Dynamical Entities

A working definition for a cognitive phenotype used in this book is a discrete cognitive or behavioral feature that can be specified with some degree of precision and quantitatively measured. The features of interest are those of most relevance for a classification of neuropsychiatric illness or the indicators of normal neuropsychological functioning. Examples include the language deficiency subtypes associated with autism (Charman et al. 2010; Tager-Flusberg and Joseph 2003), especially response inhibition and contingency detection.

Although cognitive and behavioral phenotypes are described as static entities, they are in fact *processes*—sequences of actions by a person that recur in a recognizably repeatable fashion and can thus be reliably measured in some fashion. As described in Chap. 1, the Society for the Study of Behavioural Phenotype (SSBP), http://www.ssbp.co.uk, has proposed a working definition for the phrase

behavioral phenotype as "a characteristic pattern of motor, cognitive, linguistic and/or social abnormalities which is consistently associated with a biological disorder" (Fletcher et al. 2007). An important aspect of this definition is that it describes a *pattern of actions*, not a static structure.

A cognitive or behavioral phenotype may be analogous to units in spoken language. Spoken language is a continuous stream or a trajectory of sound units—phonemes—in time. Combinations of phonemes create words, which in defined combinations create fundamental units of meaning. Translators know that languages cannot be translated word for word, but meaningful units must be conveyed in another language. Similarly, cognitive processes and behaviors are continuous processes in time that can be observed or assessed by delineating the continuous flow of behavior or thought at appropriate points. Identification of fundamental units of behavior requires isolation of a particular sequence of movements or thoughts in the context of a particular situation.

This clarification is important for identifying relationships between cognitive or behavioral phenotypes and dynamic processes in the brain. Cognitive activity that directly reflects (and is in fact caused by) sequences of electrical firing patterns in the brain must be treated as a dynamic process. The relationship is not between static entities, but between a well-defined series of observable behaviors or measurable cognitive activities, and measurable brain dynamics.

14.2.2 What is a Complex Dynamical System?

Dynamical systems theory, sometimes called chaos theory, is a branch of mathematical physics that deals with the qualitative and quantitative characterization of long-term properties of complex dynamical systems. A complex dynamical system is a collection of N components or variables, each described by a single real number value, that are mathematically or physically coupled and whose values change over time. A convenient way to represent the values of all components is a single vector in N-dimensional space, usually written as a "state vector" of real numbers. Discrete dynamical systems are analogous entities composed of vectors of discrete (integer) values. An example of a state vector is shown:

$$\begin{array}{c|c} \uparrow & \\ \vdots & \\ N & \begin{bmatrix} 1.487 \\ 0.984 \\ 0.877 \\ \vdots \\ 0.391 \end{bmatrix} \\ \vdots & \\ \downarrow & \end{array} \tag{14.1}$$

The state of a complex dynamical system at any given time is represented by the value of the state vector, which is an array of n values at each time. The sequence of state vectors through time comprises the trajectory of the system. The state space of

the system is also called the phase space. Isaac Newton carried out the earliest studies of dynamical systems when he formulated his fundamental laws of motion. For a physical system such as the solar system, composed of 10 component elements (8 planets, the sun, and the moon—Pluto is no longer considered a planet), the state of the system is given by the three-dimensional location of each body and the three-dimensional velocity of each body at each moment. Thus, the state of the entire 10-body system is completely specified by 60 real numbers. The phase space for this system is the space of all possible values of each of the 60 numbers, which is represented by a 60-dimensional vector. If the position and velocity at every moment could be plotted, these would trace out a trajectory in 60-dimensional phase space. An example of a trajectory in three-dimensional phase space is shown in Fig. 14.1. Visual representations of phase space with more than three dimensions are not possible.

The principle activity of neurons for information processing is their continually changing electrical potential, usually, which is continually oscillating or "spiking," with a spiking rate that is believed to be the method used to encode information in single neurons (Kello et al. 2012). Thus, the basic quantitative unit of the dynamic brain might be considered the spiking rate of each neuron in the brain at any given time is a snapshot of the brain's state at that time. The electrical potential of each neuron could equally be chosen to quantify neural state without changing the following argument. More generally, the entire brain could be represented as a continuous electrical field. This would complicate the mathematical argument here by replacing the discrete dynamical field with a continuous field, but the fundamental argument would remain the same.

In the most general sense, the trajectory of neural electrical values, whether the spiking rate or potential of each neuron, must directly reflect all of the movements and behaviors that may be observed in a person. As suggested by Fig. 14.2, a correspondence between patterns in neural activity and measurable neuropsychological traits—cognitive phenotypes—exists, whether or it can actually be measured in the finest detail or not. Finally, the brain is a *coupled* dynamical system. This means that the future state of any individual neuron depends on not only its current state but also on the state of every neuron in the system.

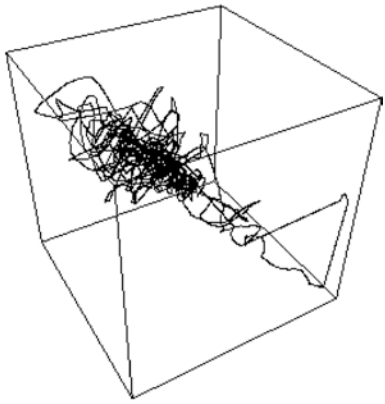

Fig. 14.1 Simple trajectory in 3-dimensional phase or state space is shown. Any single point on the line represents the state of the system or the state vector of the system, at a given time

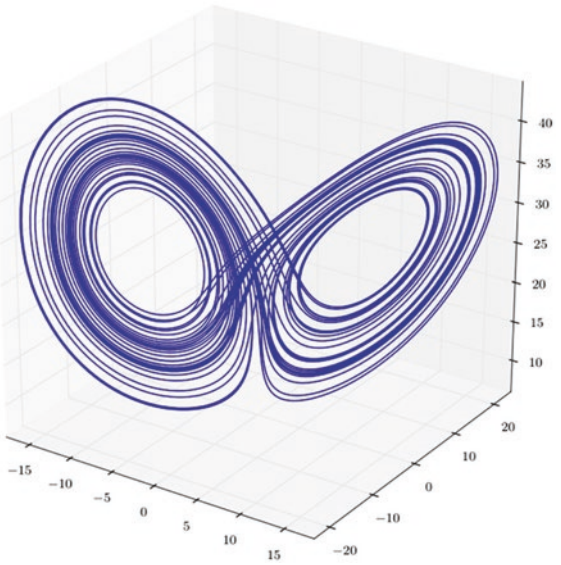

Fig. 14.2 State space plot of Lorentz attractor. For code to regenerate, see: http://www.node99.org/tutorials/ar/

14.2.3 Complex Systems Designed by Evolutionary Processes

Much of neuropsychology and biological psychiatry research has been focused on localizing brain function. While the effort to map specific brain regions with specific behavioral and cognitive deficits has been clinically useful, many higher-level cognitive functions have defied localization. For example, recent evidence demonstrates that many neurons in higher-order brain regions such as the prefrontal cortex (PFC) are not organized anatomically. Rather, they are said to exhibit mixed selectivity to multiple aspects of cognitive function (Rigotti et al. 2013). Another way of stating this is that the brain functions as a complex system.

In nature, complex systems are constructed by evolutionary-like, developmental processes or algorithms. The mammalian nervous system is not specified entirely by the genetic code but is evolved in a developmental process that is influenced by sensory input from the environment (Lu et al. 2009). An important characteristic of an evolutionary design process is that the dynamics of the resulting complex system cannot be understood from the component parts and their interactions alone. The whole is more than the sum of the parts, which also imposes a natural scale on the system, below which system functions are lost. Some system functions cannot be found in any single component but exist only when components are combined in a certain functional configuration, which may not be at all apparent from visual inspection of the network topology. However, some components may play critical roles in the system and their function is quite clear. In general, evolutionary design constructs a (complex) system that may look very different

from those that an engineer following traditional design principles would concoct (Antonsson and Cagan 2001). Although attempts have been made to view neurobiological networks in neat modular packages (Hartwell et al. 1999), many interconnections between modules prohibit the black box modularity that is a hallmark of top-down engineering design (Antonsson and Cagan 2001).

14.2.4 The Brain is a Complex Dynamical System

The state of the brain considered as a complex system composed of individual neurons may be represented succinctly at any time t as an N-dimensional vector:

$$\mathbf{B^t} = [n_1, n_2, \ldots n_N], \quad \text{where } N \sim= 10^{11} \tag{14.2}$$

where a single floating point number, n_i, roughly in the range of zero to 1000 Hz, represents the spiking rate or state of a single neuron. This could be generalized to the polarization of the neuron at any time, or even finer detail, and N would simply be larger. The superscript t refers to a specific time t. Each neuron receives input from many other neurons, perhaps many thousands, and sends its output to other neurons. The connectivity pattern determines which neurons will affect the next state of each neuron. The connections between neurons may change due to learning, and chemical influences may affect synaptic transmission, changing the effective connectivity. Together, the connectivity pattern and various influences that influence neural transmission determine the state transition rule that represents how the brain's state changes through time.

$$\mathbf{B^{t+1}} = F\left(\mathbf{B^t}\right) + \sigma^{\mathbf{t}} \tag{14.3}$$

Here, F is the function that represents how each neuron state will be updated based on the current state of every neuron and the connections to other neurons to give the new state vector at time $t + 1$. $\sigma^{\mathbf{t}}$ represents external sensory inputs, which exert an additional influence on the state of sensory neurons. If we assume healthy individual neurons and neurotransmitters, then the function F that determines brain function is essentially determined by the connectivity pattern among the neurons. Although this is a greatly simplified model of how a biological brain functions, it is nevertheless a realistic representation of how neurons change from one state to the next, and how the whole network of neurons advances through time.

In a general sense, the functioning brain is the state of all 10^{11} neurons at a given time and the trajectory of these neurons through the set of all possible states of the neurons (Kello et al. 2012), which is the phase space of the brain. Note that because neurons are connected in a highly controlled network, every neuron cannot assume an arbitrary state value at any time. Rather, the state of a neuron is constrained by the state of all neurons to which it is connected, with the influence of neighboring neurons determined by the synaptic strength and the specific neurotransmitter that mediates the connection. These may be either excitatory or

inhibitory, and this will be represented in our mathematical model by the sign on the entries in the state transition function F.

14.2.5 Phase Trajectories, Attractors, and Thought

The set of all possible brain states is called the phase space of the brain system. In our example, the phase space is represented by all points that can be written as 10^{11} dimensional vectors $\mathbf{B^t}$, with each entry n_1 having values in the physiological range of allowable spiking rates (nominally no more than 1000 Hz). In general, a dynamical system does not assume all the points in its phase space. Rather, it moves through the phase space on trajectories that fill only a small part of the phase space. Figure 14.4 illustrates a trajectory in a 3-dimensional phase space for a function called the Lorentz attractor. The phase space is delimited by Cartesian coordinates (x, y, z), where x, y, and z are any real numbers. Regardless of what starting point is chosen, the transition rule for the Lorentz system is such that the trajectory settles into the butterfly-like pattern within the phase space called the *attractor*. The blue lines are the trajectory of this system in phase space and fully describe how the system behaves under any circumstances.

The space that the trajectory occupies is determined by the transition rule F in Eq. (14.2). A very simple neural structure, such as the brain of a flatworm, will have a rather limited trajectory in phase space. Indeed, the flatworm has only 312 neurons, so its phase space is rather limited. The number of different states that such a brain may occupy is small relative to a brain made of 10^{11} neurons. The number of behaviors that the organism may exhibit in response to any given stimulus or due to self-generated impulses is therefore also rather small, since every behavior must necessarily reflect a sequence of neural firing patterns. The size of the state space increases combinatorially as the number of neurons, so that 10^{11} neurons, yields an enormous number of possible states.

If the vectors represented by Eqs. (14.2) and (14.3) were to be plotted throughout time in an N-dimensional plot, where N is the number of neurons or length of the state vector B, the trajectory would trace out the phase space of the dynamical system. In the context of a brain, we might say that each person, each brain, has a unique function F that results in a unique dynamical system that traces out its own unique path through time. It responds in different ways to different stimuli. Many people, many brains, respond to similar stimuli in the essentially the same way. For example, all the neural firing patterns—a trajectory through the brain's state space—that correspond to running or chewing, swallowing and digesting food, or the saccadic eye movements involved in watching a bird fly past are quite similar in most people. If we could trace out the pattern of the brain in an N-dimensional plot, the trajectories for each of these cognitive patterns that correspond to specific behavioral processes would be very similar in most people. Neuropsychology, the study of brain—behavior relationships, would not be possible if this was not so.

If the brain is viewed as a dynamical system, the connectivity pattern between neurons is an important determinant of the state transition function, which essentially determines how the brain functions. Research suggests that complex mental disorders are associated with abnormal brain connectivity that may vary between different regions and different scales (Noonan et al. 2009). Estimation of neural connectivity variation or differences might be a useful way to detect abnormal brain function as compared to normal function. This follows from our simple model: the trajectory that a brain follows in state space—dynamical systems language for brain function—is largely determined by network connectivity. If an *N*-dimensional plot could be made, a generalization of Fig. 14.2, we might be able to see with our eyes those regions in phase space where normal and abnormal brains differ. Differences in specific regions might correspond to differences in the way two brains respond to seeing emotion in another person's face, for example, or in how the brain responds to specific stimuli. A brain that is subject to seizure might have a region of phase space that looks decidedly unique and different from brains that do not easily have seizures. Again, in dynamical systems language, a brain subject to regular seizures will have many trajectories that fall into a seizure state. A healthy brain has few trajectories that fall into seizure attractors. These include stimuli such as strobe lights or hyperventilation.

A region of phase space into which many trajectories are attracted or move into is called, not surprisingly, an attractor. "Attractors are typical patterns of dynamical, interdependent behaviors of limited dimensionality and carved out from a much larger space of possible patterns and dimensions. These global structural patterns, which emerge from interactions among the system's components through phase space, can be characterized as emergent collectives" (Juarrero 2010). By definition, then, any situation or stimulus that evokes a similar cognitive or behavioral response must also result in a similar response in the brain; the trajectory of the neural response moves into an attractor of the dynamical system, the brain (Pascanu and Jaeger 2011). For example, if a person responds to a variety of life events by developing major depression, the "depression" is a kind of attractor—a region of phase space with certain well-defined brain-based behaviors that can be detected by behavioral assessments. Similarly, the trajectory of brain states moves into the region of phase space that causes depression behaviors. Whenever a person is depressed, parts of their brain are in an attractor region of phase space.

14.3 Measuring Complex Dynamical System Properties from Time Series

The trajectory of neural brain states at each moment of time is a trajectory through phase space and necessarily must directly cause the cognitive states and behaviors at that given moment, unless one posits that the brain alone does not cause all actions and thoughts. That is, the brain-state trajectory in time through phase

space corresponds directly through some complicated mapping to every thought and behavior at that time, even if the state of every neuron is not measurable, certainly by non-invasive means. However, it may be possible to mathematically infer certain dynamical properties of the attractors in the brain dynamical system from measurements of electrical potentials on the surface of the brain or scalp. Developments in dynamical systems theory demonstrate that such inferences are in principle possible.

14.3.1 Embedding Theorems: Reconstructing CDS from Time Series

Important theorems in mathematics were proved in the 1980s and 1990s regarding what is known as the reconstruction problem. The essence is illustrated in Fig. 14.3.

In his explanation of the diagram in Fig. 14.3, Casdagli states that the "true dynamical system *f*, its states *s*, and the measurement function *h* are unobservable, locked in a black box," as shown in the black square in the upper right. This is an accurate description of the brain as a dynamical system as well. However, the set of values in a time series *x* are measurable linear combinations of any set of fundamental components of the true dynamical system, as represented in the lower left graph of Fig. 14.3. The embedding theorems of dynamical systems assert that dynamical properties of the original *N*-dimensional system are

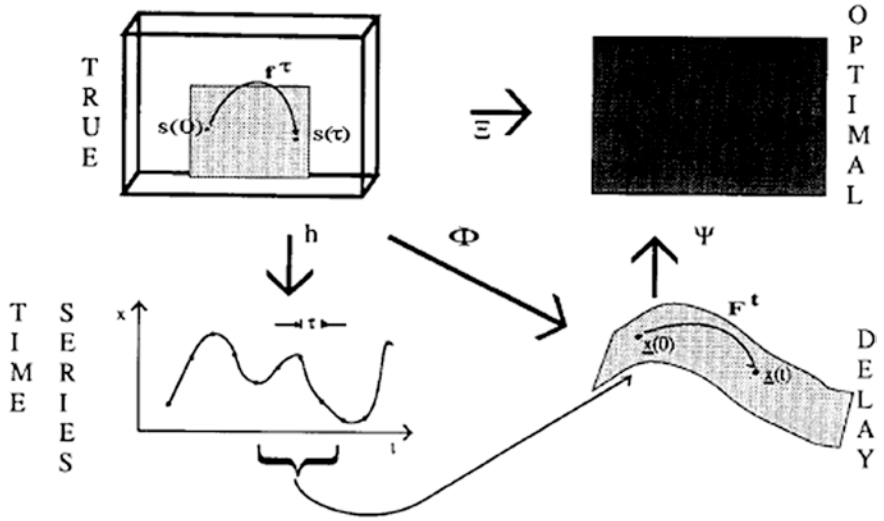

Fig. 14.3 Casdagli's illustration of how dynamical system properties may be inferred from time series measurements (Castagli et al. 1991)

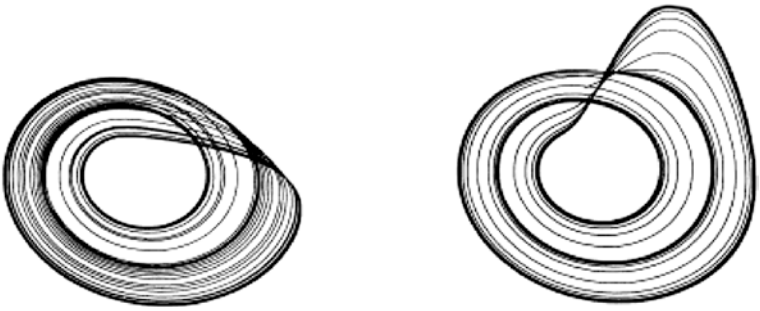

Fig. 14.4 The trajectory mapping out the two-dimensional attractor of the Rossler system is shown on the *left*. On the *right* is a reconstruction of the attractor from a single one-dimensional time series

embedded in the time series x. This property has potentially profound implications for EEG analysis. It implies that the scalp measurements of an EEG sensor, which are linear combinations of the contribution of many neurons, contain information about all neurons in the system. This does not mean that all the information about all neurons can be extracted from a finite, discrete time series measurement. Nevertheless, computational methods have been developed by which dynamical properties of the unknown dimensions may be reconstructed. The usefulness of this information for detecting pathological brain activity must be explored experimentally to determine its usefulness.

The embedding theorems have profound implications for electrophysiological measurements of groups of neurons using EEGs or other devices. For example, the actual spiking-rate states of all the neurons in the brain are not observable or measurable. If they were, then an exact representation of the state space of the brain and its moment-by-moment dynamics could be measured. The reconstruction theorems state that certain properties of the unobservable multidimensional state space of the brain as a dynamical system can be reconstructed from a one-dimensional time series measurements from EEG sensors. What this implies practically is that the EEG time series contain information about the dynamics of the entire brain. Computational methods that implement the reconstruction theorems may thus be used to compute values which represent dynamical properties.

A simple example is shown in Fig. 14.4. The phase trajectory of a nonlinear dynamical system, the Rossler equations, is shown, together with its reconstruction from a one-dimensional time series. The diagram shows the attractor of the Rossler dynamical system in two-dimensional Cartesian space. On the right is a reconstruction of the attractor using embedding and reconstruction theorems from a single time series measurement of the sum of x and y in the original system.

14.3.2 Recurrent Processes, Recurrent Behaviors

Normal behaviors can only be defined because the way most people respond to given situations is somewhat similar. A normal 2-year-old child will respond to a familiar face such as a parent or sibling with, usually, an emotional reaction, a smile and perhaps attempts to communicate. A child with autism is distinguished from the typical child—and similar to other children with autism—by a different response. These recurrent behaviors are reflected in the brain as well. In dynamical systems' language, the state vector of neurons follows a similar trajectory or pattern whenever presented with the same stimulus. The trajectory through phase space of an autistic brain is different from a typical brain in a way that is also recurrent: autistic behaviors can be identified as such because they follow a common, recurring pattern. To discover neural correlates of these autistic behaviors requires a search for patterns of quantitative neural measurements that can be mapped to some measure of the behaviors.

Recurrence Plot (*RP*) *Analysis*: A relatively recent and very general approach to nonlinear signal analysis is based on the concept of RPs, introduced by Eckmann in the late 1980s to graphically represent the dynamics of complex systems (Eckmann et al. 1987). Although RPs were originally developed as graphical devices, the concurrent growth of computers for data analysis and research in nonlinear or chaotic systems enabled quantitative statistical analysis of RPs. The methods developed for this analysis have been formalized and are collectively referred to as recurrence quantitative analysis (RAQ) (Marwan et al. 2007; Schinkel et al. 2009). RP analysis is an empirical approach to analyzing time series data and is in principle capable of characterizing all of the essential dynamics of a complex system (Webber and Marwan 2015).

RP analysis is a useful tool analyzing "real-world, noisy, high-dimensional data" (Webber and Zbilut 2005) and is a general empirical approach that can detect macroscopic properties of dynamical systems such as entropy and generalized synchronization. It has proven to be a powerful tool already in physics, geophysics, engineering, and biology (Komalapriya et al. 2008; Marwan et al. 2007). Its use in neuroscience as a method for analyzing neurophysiological time series is in the early stages. Single-trial ERP detection (Schinkel et al. 2009) and state changes before seizure onset (Acharya et al. 2011) are two recent applications.

An illustration of a RP for several different time series is shown in Fig. 14.5. The dynamics of each is revealed by a unique pattern in the RP. RAQ involves the computation of features from the RP that are a quantitative characterization of the dynamics of the system from which the parameters were derived.

Recurrence Quantitative Analysis (*RQA*): The definitions and descriptions of RP parameters given here are derived and explained more fully in the literature (Marwan et al. 2007; Webber and Marwan 2015). RP statistics are computed from structures in the RP, analogous to computing object statistics in an image. Statistical analysis of RPs or RQA is in its infancy. Several statistics have been found useful for characterizing system dynamics and are discussed below. Other relevant statistics remain to be discovered. Significant changes in some RQA

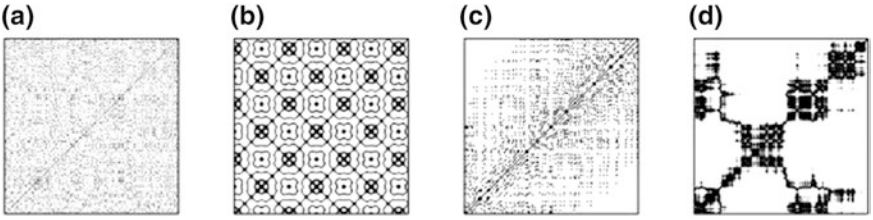

Fig. 14.5 Characteristic typology of recurrence plots: **a** uniformly distributed noise, **b** periodic, **c** drift (logistic map corrupted with a linearly increasing term) and **d** disrupted (Brownian motion). From (Marwan 2012)

values are highly correlated with and useful for detecting state transitions to new dynamical regimes, such as chaos–order or chaos–chaos transitions (Marwan et al. 2007). Others changes in RQA values are particularly sensitive to short-time transitions, for instance, those that occur during evoked response potential (ERP) experiments (Schinkel et al. 2007, 2009).

Recurrence rate (RR) is a measure of the density of recurrence points in the RP. Specifically, RR is the percentage of recurrent points falling within the specified radius parameter. In the limit $N \to \infty$, *RR* is the probability that a state recurs to its ε-neighborhood in phase space. RR has been found to be useful for detecting ERPs using single trials (Schinkel et al. 2009). RR has a high value for systems whose trajectories often visit the same phase space regions (Marwan et al. 2007), implying that RR may be useful for detecting regions of hypersynchronization.

Diagonal Measures: Determinism (DET) measures the proportion of recurrent points forming diagonal line structures. The name determinism comes from repeating patterns in the system and is an indication of its predictability. Regular, deterministic signals, such as sine waves, will give very long diagonal lines, while uncorrelated time series, like chaotic processes and random numbers, will give short or no diagonal lines. The ratio of these first two parameters, DET/RR, has been used to discover dynamical changes in physiological time series (Webber and Zbilut 1994). This will be explored further below in the context of epileptiform activity.

Line max (*L*max) is the length of the longest diagonal line segment in the plot, excluding the main diagonal line of identity. This recurrence variable is inversely related to the Lyapunov exponent that is frequently used to characterize system dynamics (Eckmann et al. 1987; Trulla et al. 1996). Positive Lyapunov exponents gauge the rate at which trajectories diverge, and are an indicator of chaos. Thus, smaller *L*max indicates a more chaotic (less predictable) signal. Conversely, larger *L*max is an indication of predictable signals, which may arise as the result of hypersynchronization of many chaotic oscillators to produce a single, high-amplitude oscillation. *L*max is the inverse of the divergence (DIV), a statistic that is sometimes computed. Div measures the exponential divergence of the phase space trajectory. Faster divergence results in shorter diagonal lines.

The above measures are computed from the length distribution of diagonal lines in the RP which encode the main properties of the system, such as predictability and measures of complexity (Marwan et al. 2007). Vertical lines in the RP are related to the presence of laminar states in the system. In contrast to the measures based on diagonal lines, these measures are able to find state transitions in chaotic systems, allowing investigation of intermittency, even for rather short and non-stationary data series.

Vertical Measures: Laminarity (LAM) represents the occurrence of laminar states in the system without describing the length of these laminar phases. LAM will decrease if the RP consists of more single recurrence points than vertical structures (Marwan et al. 2007). *Trapping Time* (TT) is the average length of vertical line structures. TT is an estimate of the time the system will remain in a current state or the length of time that the system is "trapped" in a state. It may be related to the length of transient synchronization of component oscillators that contribute to a measured EEG channel. Other statistics may be derived from RPs, some of which are discussed in (Marwan et al. 2007). By treating the RPs as shown in Fig. 14.2 as images, it may be possible to apply image classification algorithms to find new characteristic patterns that are associated with distinct time series types or dynamical regimes that have not yet been discovered using statistical measures (Daniusis and Vaitkus 2008; Norman et al. 2006).

14.4 From Complex Systems to Cognitive Phenotypes

Even if the recurrence matrix derived from scalp EEG time series contains all of the essential dynamical information about the brain as a complex dynamical system (Marwan et al. 2007), the correlation between complicated numerical data derived from the analysis, an cognitive or behavioral phenotype, may be subtle and hidden in complicated patterns. A data-driven approach is ideally suited to find the clinical correlates of cognitive phenotypes in complicated data derived from EEG time series. No a priori *model* is needed. Instead, very general machine learning algorithms are given many examples and are programmed to search for models that best fit the data and explain the phenomenon of interest.

14.4.1 Data-Driven Discovery: The Fourth Paradigm

Near the end of the twentieth century and continuing into the twenty-first century, a new approach to discovering relationships in complex data has emerged, under a variety of names, including data mining, data analytics, machine learning, or "big data" analysis (see also Chap. 15). This approach to scientific discovery is distinguished from hypothesis-driven data analysis by instead letting the data assume a primary role, then using machine learning algorithms to find the model

or hypothesis that best fits the data. It is particularly appropriate for discovering correlations between multisource or complicated sets of data and phenomena of interest when there is no foundational theory to enable models or hypotheses to be determined in advance and tested.

To cite one example, the NeoNatal Intensive care unit at the Hospital for Sick Children (SickKids) in Toronto has created a system for early identification of late-onset neonatal sepsis in newborns, a potentially fatal blood infection that occurs in infants. All physiological data that are measured, plus environmental variables, family history, and other medical conditions are all collected continuously, resulting in over 10 million data points per infant per day. The predictive analytics enables the presence of infections to be predicted before the symptoms are apparent to neonatologists, allowing early intervention and better outcomes (McGregor 2013).

Data-driven discovery can find correlations and predictive patterns in data that cannot be found by any other method. This approach requires a move away from trying to understand the deeper cause. Understanding the deeper causes is the goal of scientific research. However, for clinical application, statistically significant and reliable predictive capabilities from data can be lifesaving, long before causes are understood. This technique is saving lives, even though it is not known what combinations of variables are actually determinative. Causation, expressed in traditional medical terms, is not known. One might argue that the causal pattern is well known to the algorithm; humans are just unable to see the pattern.

In a philosophical sense, machine algorithm discovery of a model equation that describes a natural process is not so different from human discovery of a "law" of physics. Newton's law of motion, for example, that says the acceleration of a body (of constant mass) is proportional to the mass of the body and to the applied force is empirically derived. Many experiments were done to confirm its veracity under many conditions. Albert Einstein and the founders of quantum mechanics showed that Newton's laws of motion were only approximately correct, and that other laws were actually more appropriate. What is called a "law" of nature really falsely assumes that we have insight into a hidden fundamental nature of the universe, when really all we have are empirical observations. This may be controversial, but from a practical viewpoint, a learning algorithm can also discover Newton's laws of motion from very many empirical observations, as was demonstrated by (Schmidt and Lipson 2009).

14.4.2 Mapping Brain Dynamics to Cognitive Phenotypes

RQA can be used to compute a number of different nonlinear measures from each of the EEG channels as described above. The RP for each time series derived from the EEG channels contains considerable information about brain dynamics, state space trajectories, and attractors (Marwan et al. 2007). A typical EEG headset uses 19 sensors in a configuration referred to as the standard 10–20 system [for example, http://en.wikipedia.org/wiki/10-20_system_(EEG)]. Some newer research

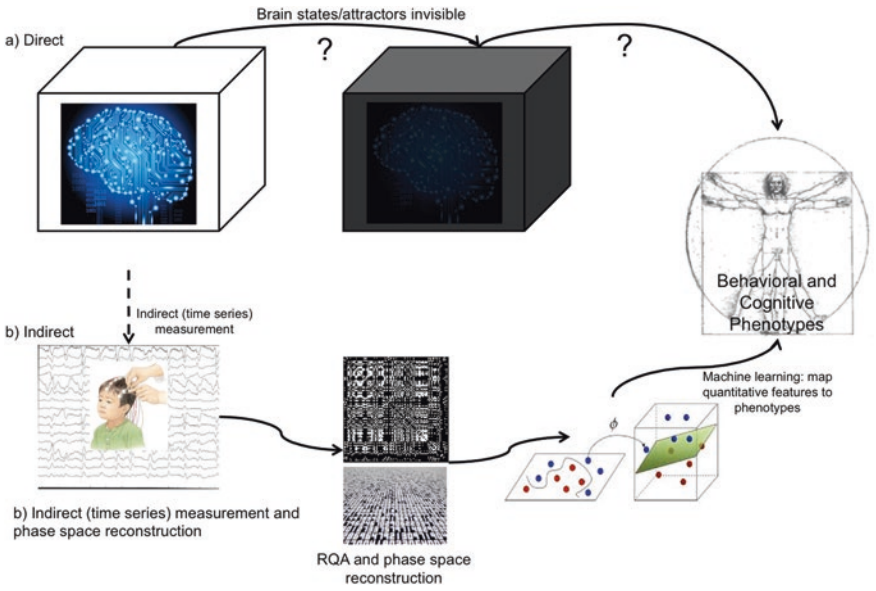

Fig. 14.6 A direct mapping from brain states to behavior, as illustrated by the upper pathway is not possible at this time, since brain states are hidden and cannot be measured directly. The lower, indirect pathway illustrates the reconstruction of dynamical features using recurrence quantitative analysis. The complicated relationship between patterns in RQA values and behavioral or cognitive measures are determined using machine learning methods

grade systems use up to 256 sensors (Yamazaki et al. 2013). If six RQA values are computed from each of 19 sensors, the result is 114 numerical values. Hierarchical methods that use wavelet transforms to compute multiresolution time series from each original time series can multiply the number of system features by many times.

Feature extraction is not just a method to enhance machine learning for classification and regression. In this context, the features include the computed nonlinear parameters from RP and cross RP analyses for each electrode. If these are arranged in a two-dimensional matrix, with rows for each channel and columns for each measure, then the values for each channel can be treated as a group to determine the location of channels that are most informative for the particular classification or regression task. In this way, the learning algorithm can be used as a discovery tool to localize neural activity that differentiates epileptic regions (Fig. 14.6).

14.5 Clinical Applications

In the introduction, we present a prescription for discovering quantitative mappings between measurable brain electrophysiology and cognitive or behavioral phenotypes. This section focuses on the results from several preliminary studies

that provide evidence that this approach will be useful for widespread, low-cost clinical application. The results shown here are derived from relatively small populations. Much larger populations may enable machine learning algorithms to uncover more subtle patterns, possibly enabling finer diagnostic capabilities.

Neuropsychiatric disorders impose the largest burden of disease of all chronic diseases in the world and are among the greatest threats to childhood health. In both developed and developing countries, brain disorders are an enormous economic and personal burden. Many mental and neurological disorders of adulthood have antecedents in childhood, but are not recognized in childhood. Studies have consistently shown that early intervention leads to better long-term outcomes for neuropsychiatric disorders. Thus, the most leveraged opportunity for intervention to prevent or reduce the severity of these diseases, in terms of both personal suffering and economic impact, is childhood. Creation of affordable, widely available technology for early detection, reporting and monitoring, particularly in underserved populations, is the key to significantly reducing the burden of neurological impairments and developmental disorders.

14.5.1 Autism Spectrum Disorder

Bosl et al. (2011) computed multiscale entropy (MSE) values for 41 infants at high risk for autism (HRA) and 31 controls (CON). Using a tenfold cross-validation, subjects were classified into either control or high-risk groups using MSE values at all electrodes as input data to a machine learning algorithm. Classification accuracy approached 100% at 9 months. A follow-up study (Bosl et al., in prep.) using RQA values for a feature set showed that a 36-month outcome of autism could be predicted with high accuracy by 12 months of age using nonlinear EEG analysis alone. The severity of autism symptoms appears to be predictable as well, suggesting that RQA features taken together contain quantitative information about the severity of autistic behavioral and cognitive phenotypes.

14.5.2 Epilepsy

Epilepsy is currently defined by two independent unprovoked seizures. To date, there is no robust and easily measured test to evaluate the predisposition to have seizures. Underlying pathology in the epileptic brain may be a dynamical property that enables the transition from normal to seizure states to happen spontaneously. This dynamical property persists, independent of spiking activity on visual EEG analysis. If the propensity to have seizures, or epileptogenicity level, is a property of a dynamical system, then a potential biomarker for epilepsy might be found in a

set of parameters that characterize complex system dynamics. In symptomatic epilepsy, the epileptogenetic process often follows a traumatic or infectious insult, or is caused by a tumor. If changes in brain dynamics can be detected before the first seizure, a window of opportunity for intervention might be created.

RAQ values were computed and found to contain information that enabled a machine learning algorithm to distinguish patients with absence epilepsy from non-epileptic controls using relatively short interictal EEG segments. Machine learning classification yielded accuracies of 100% in a tenfold cross-validation (Bosl et al., in prep.).

14.5.3 Global Mental Health

Mental, neurological, and substance use (MNS) disorders, or simply "mental disorders," impose the largest burden of all chronic disease classes in the world (Collins et al. 2011; Idro et al. 2010; Prince et al. 2007). Though long overlooked as healthcare priorities globally, mental disorders are an enormous economic burden on nations, particularly low-income nations, where the lack of capacity to deal with the problem leads to personal suffering for patients, which also affects caregivers, families, and communities.

The gap in mental health services between what is already known about treating these disorders and the number of people who actually receive care is quite large and cannot be resolved by extending current approaches that have been primarily developed in western countries. It will be essential to adapt known treatments and therapeutic approaches to local cultures and empirically document their effectiveness (Becker and Kleinman 2012). Integration of mental health services into existing healthcare systems, using available healthcare workers, with a view to provision of holistic health care through the life span, will be required.

The lack of specialists with advanced training in behavioral health is often cited as the primary barrier to better mental healthcare in many regions of the world, including low- and high-income countries. A lack of trained personnel or lack of access to psychiatric and neurological services can prevent attention to the enormous burden of mental disorders on many levels. Capacity building with low-cost, easy-to-use screening and diagnostic tools is of paramount importance for overcoming all of these barriers (Bakare et al. 2014).

Innovative use of information technology can help to build capacity and overcome barriers to expanding high-quality mental healthcare among underserved populations. Objective, brain-based technology for early detection of behavioral disorders can provide a foundation for considering mental disorders in the context of comprehensive, life-course management of general health and well-being in community and primary care settings.

14.6 Discussion and Future Research

Measurements of brain electrical activity with EEG are a valuable source of information for neuroscience research, yet this low-cost resource may be underutilized for clinical applications in neurology and psychiatry (Niedermeyer 2003; Niedermeyer and Lopes da Silva 2005). When the brain is analyzed as a complex dynamical system, recently developed methods and theorems from dynamical systems theory become applicable and provide powerful new insights into the functional information contained in scalp electrophysiological measurements. RP analysis, in particular, is an empirical method for analyzing nonlinear time series that, in principle, contains all of the dynamical information about the system that produced the time series. This insight is profoundly important for scalp electrophysiology, as it posits that EEG data contain functional information about the entire brain. The difficulty is that a relationship between the nonlinear time series features and cognitive phenotypes is not readily apparent and may not be explainable in current neurophysiological terms.

The key approach to finding electrophysiological correlates of cognitive phenotypes introduced in this chapter is the employment of machine learning methods. This approach assumes that complicated patterns and relationships in the RP variables computed from the EEG times series necessarily reflect all cognitive and behavioral activity. Brain states control motor and cognitive output. Machine learning algorithms can find patterns and complex relationships that may be completely opaque to human eyes. However, large datasets may also be required to enable differences in brain function that are biomarkers of serious disorders to be distinguished from the normal range of variation in brain function and behavior that exists among people.

Developments in a number of fields over the past decade have created the possibility of monitoring brain function through the life span, recording this information in electronic health records, and monitoring the trajectories for deviations that might indicate emerging disorders. Realizing this possibility will require continued research into the neurophysics of EEG measurements. In addition, research and implementation of computational methods for extracting this information, storing it in electronic health records, and mining large population databases to discover the biomarkers associated with psychopathology will also be needed. But the tools for moving this technology from the research laboratory to the bedside are available today. The potential impact of EEG-based functional brain monitoring for pathology is great. If successively developed and implemented on a large scale, it could radically alter the practice of psychiatry, neurology, and clinical psychology, with particularly great impact on underserved populations.

References

Acharya UR, Sree SV, Chattopadhyay S et al (2011) Application of recurrence quantification analysis for the automated identification of epileptic EEG signals. Int J Neural Syst 21(3):199–211

Antonsson EK, Cagan J (eds) (2001) Formal engineering design synthesis. Cambridge University Press, Cambridge

Bakare MO, Munir KM, Bello-Mojeed M (2014) Public health and research funding for childhood neurodevelopmental disorders in Sub-Saharan Africa: a time to balance priorities. Healthc Low-Resour Settings 2:1559

Becker AE, Kleinman A (2012) An agenda for closing resource gaps in global mental health: innovation, capacity building, and partnerships. Harv Rev Psychiatry 20(1):3–5

Bosl WJ, Bates A, DeGregorio G et al (in prep) EEG recurrence plot analysis for early detection of autism spectrum disorder (Manuscript in preparation)

Bosl WJ, Bates A, Fernandez IS et al (in prep) EEG biomarkers for early identification of children with epileptic encephalopathies (Manuscript in preparation)

Bosl WJ, Tager-Flusberg H, Tierney A, Nelson CA (2011) EEG complexity as a biomarker for autism spectrum disorder. BMC Medicine 9:18

Castagli M, Eubank S, Farmer JD et al (1991) State space reconstruction in the presence of noise. Physica D 51:52–98

Charman T, Jones CR, Pickles A et al (2010) Defining the cognitive phenotype of autism. Brain Res 1380:10–21

Collins PY, Patel V, Joestl SS et al (2011) Grand challenges in global mental health. Nature 475(7354):27–30

Daniusis P, Vaitkus P (2008) Kernel regression on matrix patterns. Lith Math J 48(49):191–195

Eckmann J, Kaphorst SO, Ruelle D (1987) Recurrence plots of dynamical systems. Europhys Lett 5:973–977

Fletcher R, Loschen E, Stavrakaki C et al (eds) (2007) A textbook of diagnosis of mental disorders in persons with intellectual disability (DM-ID). NADD Press, Kingston

Hartwell LF, Hopfield JJ, Leibler S, Murray AW (1999) From molecular to modular cell biology. Nature 402:C47–52

Idro R, Newton C, Kiguli S et al (2010) Child neurology practice and neurological disorders in East Africa. J Child Neurol 25(4):518–524

Juarrero A (2010) Complex dynamical systems theory. Available via Cognitive Edge. http://cognitive-edge.com/articles/complex-dynamical-systems-theory/

Kello CT, Rodny J, Warlaumont AS et al (2012) Plasticity, learning, and complexity in spiking networks. Crit Rev Biomed Eng 40(6):501–518

Komalapriya C, Thiel M, Romano MC et al (2008) Reconstruction of a system's dynamics from short trajectories. Phys Rev E: Stat, Nonlin, Soft Matter Phys 78(6 Pt 2):066217

Lu J, Tapia JC, White OL et al (2009) The interscutularis muscle connectome. PLoS Biol 7(2):e32

Marwan, N (2012) Recurrence plots and cross recurrence plots. http://www.recurrence-plot.tk/

Marwan N, Romano MC, Thiel M et al (2007) Recurrence plots for the analysis of complex systems. Phys Rep 438:237–329

McGregor C (2013) Big data in Neonatal intensive care. Computer 46(6):54–59

Niedermeyer E (2003) The clinical relevance of EEG interpretation. Clin Electroencephalogr 34(3):93–98

Niedermeyer E, Lopes da Silva FH (2005) Electroencephalography: basic principles, clinical applications, and related fields, 5th edn. Lippincott Williams & Wilkins, Philadelphia

NIMH (2008) National institute of mental health strategic plan. National Institutes of Health, Bethesda

Noonan SK, Haist F, Muller RA (2009) Aberrant functional connectivity in autism: evidence from low-frequency BOLD signal fluctuations. Brain Res 1262:48–63

Norman KA, Polyn SM, Detre GJ et al (2006) Beyond mind-reading: multi-voxel pattern analysis of fMRI data. Trends Cogn Sci 10(9):424–430
Pascanu R, Jaeger H (2011) A neurodynamical model for working memory. Neural Netw 24(2):199–207
Prince M, Patel V, Saxena S et al (2007) No health without mental health. Lancet 370(9590):859–877
Rigotti M, Barak O, Warden M et al (2013) The importance of mixed selectivity in complex cognitive tasks. Nature 497:585–590
Rong PJ, Fang JL, Wang LP et al (2012) Transcutaneous vagus nerve stimulation for the treatment of depression: a study protocol for a double blinded randomized clinical trial. BMC Complement Altern Med 12:255
Schinkel S, Marwan N, Kurths J (2007) Order patterns recurrence plots in the analysis of ERP data. Cogn Neurodyn 1(4):317–325
Schinkel S, Marwan N, Kurths J (2009) Brain signal analysis based on recurrences. J Physiol Paris 103(6):315–323
Schmidt M, Lipson H (2009) Distilling free-form natural laws from experimental data. Science 324(5923):81–85
Tager-Flusberg H, Joseph RM (2003) Identifying neurocognitive phenotypes in autism. Philos Trans R Soc Lond B Biol Sci 358(1430):303–314
Trulla LL, Giuliani AL, Zbilut JP, Jr, CLW (1996) Recurrence quantification analysis of the logistic equation with transients. Physics Letters A 267:255–260
Webber CL, Marwan N (eds) (2015) Recurrence quantification analysis. Understanding complex systems. Springer, New York
Webber CL Jr, Zbilut JP (1994) Dynamical assessment of physiological systems and states using recurrence plot strategies. J Appl Physiol 76(2):965–973
Webber CL, Zbilut JP (2005) Recurrence quantitative analysis of nonlinear dynamical systems. In: Riley MA, Van Orden G (eds) Tutorials in contemporary nonlinear methods for the behavioral sciences. National Science Foundation, Virginia
Yamazaki M, Tucker DM, Terrill M et al (2013) Dense array EEG source estimation in neocortical epilepsy. Front Neurol 4:42

Chapter 15
Conclusion: Implications for Psychiatry and Neuropsychology

Susan L. Santangelo and Vinoth Jagaroo

Psychiatry, neuropsychology, and many related disciplines can no longer operate as silos, in relative independence, with a narrow and unsystematic engagement with the broader biomedical sciences. The scientific environment created by the revolution of systems biology forces integrative engagement, the benefits of which are difficult to question. Integration across subdisciplines requires that they share a common platform for data integration. The precise technical language of a discipline concerned with one or more of the strata in the G-P matrix may always be specific to the subdiscipline, e.g., molecular-genetic referents, neurobiological and neuroanatomic descriptors, and cognitive–behavioral phenomena. However, in order to relate one level of the G-P matrix to another, the levels must have a compatible set of constructs: They need to make reference to the same "thing" but from different perspectives. This is not a problem at the molecular and cellular end of the G-P matrix—where by the canonical principle in biology, genes can be tied to proteins or regulatory functions, which can then be tied to various cellular

S.L. Santangelo (✉)
Department of Psychiatry, Tufts University School of Medicine, Boston, MA, USA
e-mail: ssantangel@mmc.org

S.L. Santangelo
Maine Medical Center Research Institute, Maine Medical Center, Portland, ME, USA

S.L. Santangelo
Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA, USA

V. Jagaroo
Department of Communication Sciences and Disorders, Emerson College, Boston, MA, USA
e-mail: jagaroo@bu.edu

V. Jagaroo
Behavioral Neuroscience Program, Boston University School of Medicine, Boston, MA, USA

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5_15

elements. But at the cognitive–behavioral phenotypic end of the G-P matrix, relational constructs are almost nonexistent. That is, the constructs and, subsequently, the language of neuropsychology and psychiatry are incompatible with the universal framework in biology that serves systems-level understanding and knowledge discovery. This systems-level understanding must surely include the influence of epigenetic and environmental systems, and this only reinforces the need for precision in entity description and construct definition.

The NP approach is an operational step toward aligning neuropsychology and psychiatry with the brain sciences. This step is integrally tied with many conceptual and programmatic adjustments that the disciplines must undertake. Some examples are described.

15.1 Constraining the Endophenotype Concept

Chapters 4 and 5 in this volume have reviewed the endophenotype concept. The historical influence and inertia of the concept inevitably influences the discourse in NPs. Many chapters in this volume have noted that the endophenotype concept, as an intermediate biological feature, hidden from the visible phenotype, was introduced to psychiatry (psychiatric genetics) in a seminal paper by Shields and Gottesman (1972) and has remained a robust concept ever since (the endophenotype concept was first described by John and Lewis (1966) in the context of insect biology). It saw a strong re-emergence during the period of the mid-1990s to the late 2000s, marked by extensive discussions in the literature (e.g., Bearden and Freimer 2006; Flint and Munafo 2007; Gottesman and Gould 2003; Ritsner and Strous 2010), especially with reference to neuropsychiatry and schizophrenia (see *Schizophrenia Bulletin*, November 2006, special issue on the EP strategy). It is now easily recognized for its reference to objectively measured discrete, heritable, phenotypic-like markers, with tractable proximity to an underlying genotype—hence constituting a strategy by which phenotypes can be bridged to genotypes. That the endophenotype is heritable is a key criterion in the definition and distinction of the concept (see Chap. 4). With this criterion and a few others, the endophenotype is conceived as being a special type of biomarker, distinct from other forms. In brain-behavioral studies, the EP concept must be credited for reinforcing an approach to cognitive processes that dissect them into features that can be operationally tied to discrete neural systems.

Advocating the utility of a broader "neurophenotype" concept is not merely a shift of emphasis to a broader set of biomarkers nor a mere change of terminology. The preceding subsections on neural dynamics and neuromodulation suggest clearly that neural markers, including subcellular forms, can be shaped by intrinsic neural dynamics and the dynamics of circuits at large. This could account for the generally weak and tenuous links between synaptic phenotypes and candidate genes. The presumption that the EP concept is firmly relevant to neural systems is a problematic one. Forced or default application of the concept because

of its influential bearing, it can be argued, can have potentially confounding effects on understanding brain–behavior relationships via a G-P framework. The EP construct, initially proposed as a useful strategy, has become reified. It is now rendered with a definite, factual quality. And lack of progress in uncovering genetic correlates of psychiatric illness via the EP strategy has been pinned on inadequate focus on quantitative phenotypes (Glahn et al. 2007). Instead, questions can be raised about the extent to which it is applicable to neural systems.

Untenable situations arise when the EP concept/strategy is forced into G-P associative frameworks tied to brain and behavior. The heritability factor becomes a source of major complication, and subsequent rationalization of the complication only exposes the problem. The untenable if not impossible picture that arises has been well described (albeit unwittingly) by Congdon et al. (2010) in a detailed discussion on EP definition: They suggest that in the sifting out of potential EPs from the vastness of "phenomic space," the criteria that should be applied are those that show the greatest potential in bearing genetic associations through statistical significance (noting the difficulty of achieving statistical significance in GWAS studies tied to neuropsychiatric disorders). However, they also highlight the tenuous and problematic nature of data used to infer heritability factors in cognitive and neuroanatomic measures. They go on to suggest that while the heritability factor in efforts to link an endophenotype to a genotype should be seen as "necessary but not sufficient," they also suggest that "characteristics of phenotype definition and measurement—such as precision, reliability, objectivity, and the relationship to specific mental constructs—are more practically relevant criteria" (p. 221). This can be read as an instance where a construct that is not panning out is being clung to while searching for other constructs. There is also the rare instance where exceptionally good recognition is given to the need for EP definition in the context of phenomics as discussed above, but where EP exploration defaults to profiles generated by existing psychometric instruments (Bloss et al. 2010). This is in contrast to the more typical interpretation of cognitive EPs where a singular cognitive operation that can be tied to a discrete neural system aids in G-P mapping (see Sect. 15.2).

The EP concept has been imported by default into the discourse on cognitive and neural markers (neurophenotypes). Its inertial influence is inseparable from the G-P initiative as applied to neural systems and the behaviors they mediate. Yet again, neurons and neural systems are shaped by many other factors in addition to the genome. Comprehensive and accurate models of causative factors behind normal and aberrant neural systems have to take into account *all* variables and dynamic interactions that shape the form. The EP concept, limited by a narrow genome-only-driven perspective, is then only applicable where a neural element or pattern can be definitively tied to a gene or to polygenic effects. But the emergent properties of cognition and behavior appear to arise from larger functional assemblies (neural circuits and networks) which by their intrinsic modulatory dynamics also shape the physical form. In this regard, the EP concept has very circumscribed value in brain science. Overextending its utility may actually hinder the discovery of multiple forces that shape neural systems.

It is worth remembering some of the cautions and questions raised when the first systematic call for phenomics was made by Schork (1997). Given that for complex diseases, the effect of a single gene on outward phenotype may be small, one can envisage a scenario where the influence of a gene can be computed to be 50% on an intermediate phenotype and 5% on the outward phenotype. In going from a 50% effect (intermediate phenotype) to a 5% effect (outward phenotype), does the intermediate phenotype necessarily have any practical bearing on the phenotype of interest? "[T]he value of an intermediate phenotype is only as good as the physiologic insights that make the connection between it and the more remote phenotype in question." (Schork 1997). The very same may apply to NPs—they need to be accompanied by a set of conditional rules.

15.2 Refinement of Cognitive and Behavioral Constructs

In the G-P research/knowledge environment, cognitive processes need to be tied to neural systems. This requires that cognitive concepts and constructs be finely specified as discrete or distinct operations. In this context, the validity of constructs of cognition and emotion in psychiatry and neuropsychology faces critical examination: How well do the constructs align with G-P linkage frameworks? Recent calls for G-P compatible specification of cognitive constructs have come from those leading the initiative on cognitive phenomics (Bilder et al. 2009a, b; Sabb et al. 2008) and those calling for a neuropsychology that is compatible with neuroinformatics (Jagaroo 2009; Jagaroo and Berman 2005; Jagaroo et al. 2013). These discussions and the issues they raise, with particular bearing on neuropsychology, can be summed up as follows.

"Cognitive processes" represented by conventional neuropsychological tests contain numerous latent constructs, compound constructs, or multivariate operations. Through normative usage in clinical practice (also maintained by commercial interests of proprietorship), the tests have become established as standard bearers. And their inherent problematic constructs have become internalized as cognitive/neuropsychological reality. They often represent poor correspondence with distinct neural systems. The perpetuation of latent constructs can be seen in the historical trajectories of many major neuropsychological and cognitive tests, often rooted in psychometry. For a particularly well-dissected example, see Boake (2002)—where the history of the Digit Span subtest of the Wechsler Adult Intelligence Scale is systematically traced to the latter half of the nineteenth century, marking some of the constructs inherited along its genealogical roots.

A case can be made that cognitive behavior occurs as distinct subsets of processes and, therefore, assessment tools that amalgamate sets of processes reflect clinical reality—however, that is not a point of dispute. The issue is that knowledge imparted by conventional cognitive assessment/psychometric tools does not serve sophisticated linkage of brain to behavior as called for in the current era. The format of these tests marks a dead end in terms of knowledge that can usefully

be integrated with contemporary neuroscience operating in the genomics and other omics-driven landscapes. Conventional tests do not represent cognitive processes as these processes are currently understood in the realm of neuroscience. Their construct definition and construct validity are lacking or have been rendered unsuitable by the standards of contemporary brain science.

> Latent phenotypes used in neuroscience are often ill-defined in the literature and reflect a combination of folk psychology and popular buzz-words, often lacking detailed construct validity. For instance, we previously found (Sabb et al. 2008) that usage of the term "cognitive control" in the literature grew exponentially over the last 10 years, even while it was being measured with the same cognitive tasks used to describe other cognitive concepts (such as working memory, task switching/set shifting, response inhibition, and response selection (Bilder et al. 2009a, b, p. 2).

Instead, what is called for is a systematic, operationalized account of behavioral phenotypes relevant to neurocognitive and neuropsychiatric domains. The goal is to achieve high-dimensional, high-definition behavioral phenotypic data—quantitative representations which are hypothesized to represent the complexity of a particular phenotypic domain. Ideally, the data need also be rendered in a format that enables automatic (informatics-driven) associations with other levels in the G-P matrix.

What such phenotypes may look like, the various forms and classes by which they may categorized, how they may be determined by different neural systems, and the different scales (levels of granularity) at which they could possibly be operationalized, and how are they to be validated are all questions ripe and open for exploration (and are questions that motivated this volume). Proposing any specific framework at this stage in the effort to try to constrain these questions may be premature. It is not clear that the forces driving this priority are fully appreciated in neuropsychology and psychiatry (yet another purpose served by this volume). The reframing of neuropsychological and neuropsychiatric functional domains, although necessary and inevitable, is still in its infancy. Researchers are bound to have divergent views of what the relevant phenotypes may look like, and through these divergent views, it may be possible to frame different scales by which the phenotypes are categorized. A taxonomy of meaningful cognitive phenotypes may be far off, but the movement toward this vision has begun.

As spelled out, the endophenotype concept if brought to bear too heavily on the NP concept may be more confounding than helpful. It has, though, been usefully applied in earlier discussions—where the prospect of cognitive endophenotypes was examined (see, e.g., Bearden and Freimer (2006)). And in that context, some suggestions about the form that cognitive endophenotypes may take marked great insights into what discrete, constituent cognitive operations may look like. Castellanos and Tannock (2002) considered various surface behaviors (behavioral phenotypes) in attention-deficit and hyperactivity disorder. For each of many manifest features such as delay aversion, time estimation, and phonemic awareness deficits, they traced sets of possible underlying cognitive operations: Shortened delay gradients may drive the surface feature of delay aversion and temporal processing deficits may underlie the surface manifestation deficits in time

estimation and phonemic awareness. And since each of these underlying operations was mapped to more than one neural system, different permutations of the neural mechanisms could explain the variability of ADHD manifestations across individuals. Yet again, this is but one of many possible forms of useful phenotypes; chapters in this volume have suggested various types.

Ontologies for Cognition: The goal of achieving refined and validated cognitive phenotypes requires standardization in terminology describing a concept and consistency across research groups in usage of the terminology. That is, cognitive phenotype discovery is predicated on the field using a standard, controlled vocabulary. Ontology refers to the specification of a concept, subject area, or body of knowledge—a formal characterization and specification of a knowledge domain through the use of a controlled vocabulary. It specifies a body of knowledge based on concepts and their relationships specific to that knowledge domain. The critical role of cognitive ontologies in phenomics has been detailed by Bilder et al. (2009b). An ontological system is crucial to the "curation" of a knowledge domain. Standardization of concepts, terminology, and constructs enables data aggregation that is amenable to knowledge discovery through data mining. Across biomedical disciplines, formal knowledge bases are often complemented by formal databases in which the knowledge is laid out in standardized fashion (see Sect. 15.3).

When one looks at functional domains in neuropsychology, there are stark differences in nosological structures across the different domains (see Jagaroo 2009 for expanded discussion). Language is one of the better and possibly easier structured functional domains: phonemes—morphemes—words—phrases—sentences, extending all the way to discourse structure. Assessment of speech and language is for the most part aligned with this structure. In the study of vision and spatial processing in neuroscience, the basic layout of the visual system—retino-geniculo-striate-extrastriate systems—has long been established, and considerable gains have been made in understanding temporal, posterior parietal, and prefrontal cortical parcellations involved with high-level visual and spatial processing. Yet, none of this has been translated into a concomitant neuropsychological battery for discrete visual and spatial processes across the hierarchy of the visual system. Primary visual perception is the exception—its assessment is systematized in optometry and ophthalmology. A neuropsychological battery for high-level vision, informed by the gains of visual neuroscience over the past three decades, is long overdue. The same argument can be made for processes of emotion and motivation.

Issues and Solutions: The initiative toward a new ontology/construct specification in cognition will inevitably face many hurdles, among them, the issue of consensus: What constitutes valid constructs and who decides the process of validation? Current efforts toward cognitive construct definition provide a view to workable models: Novel informatics tools that harness the power of collaborative community input have been developed for the purpose of "phenotype interrogation, annotation, evaluation, and selection" (Sabb et al. 2009). Such tools, e.g., PubAtlas (http://pubatlas.org), geared toward cognitive ontology/knowledge representation, and Phenowiki (http://phenowiki.org), aimed at quantitative validation of cognitive/behavioral tasks used in research, have been pioneered by the

Consortium for Neuropsychiatric Phenomics at UCLA. These tools that allow for collaborative examination of cognitive constructs and visualization of associated constructs serve the development and refinement of cognitive ontologies (for a more detailed account of such tools in neuroscience and neuropsychology, see Jagaroo 2009).

Among the more challenging issues are likely to be those concerning (a) the different levels at which cognitive-neural processes can be described and (b) the incorporation of multi-level neural systems. As an example of the former, the constituent operations commonly posited to constitute "executive functioning" include the processes of abstraction, planning, sequencing, working memory, set selection, set shifting, and response inhibition. Each of these processes can be further dissected (as illustrated by some of the chapters in this volume). In theory, there are no bounds to how fine the dissection can reach, and at some point in the future, it may be possible to compute the cognitive contribution of a single neuron in a circuit as related to these processes. At what point does fineness of resolution lose usefulness in terms of understanding brain–behavior associations? What about the issues of domain specificity and domain generality: Prefrontal cortical regions, for example, have been extensively mapped, functionally and histologically in the primate brain (Barbas and Pandya 1989; Ongur and Price 2000). However, this has not necessarily resolved some classic questions concerning the interpretation of its functional organization (see Goldman-Rakic 1996): Does each of the so-called executive functions (inhibition, working memory/set manipulation, etc.) correspond exclusively to the prefrontal architectonic divisions (domain specific)? or Do they correspond more broadly to the divisions, making them general purpose sequencing and control functions that affect all prefrontal informational domains (domain general)?

In neuropsychology and psychiatry, the dominant models of "neural systems" are arguably (a) interconnected cortical and subcortical functional loci and (b) synaptic neurotransmitter systems. Further, there is still a problematic, dominant cortical-centered view of cognition in neuropsychology. While the cortico-centric view shaped by many historical forces was somewhat ameliorated by the late twentieth century, the rise of functional neuroimaging (laying out an array of cortical nodes) shaped a new cortico-centric view. But cognitive processes are not simply the result of activation of a cortical node or network of nodes. Dynamic properties of neural systems, such as excitation, inhibition, tonic states, phasic activation, transition between phasic and tonic states, and adaptive gain, are significant variables in neural states directly involved with cognition and emotion. And complex subcortical systems, such as the cerebellum and the basal ganglia, are now considered just as central to cognition, emotion, and motivation as are cortical centers (see, e.g., De Smet et al. 2013; Soghomonian 2016). Highly integrative novel models grounded in rigorous neuroscience have been emerging in attempts to articulate the dynamic cortical–subcortical interactions as related to fundamentally cognitive functions. Consider two examples: (a) How phase response patterns of the locus coeruleus modulates cortical centers for attention—"[T]he timing of the LC phasic response suggests that it can be thought of as a temporal attentional

filter, much as cortical attentional mechanisms act as a content filter … by increasing gain of cortical representations, the LC phasic response may also enhance the effects of attentional selection …" (Aston-Jones and Cohen 2005). (b) How the basal ganglia may implement transition control in motor and perceptual behavior by using input representing the actual rates of change and comparing them to a representation of the desired rates: A difference in signal (transition error) is used to modulate brainstem control nuclei in order to achieve the correct behavior (Yin 2014, 2016). Such neural processes may provide good examples of cognitive phenotypes represented at a fine neural level—articulated in these examples at the single-cell/neuron population levels, cortico–subcortical networks, and in terms of dynamic neural mechanisms. They also include timescale variables—prefrontal cortical divisions may integrate information on different timescales (Aston-Jones and Cohen 2005)—which in theory could be a deeper level cognitive phenotype while not necessarily a cognitive endophenotype. Plausible cognitive phenotypes of this variety may be found in widespread systems across the brain and could well constitute a subtype of NPs.

Another potential class of NPs are those that are computationally derived. Computational NPs are numerous in possibilities and are just on the cusp of being explored. Quantitative NPs emerging as the result of the ever-advancing technological landscape of neuroscience has been acknowledged largely with reference to functional MRI (see Congdon et al. 2010). Functional MRI profiles corresponding to a particular disorder or its subtypes can be computed across many subjects (see Chap. 2). Other forms of computationally derived NPs hold huge potential as highly sensitive markers of dynamical brain states. A very clear example, one on EEG-derived NPs, has been given by Bosl in this volume (see Chap. 14). It is also an example of a NP that can manifest in the developing brain (at postnatal stages less than 24 months) while, again, not fitting the endophenotype definition along a G-P axis.

NPs represent the language by which brain-behavioral processes are described so as to be meaningful in a systems biology environment. In neuropsychology and psychiatry, this calls for a refinement of behavioral constructs so that they can be precisely associated with neural systems. The legacy of the endophenotype concept may have been rich and useful in providing the prompt for the turn to cognitive and neural markers. But neural systems, cognitive processes, and the nature of their association take multiple forms. NPs address all possible forms and also challenge us to specify brain-behavioral processes as discrete operations or dynamical neural states.

15.3 Neuroinformatics and a Data-Driven Knowledge Environment

The contemporary phenomenon of "big data" has been the subject of extensive discussion in biological and medical sciences (see Howe et al. 2008). Neuroscience has been no exception in facing big data and all the inherent

challenges. By 2005, scientific publications across the subdisciplines of biology and medicine had clustered more heavily around neuroscience than any of the other subdisciplines (see Rosvall and Bergstrom 2010). The endlessly burgeoning data volume can be summed up as large, diverse datasets, containing immense data complexity, generated by high-throughput profiling and analysis, and innovative informatics platforms (see Stein 2008). The data are organized into manageable dimensions through systematic annotation and aggregation. The curated data held in databases can then be fully exploited via bioinformatics methods. Bioinformatics plays a crucial role in all stages of the data environment—data acquisition, data representation, analysis, modeling, and discovery.

The first of the G-P strata to generate complex datasets via high-throughput methods was molecular biology/genetics, and this then prompted neuroscience in the direction of high-dimensional data via bioinformatics. The cognitive–behavioral end of neuroscience as related to neuropsychology and psychiatry has been relatively slow in adopting a data format that facilitates integration with the data stream in the underlying G-P strata. However, there have been a small and growing number of calls for integration of neuropsychology with this new data environment (Jagaroo 2009; Jagaroo et al. 2013). The rendering of neurophenotypes marks one of the strategies by which this can be achieved.

Knowledge Discovery: Big data in the scientific realm has also ushered in the era of "discovery science." The scale and complexity of data and the rate at which it is generated defy conventional forms of analysis. Omics data, for instance, can be so inordinate in volume that they cannot feasibly be grasped by traditional means where researchers read though published articles and mentally integrate ideas in the form of hypotheses and conclusions. Conventional statistical analyses are also unsuitable. Instead, bioinformatics takes center place. Beyond a mere supporting role in data processing and data logistics, bioinformatics, incorporating data mining and computational data integration, now plays a primary role in resolving complex research questions (see Butte 2009). This is predicated though on informatics-compatible data formats and platforms, that is, digitized data and databases, all making for data-driven frameworks.

When data mining yields valuable patterns and relationships among the data, the result is described as "knowledge discovery through data mining" (KDD). This form of research is in stark contrast to the canon of hypothesis-driven (hypothesis first) research: Discovery science is hypothesis generating. It starts with large datasets to which pattern-seeking algorithms are applied. The patterns generated could be completely novel and unexpected. Statistical modeling simulation tools may also be used toward the process of knowledge discovery via databases. This form of scientific discovery—a data-driven science, not reliant on hypotheses—has been described by various terms such as "data intensive discovery," "4th dimension research," or the "fourth paradigm." Skepticism that views data discovery and omics approaches as "fishing expeditions" need only be informed of the many recent examples in neuroscience where novel discoveries have been achieved via this approach (see, e.g., Mirnics et al. 2000).

G-P Integration Data Platforms: Integrative mapping within and across the G-P strata is no easy task, but bioinformatics is the enabling tool. It enables a multi-omics approach—to disentangle and integrate G-P data. G-P associations are refined with mathematical models that describe structural–functional relationships—the "interactome." Amount and quality of phenotype information in data repositories is crucial to successful data-driven discoveries made on such data. The scope of these databases, it must be emphasized, is not merely to serve as repositories for G-P data. The data actually enable G-P associations or even phenotype–phenotype associations. However, the data must first be formatted in a way that is compatible with the kinds of analysis required (see Webb et al. 2011). One key requisite factor in the success of genomics has been the systematic annotation of genes using a controlled vocabulary (Ashburner et al. 2000). This has enabled various kinds of informatics-driven associations in genomics. Similarly, the representation of NPs needs to follow a universal format, which will enable universal databases of NPs, which will then enable the filtering and extraction of meaningful NP relationships. Cognition and neural systems need to be uniformly phenotyped if they are going to be better understood in the context of a larger omics environment. Yet they still lack a standardized system for phenotype ontology and annotation. The general problem of how to annotate and integrate diverse biological data within the framework of bioinformatics has been given much more attention in other areas of the biological sciences where it is also met with numerous models (see, e.g., Jenkinson et al. 2008 for an integrative bioinformatics model for diverse phenotypes).

Knowledge discovery and informatics-driven G-P associations are made possible with data-driven frameworks that foster data integration or data sharing. Discovery science is often tuned to the discovery of biomarkers. The realization of the unique potential of data-driven science has led to a fundamental change in scientific practice: Data sharing has become normative. The pooling of scientific resources makes for greater scientific capital. This has taken the forms of shared databases, open source research tools, open source journals, and, quite tellingly, the requirement by major funding agencies such as the NIH that data and publications stemming from agency-funded work be open source (see Bourne 2010; Cheung et al. 2009; Kaye et al. 2009; Nielsen 2012 for in-depth discussion). Shared data repositories, discovery tools, and neuroinformatics platforms are now commonplace in the neuroscience community. The following are a few representative examples (see Jagaroo (2009) for example specific to neuropsychology).

The Virtual Brain (Ritter et al. 2013) is an open source neuroinformatics platform/computational framework for the virtual representation and simulation of the brain along different scales of anatomic density. It uses multiple scales of connectivity data subjected to methods of graph/network theory in order to understand brain function at multiple spatiotemporal scales.

Data sharing resources in the MRI community have been phenomena unto themselves. Major open source initiatives such as the *1000 Functional Connectomes Project* (FCP) (see Biswal et al. 2010; Craddock et al. 2015; Milham 2012) have proven the feasibility of the open neuroscience model—FCP pools

thousands of fMRI datasets and makes the resulting dataset freely accessible to the neuroscience community. Analysis of its large aggregated connectome dataset has led to discoveries about global patterns in the functional architecture (see Biswal et al. 2010). The *International Neuroimaging Data Sharing Initiative* (INDI) that oversees the FCP continues to enhance the data through initiatives aimed at the addition of comprehensive phenotypic data. Online repositories and computational platforms that facilitate connectome analysis "in the cloud" are powerfully emerging developments and are served by one-stop sources such as https://www.nitrc.org—offering various neuroinformatics tools and resources for neuroimaging data repositories and cloud computing. CPAC (*Configurable Pipeline for the Analysis of Connectomes*), one of the many tools it offers, provides a pipeline for automated preprocessing and analysis of large datasets, and algorithms for connectivity analysis. Some of these tools can also be interfaced with NIMH Data Repositories.

The *Open Connectome Project* (Vogelstein 2011) is another open repository of connectome data. It enables researchers to submit their data to a centralized data cluster while providing data management and analysis tools. It also accommodates data from the microscale (EM) to the macroscale (fMRI), gathered from various model organisms. Its key provision is scalable, parallel processing computer vision technology that can render large connectomic datasets as images.

Data sharing as the new currency for discovery in neuroscience has long been endorsed by major federal agencies such the US National Institutes of Health (see Insel et al. 2003). The emergence of NIH sponsored and coordinated data repositories and pipelines will hasten the shift to collaborative, discovery science: The National Database for Autism Research (NDAR), National Database for Clinical Trials Related to Mental Illness (NDCT), the Research Domain Criteria Database (RDoCdb), and the (ABCD) Adolescent Brain Cognitive Development Study offer de-identified (human subjects) data and use a common data standard. These repositories are accessible via a central portal, the NIHM Data Archive (NDA) (http://data-archive.nimh.nih.gov/). Research results based entirely on the analysis of repository (NDAR) data are already starting to appear (Hall 2014).

The behavioral neurosciences are seeing significant inroads in neuroinformatics and discovery science platforms. These approaches are dependent on common data standards and informatics protocols. They are also crucially dependent on standardized, unified, harmonized systems for data description. Since the data in question are cognitive and neural phenotypes data, the specification of cognition and neural systems as NPs is necessarily highlighted as a priority. NPs facilitate data units that can constitute the database fields, and with databases come the potential for data mining and associated discovery.

The imperative of neurophenotypes in the context of the new omics-driven biomedical environment has implications well beyond the theoretical–conceptual domain. It also implies that the training curricula of neuropsychology and psychiatry need to rapidly adapt or fall further out of step with the science of the brain. The conventions of teaching mastery of neurotransmitter and psychopharmacological mechanisms, DSM axes, diagnostic categories, neuropsychological batteries,

neurobehavioral clinical profiles, and so on, while still valuable, are rooted in very different eras. The cannons of research methods and statistics in the behavioral neurosciences, while still necessary, are no more sufficient. In their early call for a "human phenome project," Freimer and Sabatti (2003) stressed the importance of training "phenomicists" for the mission of phenomics, and Jagaroo (2009) suggested that neuroinformatics be incorporated in the training of neuropsychologists. In the omics era, all neuropsychologists, psychiatrists, and psychiatric epidemiologists should have a phenomics component added to their training. This is a crucially needed adjustment if the disciplines are to be aligned with brain-behavioral sciences in the omics era.

References

Ashburner M, Ball CA, Blake JA et al (2000) Gene ontology: tool for the unification of biology. The gene ontology consortium. Nat Genet 25(1):25–29

Aston-Jones G, Cohen JD (2005) An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci 28:403–450

Barbas H, Pandya DN (1989) Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. J Comp Neurol 286(3):353–375

Bearden CE, Freimer NB (2006) Endophenotypes for psychiatric disorders: ready for primetime? Trends Genet 22(6):306–313

Bilder RM, Poldrack RA, Stott PD et al (2009a) Cognitive phenomics. The neuropsychology of mental illness. Cambridge University Press, Cambridge, pp 271–284

Bilder RM, Sabb FW, Parker DS et al (2009b) Cognitive ontologies for neuropsychiatric phenomics research. Cogn Neuropsychiatry 14(4–5):419–450

Biswal BB, Mennes M, Zuo XN et al (2010) Toward discovery science of human brain function. Proc Natl Acad Sci USA 107(10):4734–4739

Bloss CS, Schiabor KM, Schork NJ (2010) Human behavioral informatics in genetic studies of neuropsychiatric disease: multivariate profile-based analysis. Brain Res Bull 83(3–4):177–188

Boake C (2002) From the Binet-Simon to the Wechsler-Bellevue: tracing the history of intelligence testing. J Clin Exp Neuropsychol 24(3):383–405

Bourne PE (2010) What do I want from the publisher of the future? PLoS Comput Biol 6(5):e1000787

Butte AJ (2009) Translational bioinformatics applications in genome medicine. Genome Med 1(6):64

Castellanos FX, Tannock R (2002) Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. Nat Rev Neurosci 3(8):617–628

Cheung KH, Lim E, Samwald M et al (2009) Approaches to neuroscience data integration. Brief Bioinform 10(4):345–353

Congdon E, Poldrack RA, Freimer NB (2010) Neurocognitive phenotypes and genetic dissection of disorders of brain and behavior. Neuron 68(2):218–230

Craddock RC, Tungaraza RL, Milham MP (2015) Connectomics and new approaches for analyzing human brain functional connectivity. Gigascience 4:13

De Smet HJ, Paquier P, Verhoeven J et al (2013) The cerebellum: its role in language and related cognitive and affective functions. Brain Lang 127(3):334–342

Flint J, Munafo MR (2007) The endophenotype concept in psychiatric genetics. Psychol Med 37(2):163–180

Freimer N, Sabatti C (2003) The human phenome project. Nat Genet 34(1):15–21

Glahn DC, Thompson PM, Blangero J (2007) Neuroimaging endophenotypes: strategies for finding genes influencing brain structure and function. Hum Brain Mapp 28(6):488–501
Goldman-Rakic PS (1996) The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. Philos Trans R Soc Lond B Biol Sci 351(1346):1445–1453
Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160(4):636–645
Hall D (2014) Big data opportunities using NIH/NIMH data repositories. https://www.hhs.gov/sites/default/files/advcomcfs/meetings/presentatons/cfs-workshop-danhall-june-2014.pdf
Howe D, Costanzo M, Fey P et al (2008) Big data: the future of biocuration. Nature 455(7209):47–50
Insel TR, Volkow ND, Li TK et al (2003) Neuroscience networks: data-sharing in an information age. PLoS Biol 1(1):E17
Jagaroo V (2009) Neuroinformatics for neuropsychology. Springer, New York
Jagaroo V, Berman MO (2005) Neuroinformatics: how it will reinnervate the field of neuropsychology. Paper presented at the annual meeting of the international neuropsychological society, St. Louis, Missouri
Jagaroo V, Bosl W, Blider R (2013) Neuroinformatics: the shaping of neuropsychology for the 21st century. Paper presented at the symposium at the annual meeting of the international neuropsychological society, Hawaii, February 2013
Jenkinson AM, Albrecht M, Birney E et al (2008) Integrating biological data–the distributed annotation system. BMC Bioinform 9(Suppl 8):S3
John B, Lewis KR (1966) Chromosome variability and geographic distribution in insects. Science 152(3723):711–721
Kaye J, Heeney C, Hawkins N et al (2009) Data sharing in genomics–re-shaping scientific practice. Nat Rev Genet 10(5):331–335
Milham MP (2012) Open neuroscience solutions for the connectome-wide association era. Neuron 73(2):214–218
Mirnics K, Middleton FA, Marquez A et al (2000) Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. Neuron 28(1):53–67
Nielsen MA (2012) Reinventing discovery: the new era of networked science. Princeton University Press, Princeton, NJ
Ongur D, Price JL (2000) The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cereb Cortex 10(3):206–219
Ritsner MS, Strous RD (2010) Neurocognitive deficits in schizophrenia are associated with alterations in blood levels of neurosteroids: a multiple regression analysis of findings from a double-blind, randomized, placebo-controlled, crossover trial with DHEA. J Psychiatr Res 44(2):75–80
Ritter P, Schirner M, McIntosh AR et al (2013) The virtual brain integrates computational modeling and multimodal neuroimaging. Brain Connect 3(2):121–145
Rosvall M, Bergstrom CT (2010) Mapping change in large networks. PLoS ONE 5(1):e8694
Sabb FW, Bearden CE, Glahn DC et al (2008) A collaborative knowledge base for cognitive phenomics. Mol Psychiatry 13(4):350–360
Sabb FW, Burggren AC, Higier RG et al (2009) Challenges in phenotype definition in the whole-genome era: multivariate models of memory and intelligence. Neuroscience 164(1):88–107
Schork NJ (1997) Genetics of complex disease: approaches, problems, and solutions. Am J Respir Crit Care Med 156(4 Pt 2):S103–S109
Shields J, Gottesman II (1972) Cross-national diagnosis of schizophrenia in twins. The heritability and specificity of schizophrenia. Arch Gen Psychiatry 27(6):725–730
Soghomonian J-J (2016) The basal ganglia—novel perspectives on motor and cognitive functions. Innovations in cognitive neuroscience, 1st edn. Springer International Publishing, Switzerland
Stein LD (2008) Towards a cyberinfrastructure for the biological sciences: progress, visions and challenges. Nat Rev Genet 9(9):678–688

Vogelstein JT (2011) Q&A: what is the open connectome project? Neural Syst Circuits 1(1):16
Webb AJ, Thorisson GA, Brookes AJ et al (2011) An informatics project and online "Knowledge Centre" supporting modern genotype-to-phenotype research. Hum Mutat 32(5):543–550
Yin HH (2014) Action, time and the basal ganglia. Philos Trans R Soc Lond B Biol Sci 369(1637):20120473
Yin HH (2016) The basal ganglia and hierarchical control in voluntary behavior. In: Soghomonian J-J (ed) The basal ganglia—novel perspectives on motor and cognitive functions. Springer International Publishing, Switzerland

Index

Note: **Page numbers followed by *f* and *t* indicate figures and tables respectively**

V. Jagaroo and S.L. Santangelo (eds.), *Neurophenotypes*,
Innovations in Cognitive Neuroscience, DOI 10.1007/978-1-4614-3846-5