Imaging in CNS Drug Discovery and Development

Implications for Disease and Therapy

David Borsook
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Editors

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Preface

The last decade has seen a loss of confidence in the big pharma model for the development of new drugs. Despite unprecedented development costs, only about 10% of molecules entering Phase I were registered as drugs between 1991 and 2000 (Kola and Landis 2004). More concerning for the industry is that a significant proportion of the molecules failed in late phase development, after major investments already had been made. Two problems dominated these late stage failures: lack of efficacy and unanticipated safety risks.

Several reasons for this high attrition are suggested by the observation that critical issues related to efficacy often were not answered early in development. For example: Does the molecule reach its target? Is there evidence for the desired pharmacological effect in vivo? What is the dose-response relationship? In cases in which some of this information was established in preclinical models, the models did not necessarily reflect the critical biology in humans or for the human disease. Drug development needs to incorporate approaches for more direct in vivo pharmacology in humans.

In addition, because so much of the safety evaluation either relies on short term outcomes in humans or preclinical studies using high compound concentrations, more slowly developing pathologies or pathologies idiosyncratic to humans or particular populations can escape detection until large numbers of patients are treated for long periods in Phase III studies. Better ways of bridging between preclinical toxicology and clinical toxicology studies are needed. More sensitive measures for toxicology are desired in clinical studies. Safety assessment also can benefit from in vivo physiological measures in humans.

The primary limitations of conventional clinical development for many current major disease targets (e.g., in CNS, metabolic and cardiovascular indications) relate to requirements for long periods of evaluation and the modest sensitivity of usual, clinically based measures of outcome. While these clinical measures of outcome may have ecological validity in terms of ultimate clinical impact, they typically are only indirectly related to pharmacology and rarely address toxicology in particular. A compelling new approach to addressing this challenge is the aggressive application early in development of experimental medicine approaches designed to test specific pharmacological or toxicological hypotheses. Using biochemical, structural, or physiological measures that report on changes reflecting distribution or

direct consequences of drug action, the kinds of critical questions posed above can begin to be answered translationally in a coordinated strategy extending from preclinical to clinical studies. The translational element involves initial qualification of biomarkers in preclinical experiments, where they can be related directly to a broad range of well-accepted outcomes. When combined with patient populations in which the disease mechanisms are well characterized, the interaction between pharmacology and disease mechanisms can be elucidated more powerfully in shorter studies with more precisely defined and sensitive measures of response.

Such short term biomarker measures of drug distribution of pharmacological response may or may not be predictive of ultimate clinical response for any indication. However, they constitute direct tests of the fundamental hypotheses that are driving development of a molecule. Strict criteria for progression can be defined, making proof of pharmacology a critical part of a decision to progress development from early stages.

While some may argue that there are many examples of useful drugs with activity in disease that was not well predicted by the initial pharmacological hypothesis, set against this is the sad prior (for a rigorous, Bayesian view of drug development): most molecules will fail to make suitable drugs. The prior probability of not developing a potentially important therapeutic molecule because of failure at an early, direct test of pharmacology is therefore low.

*Imaging in **CNS** Drug Discovery and Development* provides a primer to the emerging potential of imaging as a general biomarker particularly for CNS drug development. The Editors have gathered together an internationally respected group of experts. Both academic and industry leaders are included. Together, they have produced a unique volume introducing the major tools, approaches, and challenges.

Important themes of integration run through the book. The selection of chapter topics emphasizes the need to integrate clinical and preclinical investigations of pharmacology. Preclinical investigations provide a fundamentally important way of relating imaging measures directly to conventional pharmacological and neurobiological response indices. It is not just through biomarker qualification that preclinical imaging provides an important tool to drive more effective clinical investigations. Preclinical studies also provide an opportunity to more completely define response relations and to push the range of such studies over a broader range, providing hypotheses that can later be explored in human toxicologically focused investigations. Preclinical imaging also allows the similar measures used for candidate selection to be applied to the initial proof of pharmacology in humans. At the same time, applications of imaging to preclinical investigations address the three R's of *reduction*, *refinement*, and, by extension to the clinical studies, an emphasis on *replacement* of use of animals by human experimental medicine in drug development.

A second theme addressed very directly in the concluding section of the book is the importance of integration of imaging and other biomarker information to provide multivariate measures of response. The neurobiology of disease and related neuropharmacology are complex. There is increasing evidence that multivariate

approaches provide new ways of enhancing precision of outcome measures and sensitivity. Computational power now should not be limiting. It is imperative that we use the full range of data available more effectively.

Applications of imaging to drug development have been growing rapidly in number over the last few years. In this exciting environment, it would not be possible to create a volume that remains fully current with the state-of-the-art. The Editors therefore have included chapters from experts providing paradigmatic examples that establish a "blueprint" for a way forward. Key therapeutic areas that illustrate the major problems have been identified. The use of functional imaging-based measures to objectify subjective experience is described in the chapter on pain, illustrating how sensitivity to the range of responses to a complex illness can be captured powerfully by imaging. The description of initial studies with post-traumatic stress disorder highlights the role of imaging in diseases of mind. Examples also are chosen from disorders in which there is a more complete understanding of disease neurophysiology, such as addiction and anxiety, illustrating how knowledge of the underlying cognitive systems can be coupled with imaging to drive stronger pharmacological hypotheses. Finally, the discussion of plasticity highlights one of the most important characteristics of noninvasive imaging approaches: the potential to follow the dynamics of change over time.

Recent commentators have looked to major changes in industry structure as a solution to the problems of innovation and high attrition in pharma. *Imaging in **CNS** Drug Discovery and Development* is part of a fundamentally optimistic alternative future scenario: the idea that drug development can be made better by becoming smarter. Implicitly, the Editors make a strong case that, using a science-based strategy, the paradigm for drug development can be improved. All of us must hope that this promising path forward will have a substantial impact on getting better medicines to the right patients more quickly. This volume contributes substantially to accelerating this grand experiment.

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Reference

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About the Editors

David Borsook, MD, PhD, trained in medicine and neurobiology at the University of the Witwatersrand, Medical School, Johannesburg, South Africa. He graduated in 1980. Following his internship, he trained in Neurology at Boston City Hospital and then was the first Pain Fellow at the Massachusetts General Hospital, Department of Neurology. He subsequently was the Director of the Pain Center at the Hospital from 1994 to 2004. He has completed doctoral studies in Neurobiology and later started the Pain Imaging Program in the Department of Radiology at Massachusetts General Hospital. In 2002, he led an effort to cofound a Biotech – Descartes Therapeutics Inc., with his colleague Lino Becerra PhD to use imaging in drug development, where he was Senior Vice President and Chief Scientific Officer. He currently directs an integrated imaging program – Pain & Analgesia Imaging Neuroscience (P.A.I.N.) Group at three Harvard Medical School Affiliated Hospitals, Massachusetts General Hospital, McLean Hospital and Children's Hospital Boston. A component of this is a consortium of pharmaceutical and academic centers involved in the evaluation of fMRI in drug development known as ICD (Imaging Consortium for Drug Development). He has participated in a number of NIH meetings on future directions of pain research. His research is supported by grants from the National Institutes of Health, Foundations and Pharmaceutical Companies interested in the use of imaging in defining pain phenotype. He has published over 85 papers that include various aspects of pain, imaging in pain, and analgesia.

Dr. Lino Becerra, PhD, is Lecturer in Psychiatry at Harvard Medical School, he has co-appointments in the Departments of Psychiatry at McLean Hospital and Massachusetts General Hospital (MGH), and Radiology at MGH. He is the Director of the Imaging and Analysis Group at the Brain Imaging Center, McLean Hospital; and Co-Director of the Imaging Consortium for Drug Development (ICD) and the Pain Imaging and Analgesics Neuroscience Group (P.A.I.N. Group) at the same institution. Dr. Becerra was a cofounder of Descartes Therapeutics Inc., a biotech company dedicated to the development of drugs for chronic pain patients. His research interests are focused on the optimization of functional imaging for its utilization in drug development, in particular for chronic pain. Translational aspects of drug development through the study of preclinical and clinical early phase trials with the aid of

neuroimaging have been his main interest. Dr. Becerra is the author of over 50 publications, reviews, and book chapters appearing in journals such as *Neuron*, *Neuroscience*, *Journal of Neuroscience*, *Journal of Neurophysiology*, *NeuroImage*, and *European Journal of Pain*. He is a reviewer for these Journals, as well as for *Biological Psychiatry* and *Archives of General Psychiatry*.

Edward Bullmore, MD, PhD, trained in medicine at Oxford and St Bartholomew's Hospital, London, graduated in 1985. Following a period of further medical training as a Lecturer in Medicine at the University of Hong Kong (MRCP 1989), he started specialist training in psychiatry at St George's Hospital, London, and then at the Bethlem Royal & Maudsley Hospital as a registrar from 1990 (MRCPsych 1992). From 1993, he was supported by the Wellcome Trust as a Research Training Fellow (then as an Advanced Research Training Fellow 1996–1999) at the Institute of Psychiatry in London, where he completed doctoral studies on statistical analysis of magnetic resonance imaging data (PhD 1997). In 1999, he moved to the University of Cambridge as a Professor of Psychiatry and since 2005 he has been Clinical Director of the Behavioural & Clinical Neurosciences Institute at Cambridge. Also since 2005, he has combined his academic roles with a 50% secondment to GlaxoSmithKline as Vice-President for Experimental Medicine and head of GSK's Clinical Unit in Cambridge (CUC). His research in Cambridge has been supported by grants from the National Institutes of Health (Human Brain Project), the Wellcome Trust and the MRC; he has published more than 200 papers on various aspects of neuroimaging, neuroscience, and psychiatry. In 2008, he was elected a Fellow of the Academy of Medical Sciences.

Richard Hargreaves, PhD, trained at Chelsea College, London University in the UK where he obtained a First class honors degree in pharmacology. After completing his doctorate through the Physiology Department at King's College London University UK, he joined Merck's Neuroscience Research Center in Harlow UK in 1988 where he occupied positions of increasing seniority. Richard led the discovery biology teams that contributed to the development of MAXALT® (rizatriptan) for the treatment of migraine and EMEND® (aprepitant) and IVEMEND® (fosaprepitant), novel agents that advance the protective pharmacotherapy of acute and delayed chemotherapy-induced nausea and vomiting and postoperative nausea and vomiting. In 1999, Richard moved to the USA to establish and lead a worldwide imaging research strategy for Merck Research Laboratories. Since that time, he built a Global Multimodality Imaging Group that supports decision making in drug discovery and development across Merck's key therapeutic areas. A key component of this imaging strategy has been the use of precompetitive initiatives to combine expertise and share the costs of developing and characterizing new imaging tools and technologies that can be used to improve the evaluation of the safety and efficacy of novel drug candidates. Richard was awarded the 2007 Gary Neill Award for "Innovation in Drug Development" by the American Society of Clinical Pharmacology and Therapeutics (ASCPT) for his work on imaging in drug discovery and development. In February 2008, he was named Worldwide Head of Basic Research, Neuroscience for Merck Research Laboratories.

Part I
Background

The Challenges and Opportunities

David Borsook, Edward Bullmore, Lino Becerra, and Richard Hargreaves

Introduction

The global burden of neurological disease is high (Macdonald et al. 2000) and is expected to continue to increase dramatically in the future, given the increase in the elderly population. This medical need is reflected in the intense research and development activity; in the USA alone, there may be as many as 300 neuroscience drugs in development. Between 2003 and 2005, the global market for CNS therapies grew by nearly 20% (~10% of the total pharmaceutical sales) (Market Trends 2007; Palmer and Stephenson 2005) and is predicted to expand to nearly $64 billion by 2010. In addition, new insights into the complex interplay between peripheral and central mechanisms involved in metabolic diseases (Elmquist and Flier 2004; Theander-Carrillo et al. 2006; Obici et al. 2002) have revealed new CNS therapeutic targets for drug development. Drug development is an expensive venture with the average costs of developing a new chemical entity exceeding $800 million. Therefore, with such a large number of opportunities in the CNS field, there is a pressing need to find ways to improve the speed and reduce the cost of decision making, so that only the best molecules and hypotheses are taken into consideration in the later stages of drug development. It is important to identify likely "losers" early and make clear 'no go' decisions, and to identify likely winners quickly and enable them to increase their probability of success in development. New CNS imaging technologies have become a focus of attention as they provide fast, efficient and objective ways to evaluate the direct wanted and unwanted effects of centrally acting drug candidates on the brain. Indeed, imaging is now an integral part of most conferences on CNS drug development, and the pharmaceutical industry has invested in internal and external imaging programs to support CNS drug discovery (for current reviews see (Borsook et al. 2002; Borsook et al. 2006; Wise and Tracey 2006; Matthews and Honey GD Bullmore 2006)).

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Failure: A Driving Force for Improved Approaches

In 2006, the number of drugs discontinued for peripheral and CNS disease (including the treatment of a range of neurological disorders, including chronic pain, Alzheimer's disease, Parkinson's disease, multiple sclerosis, epilepsy and anxiety) is significant (>50%) (Collins 2007). Unlike many other therapeutic areas, there is a paucity of scientifically validated and clinically qualified biomarkers predictive of central activity, and this contributes to the low probability of success for novel CNS drug discovery and development programs directed against unprecedented targets. The prolonged timelines, the expense, and the uncertainty of decision making in CNS therapeutic evaluation also raises the distinct possibility that drugs are "put on the shelf" with missed opportunities because the initial indications explored were misdirected. Clearly, new indications could be rationally explored if functional insights into the effects of a drug on CNS circuits could be objectively assayed.

New Approaches and Indications

Many CNS drugs have been found serendipitously from astute clinical observations in humans that have spawned drug development programs. A more rational approach is to use neuroimaging techniques to define the disease condition in humans first. In addition, while there is a focus on developing drugs with specific MOA, the hypothesis driven mechanism of action (MOA) approach to drug development may have limited utility as many successful CNS therapeutics have no clear primary MOA, and others may provide benefit through actions at multiple sites. In such cases, looking at the functional CNS fingerprints of active drugs and using them, much like RNA expression profiling, to identify novel therapeutics is a rational and mechanistically unconstrained approach. Clearly, new chemical entities have specific effects on a disease process. In addition, by assaying the direct effects of a drug on CNS function through the mapping and interpretation of the specific circuits affected, it may be possible to evaluate new indications for specific drugs.

Integration of Processes in CNS Drug Development

The key theme for this book is whether and how neuroimaging could improve the success rates in CNS drug development. The neuroimaging approaches considered in the chapters that follow cover four main areas: (1) *Functional Imaging*; (2) *Anatomical Imaging;* (3) *Molecular Imaging*; and (4) *Chemical Imaging.* (Beckmann et al. 2001; Rudin et al. 2003; Silva and Chandra 2006; Beckmann et al. 2007). Obviously, it is appreciated that neuroimaging is not a stand-alone answer to all the challenges of CNS drug discovery; it needs to be carefully integrated with traditional

and emerging biomarker driven decision making processes (Gomez-Mancilla et al. 2005). The key challenges to overcome have some commonality with other therapeutic areas, and some are unique to neuroscience:

1. *Discovery and Development Hurdles*: Drug development for CNS disorders faces issues similar to those that are encountered by other therapeutic areas: increasing development costs; development of novel drug targets with unproven therapeutic potential; and health care systems and regulatory agencies demanding more compelling demonstrations of the value of new drug products.
2. *Clinical Testing*: Clinical testing remains the core area for the registration of any new drug. Traditional clinical trial methods are expensive and difficult, and they frequently fail. Many CNS disorders are chronic, slow processes manifested by highly subjective and context dependent signs and symptoms are late onset (exceptions degenerative disorders) with ill-defined or undefined pathophysiology. Thus, patient populations selected for treatment trials using clinical criteria are inevitably heterogeneous, and dependence on traditional endpoints results in early proof-of-concept trials being long and large, with very poor signal to noise.
3. *Integration of New Technologies*: With the relative failure of preclinical models, more focus is being placed on accessing information from human "material", including human surrogate models, genetics, proteomics tissue samples and imaging. Biomarkers are being targeted as part of the decision-making process as a means of rationalizing CNS drug development and reducing the cost of failure.

Chasing the Ideal: Can Neuroimaging Help?

Figure 1 summarizes the questions that could potentially be addressed with the help of imaging datasets. It is important to note, however, that for many approaches, there is still some way to go before their potential benefits and limitations in the context of drug discovery decision making are determined. Failure in drug development has been suggested (Hurko and Ryan 2005) to result from making poor choices in several crucial areas: recognition of differences between animal assays and human disease, selection of doses sufficient to test clinical hypotheses, selection of objective surrogate models to obtain proof of biology, specific measures of disease, selection of subjects for proof of clinical concept testing, and sensitive and early detection of therapeutic response. Developing imaging strategies that may help evaluations in these domains may impact the speed and cost effectiveness of CNS drug development significantly.

Animal–Human Translation

Non-invasive functional imaging allows specific insights into drug and disease phenotype in humans that can be used to select and align preclinical CNS models and

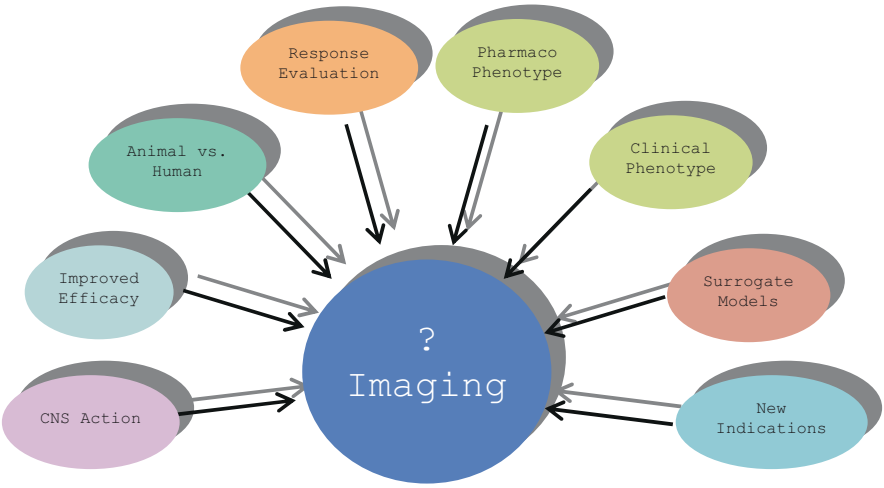

Fig. 1 Profiling disease state and drug effects: objective measures

characterize effective and ineffective drug therapies, thereby improving early decision-making on novel candidates. Insights garnered during preclinical development can sometimes be "lost in translation", and drug candidates that look excellent in the laboratory, fail in early phase clinical trials. Improvements in our ability to translate from animal to human or vice versa will advance traditional approaches where animal models are often developed with no clear path to evaluating their equivalence in the human condition. Such a notion of course implies that the "language of translation" is the pattern of activations across the neuro-circuitry of the brain.

CNS Target Engagement and Dosing

Direct measurement of CNS target engagement by drug candidates is critical for CNS drug discovery and development. PET molecular imaging can confirm that drugs reach their target in sufficient amounts at safe and well tolerated doses to make clinical studies worthwhile and to reject hypotheses with certainty if data are negative. It is important to remember, however, that occupancy is not efficacy; knowing target engagement allows rational dose selection for clinical proof of concept testing trials. PET radioligand binding studies do not, however, provide any insight into the functional aspects of a particular drug. Functional imaging has advanced from PET based tracer studies using [18F] FDG glucose metabolism and 15O – water cerebral flow to using a range of functional MRI techniques (rsfMRI, BOLD, ASL) with different experimental designs. These fMRI techniques can provide data on dose-responsiveness by studying increasing activation (or deactivation) in specific brain regions of interest, particularly those hypothesized to be involved

in the desired therapeutic action (for example, activation in the periaqueductal gray in pain, or the hippocampus or amygdala in anxiety).

Human Surrogate Models

Activation in specific brain circuits defines the behavioral consequences of a drug effect or disease process. Patterns of neuro-activation and their changes are, therefore, potential markers of disease state or drug efficacy. An objective evaluation of the CNS processing involved in disease states in humans will allow for a top down approach to the evaluation of drug effects on disease state for most functional CNS diseases. Thus, functional neuroimaging provides an objective readout of CNS activity (neuroinformatics) that can inform neurobehavioral studies of CNS disorders and provide a novel framework to evaluate therapeutic hypotheses rapidly.

The use of healthy human subjects for the evaluation of a drug may provide information about safety and tolerability, but may not provide helpful information about efficacy for a particular condition. Having appropriate healthy surrogate models or markers for the evaluation of a drug for efficacy would, of course, be extremely helpful. In the early stages of CNS drug development, the delivery of potential therapeutics and their actions are often studied in healthy individuals with normal neurocircuitry. Whilst this can have great value in ensuring target engagement and proof of biochemical mechanism, these studies usually create baselines for the study of disease states and their therapeutic modulation – the holy grail of targeted CNS therapies. In some cases, it is difficult to recapitulate key aspects of psychiatric or neurological disease in healthy individuals, but their use is a rapid step toward the development of paradigms for patients.

Clinical Phenotype

Objective indices of clinical phenotypes (anatomical, functional or chemical) would be highly useful in clinical trials, as well as in standard clinical practice for disease evaluation. Many CNS diseases evolve slowly, and their clinical manifestations may therefore, post-date changes that may have been taking place slowly over months or years. An ability to evaluate a CNS brain state using imaging may open novel prediction and prevention approaches in drug development. In a number of fields of CNS disease, there appears to be some reason to believe that imaging markers of a clinical phenotype could have real utility. Today, anatomical imaging is perhaps the most advanced in terms of brain measures in disease states such as multiple sclerosis and Azheimer's disease (see Alzheimer's Disease Neuroimaging Initiative, http://www.loni.ucla.edu/ADNI/). Interestingly too, it has recently been shown that chronic pain may affect brain function and structure (gray matter loss) and here, neuro-imaging may provide novel markers for the development of therapies

modifying new disease rather than symptomatic therapies. Using neuro-imaging to provide a clinical phenotype and monitor its progression will enable the selection of the most appropriate patients for clinical trials, especially in CNS neurodegenerative disorders, and will hopefully lead to smaller cohorts being needed to power pivotal long-term outcome studies.

Pharmaco-Phenotype

Some drugs may have the best efficacy in patients with a particular genetic constitution. Targeting drugs to subpopulations (enriched in terms of clinical phenotype or genotype) may show enhanced benefit-risk. A "pharmaco-phenotype" defined by neuroimaging may assist in the enrichment of trials or help with individualizing therapy in groups of subjects when used as a means of differential diagnosis.

Challenges in Adopting Neuroimaging Technologies

The adoption of imaging technologies requires evidence based data. While imaging holds the promise of accelerating and improving success rates in CNS Drug Development, it is clear that there is still much work to be done to define the utility, reproducibility, and harmonization of imaging protocols, data capture and analysis that are so critical to its use in decision making, particularly in longer term studies that use it to monitor progression of neurological disease. Nevertheless, we have seen revolutionary progress in the scientific validation and clinical qualification of many new neuro-imaging approaches. This book is an effort to understand the current and potential use of imaging technologies in drug development, supporting the path of bringing safe and effective medicines to patients and providing them better quality of life.

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Imaging of CNS Systems: Importance for Drug Development

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Introduction

During the twentieth century, society has experienced enormous benefits from advances in health care with a dramatic prolongation of life expectancy and life quality. While these improvements can be attributed in part to public health measures such as clean water, reduced smoking, preventive medicine, vaccination, and a reduction in the spread of infectious disease in the first half of the century, a dramatic increase in the availability of novel, efficacious and safe drugs has played a cardinal role in the latter half of the century. During this time, the pharmaceutical industry, along with academic, government-sponsored research, has materialized chemical and biological innovation contributing to all aspects of disease management, including diagnosis, prognosis and therapy. However, the pace of such medical innovation, as judged by successful approvals of new drugs, has significantly declined over the past two decades despite exponential increases of investments in research and development (Feuerstein et al. 2008; Pangalos et al. 2007).

One factor contributing to this inverse relationship between escalating drug development costs and successful new drug approvals can be attributed to the continued decreasing probability of successful transition through critical proof of concept studies in early clinical development (Feuerstein, 2007). Another contributing trend over the past decade has been the greatly increased public and political scrutiny with regard to adverse events for both marketed products and investigational drugs, resulting in an increased risk aversion by regulatory agencies around the world and an increasing demand for larger and longer late stage clinical trials. Finally, as generic drugs have become increasingly available, new investigational therapies face tougher developmental hurdles, and a greater need to demonstrate clear superiority or differentiation with regard to safety or efficacy compared to existing therapies.

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In this increasingly challenging environment, the key objective of translational medicine is to help improve the success rates of investigational drugs in clinical development. In order to accomplish this goal, translational medicine employs biomarkers to aid in the understanding of (1) the relevance of the drug target to human disease (2) the drug interaction with the target (3) the consequences of target modulation by the drug (pharmacodynamics) in respect to efficacy and safety (4) patient selection for the best medical outcome and (5) new disease biomarkers. The use of such evidence-based biomarkers can increase confidence during early development, improve the ability to prioritize clinical drug candidates across a broad portfolio and yield better and more cost effective decision making for the advancement of compounds through the development process. For convenience and to achieve a uniform lexicon for the wide array of potential biomarkers, we group biomarkers into the following categories (see Fig. 1):

1. Target Validation Biomarkers provide scientific evidence on the role of the target in human diseases and its potential to be exploited in drug discovery and development campaigns.
2. Target-Compound Interaction Biomarkers provide evidence on the physico–chemical interaction of the drug with its intended target.
3. Pharmacodynamic (PD) Biomarkers report on the biological consequences of drug action in the exposed organism or patient. These include biomarkers of efficacy and safety.

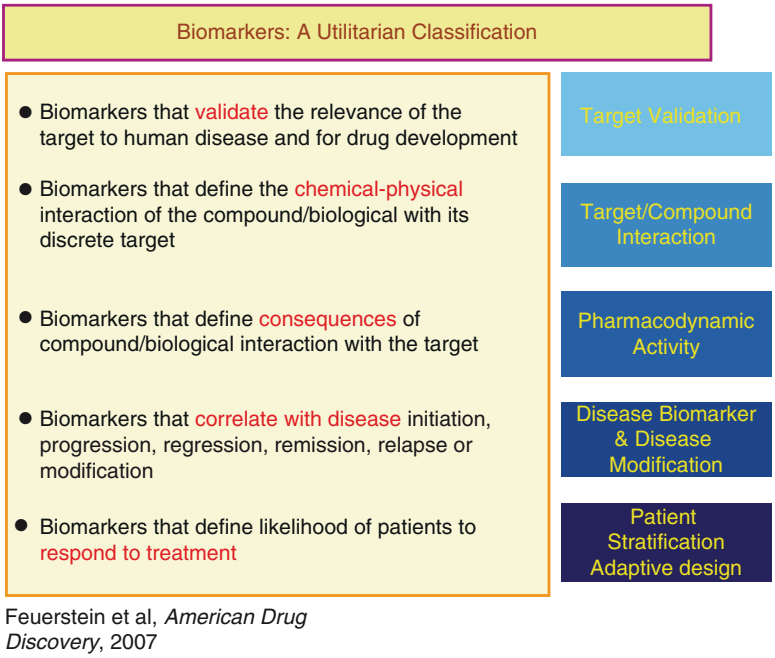

Fig. 1 Classification of biomarkers according to utility

4. Disease biomarkers report on elements of disease progression, regression, severity etc and provide guidance on whether a drug has the potential to fundamentally alter or modify the disease process.
5. Patient Selection, Stratification Biomarkers provide information about those patients most likely to respond (or not respond) to the treatment. Such biomarkers provide an opportunity to stratify patients for risk of disease progression and potentially enable shorter trials with higher event rates and earlier outcome assessments.

In this context, the use of imaging in drug development for CNS disorders is of particular importance, given the relative inaccessibility of the brain to a direct sampling of cells, tissues, or fluids *in vivo*. Imaging techniques offer non-invasive approaches to assess systematically both the structural and functional integrity of the CNS and can be applied to all of the biomarker categories previously defined. Furthermore, imaging techniques established in humans are now feasible in many of the key animal models that serve drug discovery and development (rodents and nonhuman primates), allowing a closer alignment of imaging biomarkers across species and an improved congruency between the laboratory and clinical settings. Some relative advantages and limitations are summarized in Table 1.

Table 1 Comparison of imaging technologies commonly used in humans for studies of drug effects on the central nervous system

Technique	Advantages	Limitations
Computed tomography (CT)	Readily available Noninvasive Can assess vasculature	Only provides structural Information Radiation exposure limits
Magnetic resonance imaging (MRI)	Multiple applications Structural imaging T1, T2, FLAIR, DWI, PWI Diffusion tensor imaging Functional imaging (regional blood flow) Metabolite measurement Cerebrovascular assessment Noninvasive Good spatial resolution	Cannot be used for ligand binding Temporal resolution limited to ~ 7 s for fMRI Sensitive to motion artifacts Some subjects cannot tolerate confinement in magnet Cost and bed-side limitation
Positron Emission Tomography (PET)	Can be used for Blood flow Metabolism Ligand binding	Requires administration of radioactivity Requires access or proximity to cyclotron, radiochemistry lab Limited temporal resolution Chemistry limitation in ligand preparation
Single Photon Emission Computed Tomography	Can be used for Blood flow Ligand binding More widely available than PET	Only semi-quantitative Limited spatial and temporal resolution Chemistry limitation in ligand preparation

Note: EEG and MEG are not considered here. Combinations of the above techniques are often used (e.g., PET and MRI, PET and CT) to take advantage of complementary features.

In the following sections, we will describe examples of imaging-based biomarkers as they have been applied in drug discovery for diseases of the CNS. These include both "neurologic" diseases such as Alzheimer's disease (AD) and stroke, characterized by macroscopic alterations in brain structure, and "psychiatric" disorders, including schizophrenia and mood disorders, that are manifest chiefly by alterations in thought, mood, and behavior. We will demonstrate the use of target-compound interaction biomarkers in the development of symptomatic therapies of AD, disease and disease modification biomarkers in developing disease modifiers in AD, patient-selection biomarkers for acute stroke and stroke recovery treatment, and pharmacodynamic and disease biomarkers in schizophrenia and Major Depressive Disorder (MDD).

Imaging Biomarkers for Target–Compound Interaction in Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common cause of age-related dementia. It is characterized clinically by a gradual deterioration of intellectual abilities concomitant with dramatic alterations in personality, affective regulation, and behavior (Bozeat et al. 2000). In its more advanced stages, AD is typified by severe and wide-ranging cognitive deficits, including gradual but inexorable memory loss, difficulty in learning, loss of language skills, impairment of judgment, a decline in the ability to perform routine tasks, and ultimately, disorientation and loss of interpersonal contact. The neurodegenerative nature of the disease eventually leads to the failure of other organ systems and death.

Treatments for AD address short-term improvement and stabilization of cognitive and functional deficits. The scientific rationale for the first symptomatic therapies was based on research showing profound degeneration of ascending cholinergic pathways from the basal forebrain to the hippocampus and cerebral cortical areas and led to symptomatic treatment strategies, aimed at boosting cholinergic function (Araujo et al. 1988; Bowen et al. 1983; Davis et al. 1982). For the cholinergic agents, imaging approaches using PET have been useful in demonstrating target engagement and pharmacodynamic activity. For example, one PET study demonstrated that donepezil treatment (3–5 mg per day) reduced AChE activity in the cerebral cortex of AD patients concomitantly with the patient's symptomatic improvement (Shinotoh et al. 2001). Similar PET studies demonstrated that donepezil (5 and 10 mg per day, 5 weeks) inhibits cortical AChE activity by 27% in the AD brain (Kuhl et al. 2006).

Preclinical studies have reported that specific 5-HT_{1A} receptor antagonists improve learning and memory in animal models, and several compounds have been advanced into clinical testing in AD patients (Schechter et al. 2005). In the early clinical development program, PET or SPECT imaging using specific radioligands for these target receptors provided information about the degree and duration of receptor occupancy (RO) as a target-compound interaction biomarker for confirming CNS target engagement. For example, in the early development of lecozotan, a 5-HT_{1A} antagonist, a PET study was conducted to assess the 5-HT_{1A} RO of the drug in healthy, young, elderly and AD subjects (Raje et al. 2008) (Fig. 2). This work

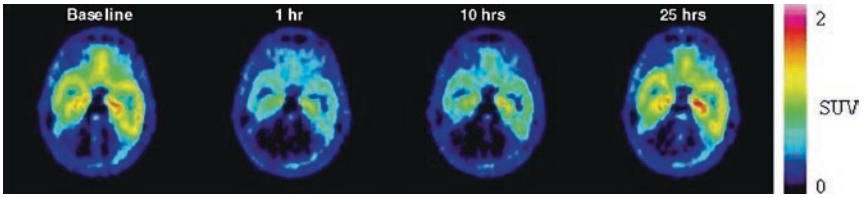

Fig. 2 [^{11}C]-WAY-100635 uptake in human temporal cortex. The scans were taken before and at different times (hours) after administration of 5 mg of lecozotan IR formulation to a young healthy subject. 5HT$_{1A}$ receptor occupancy of lecozotan, calculated based on uptake, serves as a biomarker of target-compound interaction, thus demonstrating CNS target engagement. *SUV* standardized uptake value. Figure courtesy of Dr. Sageeta Raje, Wyeth Research, Collegeville, PA

allowed a clear understanding of the relationship between lecozotan 5-HT$_{1A}$ RO and drug plasma concentrations and enabled the development of a PK/PD model which predicted peak 5-HT$_{1A}$ RO of 70–76% following a total daily dose of 10 mg. The PET data and PK/PD modeling work helped to guide the dose selection for the subsequent clinical studies in AD patients to further examine the efficacy and safety of lecozotan. This example illustrates the power of neuroimaging approaches to guide better decision making with regard to dose selection (Feuerstein et al. 2008). Several PET ligands are available for labeling the 5-HT$_{1a}$ receptor (e.g., [^{11}C]-WAY-100635), however, for newer targets, few PET or SPECT ligands are available. Furthermore, not all targets are suitable for imaging with PET or SPECT, depending on their level of expression in regions of interest in the brain relative to the surrounding areas(Ametamey and Honer 2007; Pimlott 2005). Importantly, the process of developing and validating radioligands for human use can be laborious, often taking 2 years or longer. Thus, once a molecular target has been validated as promising, it is highly desirable that PET/SPECT ligand development takes place in parallel to the drug discovery program in order to provide sufficient lead time for use in early clinical studies.

Imaging Biomarkers of Disease and Disease Modification in Alzherimer's Disease

Disease modification in AD refers to the ability of a drug to slow or halt the disease process by, for example, modulating the deposition of beta amyloid or the hyperphosphorylation of tau. The majority of disease modifying investigational drug treatments target either the production of beta amyloid by inhibiting beta (Hussain et al. 2007) or gamma secretase(Best et al. 2007), or the enhancement of beta amyloid clearance by active or passive immunization(Solomon 2007).

Several neuroimaging approaches have been explored in AD and some may hold promise as biomarkers of disease progression. Independent studies have shown that progressive brain atrophy, as measured by serial MRI, can be detected longitudinally in AD patients (de Leon et al. 2006; Jack et al. 1998; Xu et al. 2000). Changes in

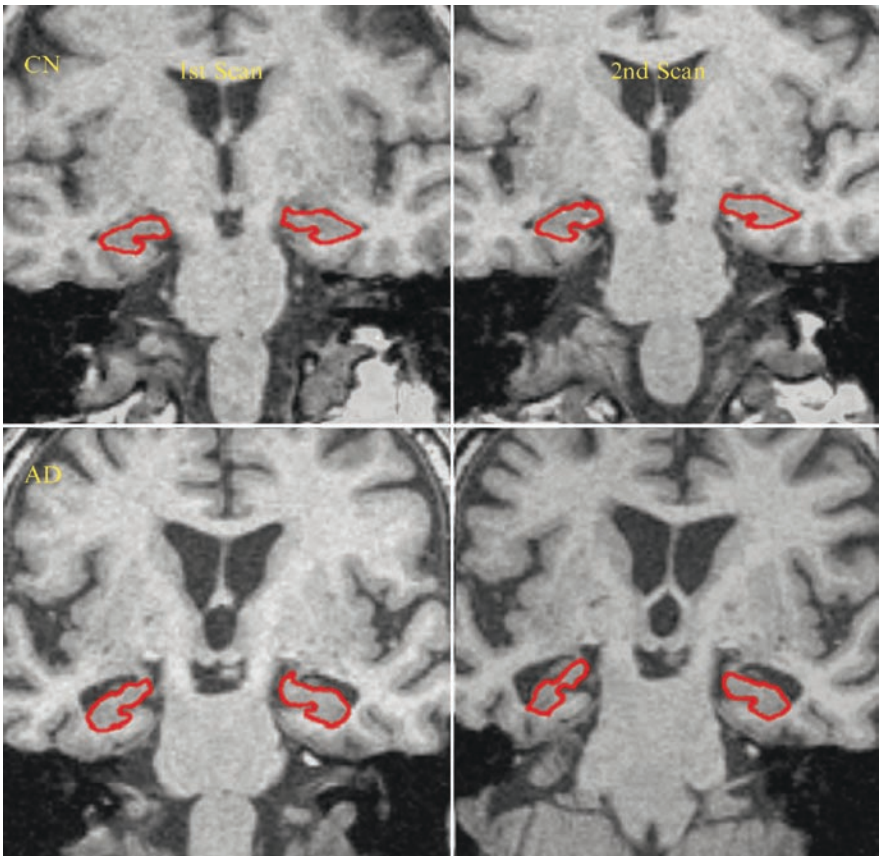

Fig. 3 Hippocampal atrophy detected in AD patients. Two MRI scans 1 year apart in the same individuals are shown. Hippocampi are indicated by the red trace on coronal sections. Marked atrophy (reduction of hippocampus area) is observed in an AD patient compared to an age-matched control subject. The area of hippocampus can be quantified as a biomarker of disease progression. Figure courtesy of Dr. Michael W. Weiner, University of California, San Francisco, CA

MR-based regional changes (hippocampus, entorhinal cortex, and corpus callosum) may be even more specific to the pathological process of AD than global (whole brain and ventricles) brain volume measures (Fox et al. 2005; Jack et al. 2003) (Fig. 3). These studies consistently show loss of brain volume in AD patients that is at least twice the rate of loss seen in age-matched control subjects. These imaging biomarkers have been piloted in several clinical trials with candidate disease modifying agents. For example, volumetric MRI measures of whole brain atrophy and hippocampus atrophy have been measured in clinical trials with anti-amyloid immunotherapy as well as small molecule amyloid modulators (Fox et al. 2005).

FDG-PET (fluorodeoxyglucose positron emission tomography) is an imaging method that provides a global measure of brain glucose metabolism.

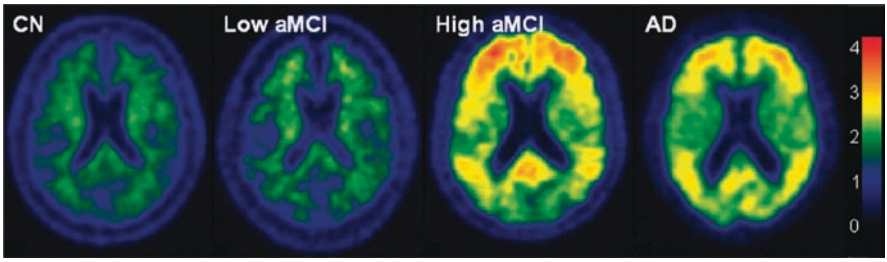

Fig. 4 Amyloid deposits detected with ^{11}C-PIB PET. Increased retention of PIB signal in cortical and temporal areas is observed in AD patients and subset of amnestic MCI patient compared to cognitive normal controls. The global PIB retention ratio can be used as a biomarker of amyloid plaques and may have potential as a biomarker of disease progression and therapeutic activity of amyloid-targeting agents. Figure courtesy of Dr. Chester A. Mathis, University of Pittsburgh, Pittsburgh, PA

In patients with AD and mild cognitive impairment (MCI), the cerebral metabolic rate for glucose (CMRgl) reductions in the posterior cingulate, parietal, temporal, and prefrontal cortex are correlated with dementia severity and progression (Mega et al. 1997; Mosconi et al. 2005). Molecular imaging also promises to provide specific information about the neuropathology of AD. The most advanced PET imaging ligand, the Pittsburgh Compound-B (PIB), is a thioflavin derivative that appears to be relatively selective for Aβ plaques (Klunk et al. 2004; Klunk et al. 2005) (Fig. 4). A few other amyloid imaging ligands have been reported, including ^{18}F-FDDNP, a PET ligand that binds both amyloid plaques and neurofibrillary tangles (Rowe et al. 2007; Small et al. 2006). Indeed, FDDNP studies in AD and MCI patients have found binding in areas of the brain with amyloid deposits and an increased signal at longitudinal follow-up(Small et al. 2006).

For any of the briefly described neuroimaging measures to qualify as a validated biomarker of AD disease progression, a correlation with clinical symptoms over a period of time needs to be demonstrated. In this regard, a consortium of academic, industry and government investigators have embarked on the Alzheimer's Disease Neuroimaging Initiative (ADNI), a large longitudinal neuroimaging and biomarker study of MCI and AD patients(Mueller et al. 2005). In addition to the identification of the most robust imaging biomarkers for disease progression, ADNI is also expected to deliver standardized protocols and methods for the evaluation of neuroimaging biomarkers in large-scale, multicenter studies.

Imaging Biomarkers of Patient Selection in Stroke and Cerebrovascular Disease

Stroke is the leading cause of disability in adults, and represents a growing unmet medical need, particularly as the population ages. In most cases, thromboembolism is the primary event leading to cerebral infarction, and thrombolysis with tissue

plasminogen activator (tPA) is the only approved pharmacological therapy. However, in order to be effective and safe, tPA must be administered within 3 h of the onset of an ischemic event. Such rapid delivery of treatment is often not feasible due to delays in recognition of symptoms and transit to a facility where accurate diagnosis can be made and treatment administered (Cocho et al. 2005). Vessel occlusion leads to rapid and severe reduction of cerebral blood flow (CBF) within the affected vascular territory. This deficit triggers a host of cellular events that lead to apoptotic and necrotic cell death. In this area, commonly defined as the "core" of the infarct, tissue damage ensues rapidly and is generally irreversible. The surrounding tissue is also affected by metabolic, hemodynamic and neurochemical alterations; but in this region, cell death and tissue damage progress slowly because of residual collateral blood flow. This region, which is referred to as the "ischemic penumbra", contains tissue that can be potentially salvageable if an effective intervention that improves blood flow, tissue oxygenation and energy supply are restored before irreversible damage occurs(Baron and Moseley 2000; Jones et al. 1981).

Despite considerable effort and encouraging data generated in pre-clinical models of stroke, acute neuroprotective strategies have failed to show efficacy in multiple large-scale clinical trials. The implicit aim of these strategies is to rescue the neurons and other cells in the "penumbra" that remain viable and salvageable, but are compromised by the ischemic environment. With the use of rigorous criteria, PET imaging has provided evidence that, in a subset of stroke patients, a region of viable, potentially salvageable tissue exists for at least 8 h, and possibly for as long as 24 h, after the onset of ischemic stroke, whereas in others, the infarct reaches its maximal extent only a few hours after the onset of clinical symptoms (Baron and Moseley 2000). The variability in terms of penumbra duration is likely to contribute to patient heterogeneity commonly encountered in clinical trials. This is an area in which imaging strategies can have a unique impact in selecting patients who are most likely to benefit from therapy, and the implementation of these strategies should be a cornerstone in the design of future clinical trials for neuroprotective drugs.

Although PET is one of the best approaches to accurately define the penumbra based on CBF and metabolic parameters (i.e., ^{15}O-H_2O, ^{18}FDG PET imaging), it is unlikely, from a practical point of view, that PET will be a useful tool in the acute clinical setting. In this regard, computed tomography (CT) and magnetic resonance (MR) imaging are the most widely used approaches for assessing ischemic lesions and perfusion deficits (Wardlaw 2001). CT is a widely used imaging technology with several advantages, such as speed of image acquisition, widespread availability in clinical centers and the ability to depict intracerebral hemorrhage. Infarct appears as a low density (*dark*) region that corresponds to a vascular territory and is often accompanied by swelling; however, the time at which these hypodense lesions become visible varies from hours to days. In general, large infarcts are more likely to be visible than small ones, and a visible hypoattenuation on noncontrast CT scans is rarely reversible. Importantly, it was demonstrated that the presence of a visible infarct in the acute phase of stroke significantly and independently increases the risk of hemorrhagic transformation, early death and poor long term outcome (Wardlaw et al. 2003). Cerebral perfusion in patients with acute stroke can also be

evaluated by CT using the intravenous infusion of iodinated contrast material. The use of a deconvolution-based analysis of CT perfusion imaging is one method that allows a rapid generation of maps for cerebral blood volume (CBV), mean transit time (MTT), and cerebral blood flow (CBF, where CBF = CBV/MTT). These hemodynamic parameters have been used successfully to assess the extent and severity of brain tissue ischemia (Nabavi et al. 2001). A successful CT-based imaging protocol for acute stroke would allow a careful characterization of patients and may facilitate patient stratification. For instance, unenhanced CT will first tell whether a patient presents with intracranial hemorrhage. This distinction will obviously separate patients with ischemic stroke that could be considered for thrombolysis. In addition, CT angiography and CT perfusion scanning could provide sufficient information necessary to determine the vascular territory affected and the extent of tissue ischemia. Analysis of the perfusion deficit and the presence of visible hypodense lesions could indicate massive infarction with low probability of treatment response to thrombolytic therapy and perhaps, neuroprotective strategies.

Because of its many advantages, CT and CTP are likely to remain the cornerstone of imaging for acute ischemic stroke. Despite their potential for patient stratification, the variability in image acquisition, analysis and interpretation of results remains a challenge for their utility in multi-center clinical trials. Thus, proper validation of a comprehensive CT-based imaging protocol for the examination of stroke patients is essential before it could be used on a regular basis as a reliable tool for patient selection across multiple centers.

In acute stroke, echoplanar MR enables rapid, non-invasive detection of infarcted and hypoperfused but still salvageable tissue in acute human stroke by using diffusion weighted (DWI) and perfusion weighted (PWI) imaging modalities, respectively. DWI measures alterations in the diffusion of water molecules that, in the case of ischemia, are manifested by hyperintense lesions reflecting a reduction in the apparent diffusion coefficient of water (ADC). These changes are primarily due to metabolic failure leading to disruption of ion homeostasis and cytotoxic edema. It is well documented that these brain regions typically correspond to infarct tissue, although very early reversibility has been shown in animal models, and occasionally, in humans (Kidwell et al. 2003); hence, DWI lesions normally represent the non-viable ischemic core. In contrast, PWI makes use of the signal loss that occurs during the dynamic tracking of the first pass of an intravenous paramagnetic contrast agent. A signal intensity-time curve is obtained from whole brain T_2-weighted perfusion scans and used to generate maps of relative CBV, MTT and regional CBF (Fig. 5). Previous reports have shown that PWI lesion volumes correlate better with acute clinical impairment scores than DWI; but both parameters predict functional outcome and infarct size (Wardlaw et al. 2003). In addition, visualization of major arteries and branches (i.e., middle cerebral artery) is obtained via magnetic resonance angiography (MRA), performed at the same time as PWI. Together, DWI and PWI provide clinically useful information about infarct topography and pathogenesis, in acute stroke.

These approaches have also enabled the identification of potentially salvageable tissue based on the DWI/PWI mismatch that could be easily calculated in the

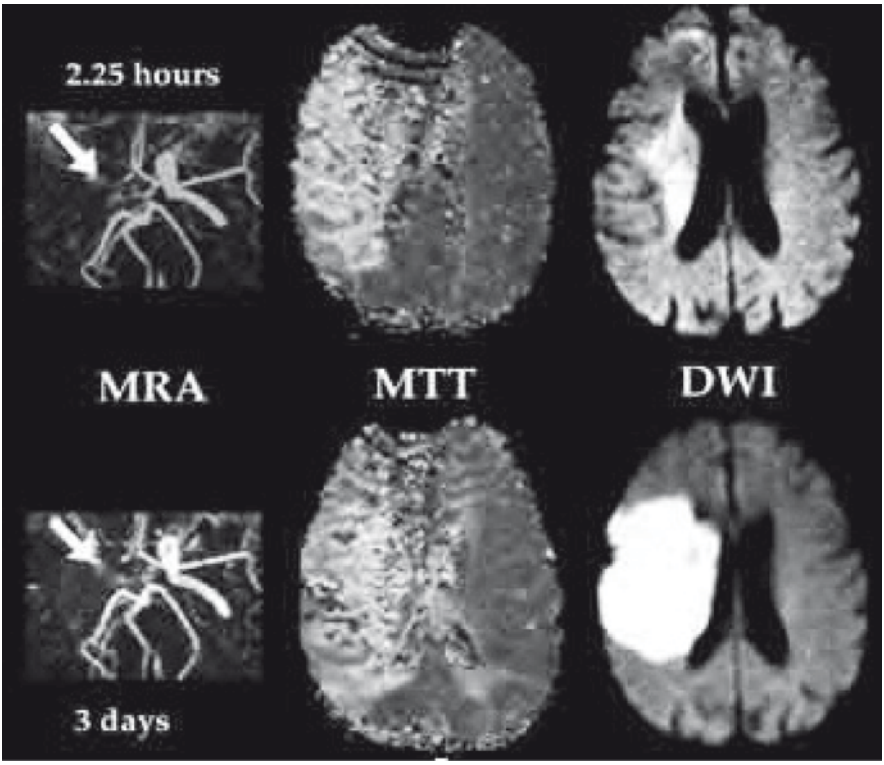

Fig. 5 PWI/DWI mismatch can be used to identify the presence of penumbral tissue. Initially (2 h post ictus) a large perfusion deficit (MTT) and small DWI lesion suggest the presence of large penumbral region. In the absence of reperfusion or other affective therapy, ischemic lesions mature into large irreversible infarcts as indicated by a significant reduction in perfusion-diffusion mismatch. Figure courtesy of Prof. Joanna Wardlaw, University of Edinburgh, Edinburgh, UK

acute clinical setting. For instance, a recent Phase II trial (Echoplanar Imaging Thrombolytic Evaluation Trial, EPITHET) used DWI/PWI mismatch as a patient selection biomarker to test the hypothesis that the presence and extent of the ischemic penumbra in acute stroke patients will predict the likelihood of response to thrombolytic therapy defined by a reduced expansion of the ischemic core (Butcher et al. 2008). This trial failed to prove any significant association between tPA use and lower infarct growth; however, the results showed that tPA was significantly associated with increased reperfusion in patients with mismatch, and this was associated with an improved clinical outcome (Davis et al. 2008). Similarly, the results of the Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE) study support the validity of the mistmatch hypothesis in patients treated with tPA in the 3–6 h window. This study showed that early reperfusion is associated with a more favorable clinical outcome in patients with significant PWI/DWI mismatch, whereas patients without mismatch did not appear to benefit from thrombolysis(Kakuda et al. 2008).

Although the results of the EPITHET and DEFUSE trials are promising, MR imaging is inherently variable in terms of protocols and analysis even within the same imaging center(Kane et al. 2007; Takasawa et al. 2008). Therefore, in order for these techniques to be used more widely, the standardization and validation of MRI based protocols for reproducible identification of penumbral tissue is needed before this approach could lead to a more rational treatment decision based on patient selection.

Imaging Biomarkers in Schizophrenia

Schizophrenia is a common and highly disabling psychiatric disorder with a population prevalence of around 1%. The manifestations of schizophrenia fall into three major domains: (1) "positive" symptoms, such as delusions, hallucinations, and disorganization of behavior; (2) "negative symptoms," including social withdrawal, lack of motivation, and reduced expression of affect; and (3) cognitive dysfunction. Of these, the positive symptoms have received the most attention in terms of drug therapies, all of which target the D_2 dopamine receptor. However, recent research has shown that cognitive dysfunction correlates most closely with psychosocial impairment, and this domain has become the subject of intense research, to better understand its neurobiology and pathophysiology, as well as to develop more effective treatments.

The recognition that most or all available antipsychotic agents interact with the D_2 receptor (Seeman 2006) predated the availability of PET ligands for this receptor (Seeman 2006). However, once the ligands (e.g., raclopride and fallypride) became available, it became possible to use them as biomarkers to correlate D_2 RO occupancy with both efficacy and safety measures for existing antipsychotic agents. Seminal studies by Kapur (REF)and others, using several available antipsychotic agents have shown that the increasing likelihood of therapeutic efficacy is associated with striatal D_2 receptor occupancy of 50–65%, while the well-known adverse effects of these agents on prolactin secretion and extrapyramidal motor functions are associated with higher levels of occupancy (e.g., ≥72% for hyperprolactinemia, and ≥78% for extrapyramidal symptoms) (Tauscher and Kapur 2001). This work has helped determine the therapeutic dosage windows for these agents in terms of maximizing beneficial effects on positive symptoms, while minimizing undesired extrapyramidal symptoms.

The dorsolateral prefrontal cortex (dlPFC) and anterior cingulate cortex are critical components of the brain circuitry underlying executive control. The failure of patients to activate the dlPFC during cognitive challenge is among the most consistent findings in schizophrenia (Bunney and Bunney 2000). This was first demonstrated with PET imaging although functional magnetic resonance imaging (fMRI) has largely replaced PET because of its relative noninvasiveness and superior temporal and spatial resolution. Most studies, including those in first-episode patients, have shown dorsolateral prefrontal cortex hypoactivation (Callicott et al. 2000;

Salgado-Pineda et al. 2004). Importantly, the "Measurement and Treatment Research to Improve Cognition in Schizophrenia" (MATRICS) initiative was established by academics, in conjunction with the NIH and FDA to identify the key domains of cognition in which a therapeutic agent should improve. The involvement of the FDA has been critical in this initiative, in order that such tests may serve as registrable endpoints. Several of the cognitive deficits observed in patients have now been correlated robustly with a reduced metabolic activity in the prefrontal cortex during task performance, raising the possibility that functional imaging will provide an accurate biomarker for cognitive impairment (Salgado-Pineda et al. 2004; Weinberger and Berman 1996).

Several lines of evidence also point to dysfunction in glutamatergic systems as a factor underlying the neurocognitive deficits associated with schizophrenia (Coyle 2006). Simple deoxyglucose imaging has been employed to correlate hypofrontality in schizophrenia to the cognitive deficits associated with the disease. Local cerebral glucose utilization with [^{18}F-] fluorodeoxyglucose (FDG-PET) has demonstrated that temporal lobe and thalamic activity correlate with positive symptoms and can be normalized with clozapine (Molina et al. 2005). However, hypofrontality in the frontal cortex has been shown to correlate with the severity of negative and cognitive deficits and is not affected by atypical antipsychotics. As such, hypofrontality may represent a useful disease biomarker of cognitive dysfunction. Imaging regional cerebral activation while patients perform tests of cognitive performance can also be used to dissect the discrete neural regions and substrates supporting cognitive performance. In contrast to other diseases (e.g., oncology), in schizophrenia it is rare that there are concrete physical entities to quantify based on the very nature of cognitive abnormalities and their basis in distributed brain systems. However, imaging techniques, such as functional MRI (fMRI) are bridging this gap. fMRI has the potential to be a powerful, sensitive and repeatable tool in our armamentarium. This technology affords the potential to distinguish patients with cognitive deficits that are driven by, for example, either medial temporal lobe or by frontal lobe dysfunction (e.g., episodic memory vs. executive function deficits) within a clinical trial (Manoach et al. 2000). Applied in the early clinical studies, one can potentially turn heterogeneous clinical populations into discrete, focused subgroups with which to answer specific and focused hypothesis about the target patient population and ultimately, increase the probability of seeing an effect with a compound whilst improving the potential for differentiation from comparators. This in turn can aid patient selection in larger Phase III studies.

Increased confidence as to whether animal models are likely to be translational in nature can only be achieved if we gain a greater understanding of the neural systems recruited in vivo during cognitive processes in rodents, and then relate these to parallel studies in humans. One means of achieving this requires that imaging biomarkers are characterized and validated using similar techniques in human and animal models. Recently, Ferris and colleagues demonstrated a methodology for fMRI scanning in conscious animals (King et al. 2005), and future development in this area will allow increased congruency between imaging biomarkers in the clinic and those applied in animal models.

Imaging Biomarkers in Mood Disorders

The major categories of mood disorders in the DSM-IV comprise MDD and the bipolar disorders, which are further divided into types 1 and 2 (American Psychiatric Association 1994). MDD has been the subject of numerous imaging studies, including structural imaging with CT and later, functional imaging using PET, SPECT, and functional MRI techniques. A smaller but substantial number of studies have also been conducted in patients with Bipolar Disorders.

Konarsky et al. have recently reviewed some 140 studies evaluating MDD and/or Bipolar Disorders. These studies show no significant differences in total brain volume in either condition relative to matched control subjects. However, there are some consistent differences in the volumes of certain brain regions between MDD and controls, including prefrontal cortex and dorsolateral, orbital, subgenual, and anterior cingulate subregions (Konarski et al. 2008). Early PET studies done with [^{18}F]-deoxyglucose showed decreased metabolic activity in the prefrontal cortical regions in patients with MDD (Goodwin 1997; Hurwitz et al. 1990). Subsequent studies with [^{15}O]-H_2O (a measure of blood flow rather than metabolic activity) confirmed these initial findings. Interestingly, the hypometabolism/ hypoperfusion in the dlPFC is also seen in patients with schizophrenia (see above), although to a greater extent than in MDD (Barch et al. 2003). Altered function in the amygdala of MDD patients was suspected for some time because of the frequent occurrence of anxiety in this illness, with Drevets being among the first to show clear evidence of altered metabolic activity in this structure (Drevets et al. 1992).

A series of PET studies by Mayberg and colleagues identified a group of structures whose activity was altered during the induction of a sad mood in healthy volunteers while they listened to a script they had created that had a sad affective theme (Liotti et al. 2000). Mayberg subsequently described a “depression circuit” in patients with MDD, composed of coordinated alterations in the activity of several cortical structures, notably the increased activity in the subgenual PFC (Cg25), the decreased activity in the dlPFC (F9), and the increased activity in the posterior cingulate structures [Brodmann areas]. Successful treatment of MDD with fluoxetine in placebo-controlled trials resulted in normalization of activity in these brain areas, while patients who did not respond, showed persistence of the abnormal activity in these regions. Furthermore, patients who responded to the placebo condition showed changes that were in the same direction as in the fluoxetine responders, but of a lower magnitude (Mayberg et al. 2000). In contrast, a different group of patients treated for their depression with cognitive-behavior therapy showed changes in the same regions, but in the opposite direction as the fluoxetine-treated patients (Goldapple et al. 2004). These apparently discrepant findings illustrate the complexity of CNS systems and the difficulties in drawing conclusions from isolated findings. Nevertheless, the consistency of the brain areas affected under these differing conditions, and the responsiveness of a small group of severely treatment-resistant MDD patients to direct stimulation of the white matter tract adjacent to the Cg25 area, does suggest that these structures play an important role in MDD and in treatment responses.

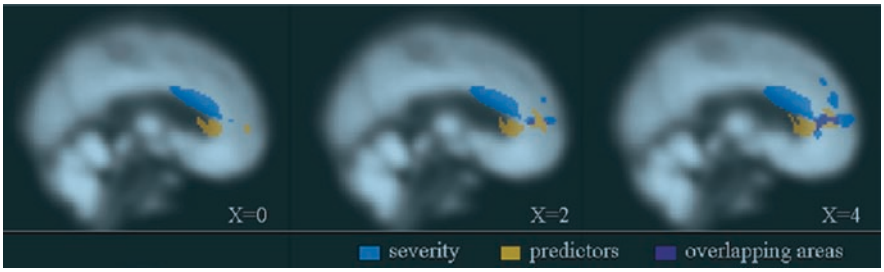

Fig. 6 Activation in pregenual cingulate cortex predicts antidepressant response independent of depression severity. This figure shows substantial dissociation between brain areas predicting antidepressant response, and those associated with severity of depressive symptoms. From Chen et al. (2007)

In recent years, fMRI has gained a wider use for assessing changes in regional cerebral blood flow because of its relative noninvasiveness and flexibility relative to PET. A recent study suggests a dissociation between the subregions of the anterior cingulate cortex that predict symptom severity versus those that predict treatment response (Chen et al. 2007), offering the possibility of using fMRI measures as a patient selection biomarker in the early clinical studies (Fig. 6). Another novel way in which fMRI techniques have been applied to drug development for depression, involves the use of surrogate populations, such as those with subclinical dysphoria. In this regard, several studies have shown that known antidepressants produce significant changes in limbic fMRI responses using emotional activation paradigms (Anderson et al. 2007; Del-Ben et al. 2005). Several companies have invested in studies being conducted by "biomarker consortia" for more extensive validation of these paradigms. If validated, these types of imaging study could be used in early drug development to provide evidence of antidepressive or anxiolytic activity, and subsequently, of more traditional proof-of-concept in clinical trials in patients (Altar et al. 2008).

Conclusions

Considerable progress has been made over the past three decades in the development of imaging technologies and their application to the study of CNS systems. A major challenge for the future is to utilize these sophisticated technologies as aids in the drug development process. Towards this end, it will be critical to achieve standardization of data acquisition procedures and analytic methods across study sites, which can contribute considerable sources of variability and a resultant loss of power to detect significant effects of drugs on brain systems. The emergence of consortia that promote cooperation among pharmaceutical companies, academic centers, and government agencies such as the NIH and FDA should facilitate the establishment

of more standardized and well-validated biomarkers to facilitate the evaluation of drugs developed for the treatment of these disabling diseases.

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Part II
Imaging Approaches

Anatomical Imaging: Volumetric Analysis

Natalie L. Voets

Introduction

Since the seminal research of Hounsfield, Lauterbur and Mansfield, the ability to directly image structures within the human brain has transformed our understanding of CNS disease. Non-invasive Magnetic Resonance Imaging (MRI) offers uniquely detailed insight into the structure of the living brain and microscopic changes in disease.

Anatomical imaging approaches vary from the gross visualisation of pathology to recent ultra-high resolution measures of specific cortical structures in the human brain (e.g. Sheperd et al. 2007). Exciting novel analysis methods are increasingly enabling assessment of disease-associated structural changes on the scale of interconnected networks in the brain. This chapter will focus on MRI-based anatomical measures of structural changes in a range of CNS diseases using various analytical approaches. In reviewing the current status of MRI anatomical imaging and its implications for CNS drug development, this chapter will track the shifting focus in analysis method developments to mirror our emerging understanding of disease pathophysiology and therapeutic challenges. As most progress has been made in the field of neurodegenerative disorders (particularly Alzheimer's disease) and schizophrenia, volumetric studies and their implications for therapeutic intervention in these areas will form the bulk of this overview. Methodological considerations of volumetric analytical approaches are discussed before reviewing potentially novel therapeutic applications derived from anatomical imaging measures.

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Early Impact of MRI on Clinical Diagnosis: Volumetric Approaches

MRI is a non-invasive technique able to provide highly detailed images of organs in the body and brain based on nuclear relaxation properties of different tissue types when subjected to a radiofrequency pulse in a static magnetic field. For an overview of MRI physics and methods, see Jezzard et al. (2001).

One of the earliest drives for introducing nuclear magnetic imaging into the medical sphere was based on preliminary demonstrations of the ability to separate tumour and benign tissue based on their respective relaxation characteristics (Damadian 1971). Following a rapid evolution in the field, MRI has made a dramatic impact on the in vivo diagnosis of diseases of the CNS.

Refining Definitions of Disease

The classification of clusters of symptoms as constituting a specific disorder has historically posed a major challenge for CNS diseases. It is well recognised, for instance, that conditions presenting as dementia (originally thought to be a psychiatric ailment) may have multiple aetiologies. As the development of tailored treatment strategies in most cases will relate to the specific pathological processes at play, a major impact of anatomical imaging on understanding CNS disease has involved the identification not only of specific structural changes in the brains of patients presenting with symptoms of dementia, but indeed specific profiles of structural change across the brain dissociating different types of dementia (e.g. Ballmaier et al. 2004).

Among the earliest breakthroughs in progressing our understanding of CNS diseases was the observation of focal atrophy on structural MRI images of Alzheimer's Disease (AD) patients (e.g. Seab et al. 1988). Early reports of hippocampal and entorhinal regional volume loss in AD as determined by manual segmentation of MRI structural images have subsequently been extended to demonstrate that (1) hippocampal and entorhinal volume loss in AD exceeds the normal rate of atrophy in the ageing brain (e.g. Jack et al. 1988), (2) constitutes a risk factor for conversion to AD in Mild Cognitive Impairment patients (Jack et al. 2004), (3) correlates with cognitive decline (e.g. Kesslak et al. 1991), and (4) is detectable prior to onset of clinical symptoms in patients at genetically higher risk for AD (Ridha et al. 2006). A number of excellent reviews discuss various imaging measures applied to the specific study of AD (Thompson et al. 2007; Apostolova and Thompson 2007).

Implications for CNS Therapy

By advancing our understanding of differential pathological processes associated with various symptom spectra, anatomical imaging has the ability to significantly

impact on CNS drug development in several ways. First, by enabling relative confidence in a diagnosis of disease (e.g. probable-AD), imaging presents an opportunity to directly assess the potential efficacy of treatments targeted at specific disease processes (e.g. demyelination in multiple sclerosis). Second, by identifying structural changes common to distinct CNS conditions presenting similar symptoms (such as psychiatric symptoms in temporal lobe epilepsy and schizophrenia), volumetric imaging measures may highlight common mechanism, and thus possible therapeutic targets. Conversely, identification of differential structural measures of pathology in the brains of patients presenting with similar clinical profiles, such as extent of hippocampal atrophy in MCI patients likely to convert or likely to remain stable (Jack et al. 2004), provides the opportunity to begin stratifying patients likely to respond to a given treatment. Recent advances in imaging of Multiple Sclerosis, for instance, have elucidated distinct patterns of cortical atrophy in secondary progressing and relapsing remitting subtypes of the disease (Ceccarelli et al. 2008), a direct relationship between grey matter atrophy and progressive white matter lesions in relapsing-remitting patients (Bendfeldt et al. 2009), and evidence for parallel pathological processes affecting grey matter and white matter independently in primary progressive cases (Bodini et al. 2009). Some of these applications will be discussed below, but for a detailed overview of (structural and functional) imaging applications in CNS drug development, Matthews and Wise (2006) and other reviews by the same first author are highly recommended.

Analytical Approaches to Assess Whole–Brain Volumetric Change

ROI approaches have offered reproducible, sensitive measures of CNS neurodegeneration in various CNS diseases (Jack et al. 1990). However, manual cortical segmentations are highly susceptible to operator bias, offer limited sensitivity to structural changes occurring within structural subregions, particularly where imaging sequences are not specifically optimised for regional contrast (e.g. deep brain nuclei), and by definition offer only a highly focal insight into neurodegeneration. Several more objective measures of grey matter volume change have emerged allowing assessment of structural changes across the brain without the need to specify a priori regions expected to show a disease or drug effect.

Voxel-Based Morphometry and Related Measures of CNS Disease

In exploratory studies aimed at further disease characterization, the most commonly reported and readily accessible automated whole-brain analysis technique is (modulated) Voxel-Based Morphometry (VBM) (Ashburner and Friston 2000)(www.fil.ion.ucl.ac.uk/spm/), and its variants (e.g. FSL-VBM, www.fmrib.ox.ac.uk/fsl/).

Since its advent, VBM has been employed in innumerable studies of whole-brain cortical atrophy in normal ageing (Tisserand et al. 2004) and disease, and has been directly compared with gold standard ROI measures (Douaud et al. 2006; Giuliani et al. 2005).

In particular relation to neurodegenerative diseases, VBM has been applied to study cortical volumetric changes in AD (Whitwell and Jack 2005), normal ageing (Smith et al. 2007), Parkinson's disease (with or without dementia) and Huntington's Disease (Douaud et al. 2006), among others, and has identified cortical changes occurring longitudinally (Ramirez-Ruiz et al. 2005; Chetelat et al. 2005), associated with performance (Grossman et al. 2004; Baxter et al. 2006), genetics (Wishart et al. 2006; Thieben et al. 2002) and pharmacological effects (Salgado-Pineda et al. 2006). More recently, there has also been a focus on discriminant or comparative studies, for example comparing VBM-MRI with FDG-PET (Kawachi et al. 2006). In psychiatry, VBM's main impact has been in the field of schizophrenia (Honea et al. 2005) and affective disorders (Nugent et al. 2006), with a similar repertoire of studies assessing longitudinal (van Haren et al. 2007), associative (behavioural: Spencer et al. 2007; genetics: McIntosh et al. 2006; pharmacological: McClure et al. 2006) and discriminant/comparative (Job et al. 2005) cortical volume changes.

Identifying specific patterns and rates of structural change occurring in individual patients may enable stratification of subjects to assess their specific therapeutic response. In the context of rapidly progressing disease, the ability to determine whether a pre-symptomatic patient is likely to experience rapid decline would offer an invaluable tool in drug development. Kinkingnehun et al. (2008) recently proposed such potential in a 3-year longitudinal study comparing volumetric changes in 'slow' and 'fast' declining Alzheimer's disease patients who at baseline presented with similar age, years of education, estimated duration of illness and disease severity. Using VBM, the authors found that despite similar cognitive performance at baseline, cortical changes were significantly greater in patients who would go on to experience a fourfold faster decline in MMSE score than patients with a 'slow' process of cognitive decline. While able to successfully discriminate between patients and controls in a given group, VBM analyses may not have the sensitivity required to assess such measures at the individual subject level, at least at an early stage of disease. More tailored analytical approaches such as SIENA (Smith et al. 2002) and Tensor-Based Morphometry may offer a useful complementary technique in these cases (e.g. Thompson et al. 2000).

The Role of Whole-Brain Atrophy Measures in CNS Drug Therapy

Objective whole-brain measures sensitive to progressive volume changes may play a central role in predicting patients likely to suffer cognitive decline among those at risk for dementia. This is particularly pertinent in the case of neurodegenerative diseases complicated by potentially multifactorial pathophysiological mechanisms,

variable clinical course even within genetically determined diseases (e.g. Huntington's Disease) and slow disease onset (see Dib 2005). As clinical symptoms in these patient populations may only emerge once a threshold of neuronal loss has been reached (Dib 2005) the development of efficacious drug treatment will depend largely on the ability to identify vulnerability factors for a given disease to halt or at least slow disease progression (Jack et al. 2003; Fox et al. 2001, see Thompson et al. 2007). This has been reflected to some extent in observations of increased grey matter volumes in bipolar patients treated with lithium (Moore et al. 2000; Sassi et al. 2002; Foland et al. 2008), and attributed to potential neurotrophic effects. While these data suggest the ability for volumetric imaging measures to detect subtle drug effects of regional brain volume, the sensitivity and specificity of these imaging measures will need to be validated in the context of drug development (see, e.g. Sormani et al. 2004; Matthews and Wise 2006). This may bear particular relevance to subtle structural changes associated with disease or therapy given observations of intra-individual variability in metabolic measures related to time of day of scanning (Soreni et al. 2006), or significant brain volume changes associated with acute dehydration (Duning et al. 2005).

VBM evidence of a direct relationship between structural changes in CNS disease and specific clinical deficits is central in the development of reliable, sensitive imaging biomarkers for use in clinical drug trials, and suggests the potential to differentiate pathophysiological mechanisms underlying distinct symptom clusters (Thompson et al. 2007). For example, a recent VBM study in Parkinson's disease patients with and without resting tremor identified a cerebellar structural change in the tremor group, offering a biological substrate for this phenotypic variability (Benninger et al. 2009). Improved understanding of disease clinical course will furthermore improve the odds of a favourable therapeutic outcome by aiding the selection of patients most likely to benefit from treatment based on their particular symptoms or stage of disease (Dib 2005). Finally, structural imaging measures predictive of disease status (e.g. MCI converters or non-converters) will enable optimal powering of clinical trials, reducing sample sizes currently limited by relatively insensitive clinical rating scales (Jack et al. 2003).

Distinguishing Measures Contributing to Cortical Density

Although highly valuable in determining cortical changes across populations and time, VBM cortical density is a combined measure of both grey matter thickness and shape. Normal variability in local surface gyrification [e.g. in Heschl's gyrus (Ide et al. 1996)] could contribute to measures of cortical density change that may offer no clinically useful information of disease pathophysiology. A recent focus on measures able to distinguish more specifically between cortical thickness and local shape changes has identified abnormal gyrification in schizophrenia (Harris et al. 2007), potentially related to disease symptomatology (Cachia et al. 2008). While still an area of active development, studies using surface-based morphometry (SBM)

approaches such as Freesurfer (http://surfer.nmr.mgh.harvard.edu) have offered evidence supporting a neurodevelopmental basis to cortical changes in this disorder. Consistent with these findings, we recently demonstrated both overlapping and spatially distinct regions of cortical change measures derived from VBM and SBM measures in the same group of adolescent-onset schizophrenic patients. In regions where VBM density change measures were not supported by local cortical thinning, examination of measures of metric distortion and locally sampled surface area measures revealed significant changes in adolescent-onset schizophrenia (Voets et al. 2008) (Fig. 1).

An emerging novel analytical approach is 3D surface reconstruction applied specifically to the shape and volume analysis of the hippocampus. These have shown sensitivity to hippocampal atrophy in temporal lobe epilepsy (Lin et al. 2005) among many other conditions, and may plausibly offer an additional potential biomarker for patient stratification. The clinical impact of such approaches resides in the ability to distinguish potentially distinct pathological processes and novel therapeutic targets where selective morphological changes relate closely to symptom onset or decline.

From Focal Atrophy to Hodology: Imaging Structural Systems in CNS Disease

With the availability of increasingly sensitive analysis tools, imaging is able to provide a window into disease-related changes occurring at the systems level (Matthews and Wise 2006), which, in turn, may describe functional network changes. In AD, it has recently been noted that the pattern of cortical atrophy appears to follow a reverse pattern to that of cortical maturation (Thompson et al. 2007) and structural volumetric changes in schizophrenia may reflect patterns of abnormal neurodevelopment (Harrison 1997). This observation offers interesting challenges for therapeutic developments targeting early processes triggering pathological cascades, and places an increased emphasis on imaging studies unravelling brain maturation processes (e.g. Dubois et al. 2006; Giorgio et al. 2008).

Cortical volume changes, particularly in the context of neurodevelopmental processes, are likely to relate directly to white matter integrity. Novel MRI-based diffusion sequences and voxel-wise analysis methods (e.g. TBSS, Smith et al. 2006)

Fig. 1 (continued) and matched controls. SBM thickness group difference maps (**b**) (FDR-corrected, $p<0.05$) identified both regions of overlap and regions differing from the VBM density result (*black circles*). Un-thresholded metric distortion maps (**c**) suggest local changes in surface area but did not survive multiple comparisons correction. Direct sampling of surface area measures within ROIs defined based on the VBM-SBM difference regions identified significant differences between patients and controls underlying VBM density results where no evidence of SBM thinning was found. Discrimination accuracy maps between patients and controls based on thickness alone (**d**), showing regions offering at least 70% discriminative power

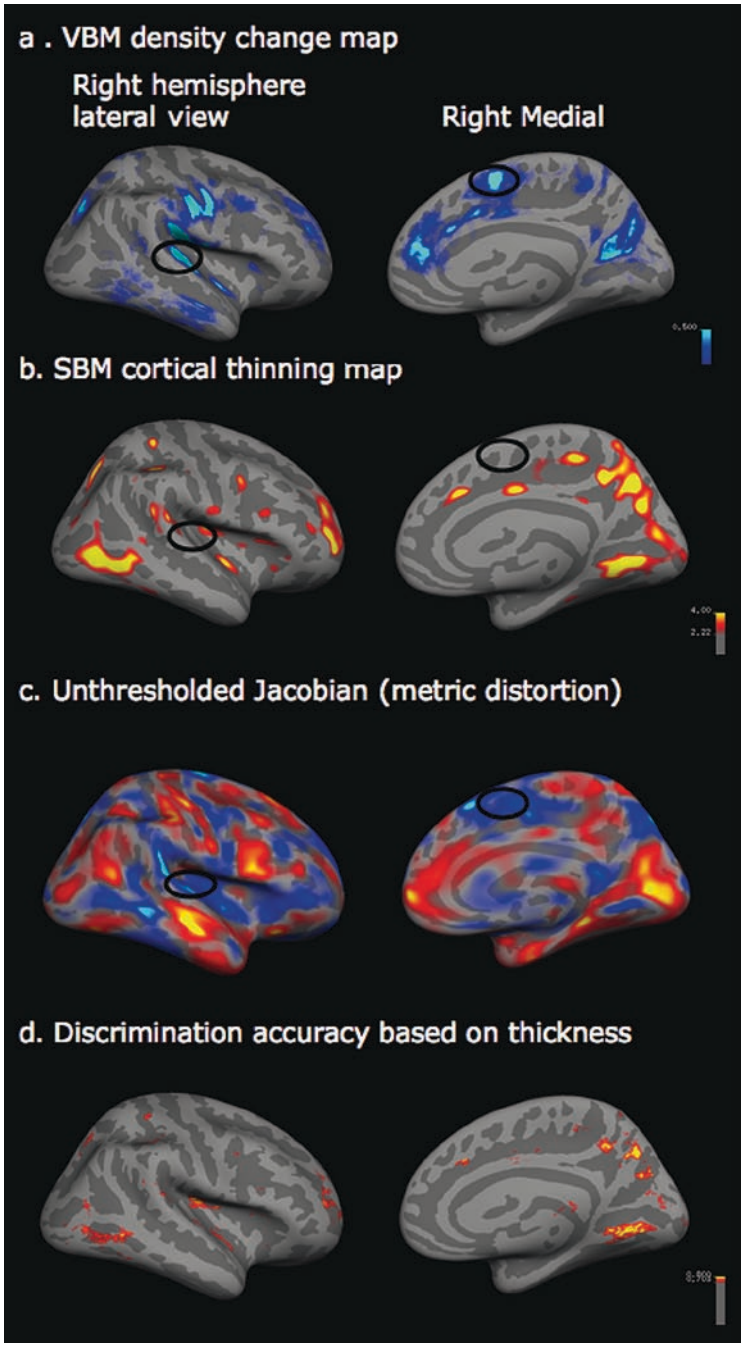

Fig. 1 Voxel-based and surface-based measures of cortical change in adolescent-onset schizophrenia. VBM analysis (**a**) projected onto the group-specific template used for surface-based analysis revealed widespread decreases in cortical density between adolescent onset schizophrenia

offer the exciting possibility to directly map cortical volume change measures to specific white matter pathways (e.g. Douaud et al. 2007; Bodini et al. 2009) and gain a global view of structural change occurring with disease in inter-connected systems. A comprehensive review by Marco Catani describes various advances and applications of these techniques (Catani 2006).

Methodological Considerations

The previous sections briefly reviewed anatomical imaging approaches in CNS disease and therapy. Volumetric analysis approaches offer a real possibility to identify biomarkers sensitive to selective disease processes and their modulation by therapeutic compounds. However, their effective development and implementation in large-scale clinical trials of novel pharmaceutical compounds, particularly those involving multiple sites employing different MRI scanners, will need to take into account the basic assumptions and limitations inherent to various analytical approaches, as well as the imaging measures themselves.

Analytical Assumptions

VBM and SBM methods are the main *automated* approaches employed in the study of brain anatomy and disease but have thus far been developed along relatively parallel avenues, with little cross-comparison.

VBM (Ashburner and Friston 2000) is a sensitive measure of global grey matter density changes and was developed with the specific aim of assessing structural differences among populations based on a comparison of "the local composition of different brain tissue types while discounting positional and other large-scale volumetric differences in gross anatomy" (Ashburner and Friston 2001). As such, the method carries specific assumptions which, if not carefully considered, may significantly affect study results (for an assessment of the effect of different processing approaches on schizophrenia VBM results, see Honea et al. 2005). A useful review of methodological considerations and suggested guidelines for using VBM is provided by Ridgeway et al. (2008). In brief, careful consideration is needed in the implementation of various VBM processing steps to avoid false results resulting from (1) suboptimal segmentations, particularly in the medial temporal lobe where automated segmentation processes are still an active area of research; (2) arbitrary or biologically implausible smoothing levels; (3) invalid statistical analysis (e.g. Gaussian smoothing applied to white matter, see Jones et al. 2005); (4) poor choice or bad alignment to the study template (see Senjem et al. 2005). This latter point perhaps constitutes the most inherent limitation of group-wise analysis approaches reliant on average templates (including surface-based methods) given the need to spatial normalise across regions that may naturally vary greatly (e.g. Heschl's gyrus duplications).

Careful cytoarthicetonic studies have questioned the direct correspondence between gyral borders and cytoarchitectonic regions (Amunts et al. 2007), further complicating the interpretation of local cortical volume change results. This may be particularly important in neurodevelopmental disorders (e.g. Guerrini et al. 2008). VBM measurements in these populations may therefore particularly benefit from supplementary local shape change and structural connectivity measures.

Interpreting Measures of Volumetric Changes

Volumetric image measures, while able to provide sensitive measures of disease-related cortical change, also need to be interpreted in the context of the basic signal measures they are based on. For example, nuclear relaxation times may be affected by specific physiological changes (Matthews and Wise 2006) and may therefore only provide a non-specific measure of, for example, water content in the atrophic epileptic hippocampus. Similarly, therapeutic compounds themselves may interact directly with the imaging measure (Salgado-Pineda et al. 2006).

It has been proposed that cortical thinning may reflect lamination changes (Makris et al. 2006), but alternatively, these could relate to "unknown vascular factors, glial cell numbers, and the extent and integrity of cortical myelination" (Thompson et al. 2007). As a powerful complement to VBM measures, SBM cortical thinning and shape change measures will, therefore, need to be validated in the context of histological evidence and sensitivity to specific pathophysiological processes before they can be readily translated into the clinical setting.

Similarly, the specific sensitivity of imaging measures applied in clinical trials needs to be understood if they are to provide a valuable biomarker of therapeutic action. Thus, greater cortical volume loss observed using a trial of antibody relative to placebo, but unrelated to symptom deterioration, could reflect sensitivity to clearance of amyloid/other confounding factors (Fox et al. 2005). Evidence of volumetric enlargement with atypical antipsychotics likewise requires further research to determine whether these cortical changes reflect true reversal of pathological processes associated with regional atrophy, or instead provide an index of therapeutically meaningless change (Thompson et al. 2007).

Therapeutic Horizons

The primary goal in imaging for CNS therapy is to identify biomarkers for accurate diagnosis, assessment of risk factors for disease, severity, progression and potential therapeutic response.

Among the most recent emerging applications of structural imaging biomarkers are methods sensitive to beta-amyloid accumulation. Emerging novel Positron Emission Tomography ligands such as PIB and FDDNP offer the potential to directly

image beta-amyloid deposits in the brains of MCI and AD patients. Cortical thinning in AD may co-localise with regions of high reported beta-amyloid load (Espeseth et al. 2008), and could therefore offer an additional, earlier biomarker for stratification of patients likely to benefit from novel treatment acting directly on beta-amyloid peptides. Intriguingly, this study, conducted in healthy volunteers either positive or negative for the APOE e4 risk allele for AD, found that although negative carriers had decreased regional cortical thickness relative to participants at increased genetic risk, the latter group may have greater progressive cortical volume loss.

Studies such as this are drawing increasing interest based on the potential to combine imaging and genetic biomarkers to provide increased sensitivity compared with neuropsychological outcome scores (Lawrie et al. 2008, Roffman et al. 2008). For example, specific polymorphisms thought to play a role in neurodevelopment, such as the Brain-Derived Neurotrophic Factor Val 66 Met Single Nucleotide Polymorphism, have been associated with medial temporal and prefrontal grey matter volume loss (Pezawas et al. 2004). Similarly, in relating structural measures, genetic susceptibility and disease symptomatology, a direct relationship has been identified between specific psychotic symptoms and a risk allele in the NGR1 gene (Hall et al. 2006), CAG triplet repeat mutation length and presymptomatic striatal volume (Henley et al. 2009) and APOE e4 load and medial temporal and temporo-frontal volume (Filippini et al. 2009). Interestingly, the latter study suggested that susceptibility to develop disease and rate of disease progression may be mediated by processes distinct from simple genetic status. By combining complementary neuroimaging measures sensitive to different aspects of disease processes occurring across the brain, it may be possible to identify biomarkers with high predictive value for future emergence of CNS disease to facilitate early assessment of novel compounds aimed at disease modification rather than symptomatic treatment. Such approaches could furthermore expand our knowledge of normal development and structure–function relationships outside of the disease context. As increasingly sophisticated analytical methods for assessing highly localised volume and shape changes become available, the potential to assess these effects in a clinical setting become increasingly more tangible.

Conclusion

The development of analytical methods (both structural and functional) for the dedicated identification and evaluation of imaging biomarkers in drug discovery is an area of active research. Anatomical imaging measures can inform both highly localised, macroscopic changes associated with disease and (potentially secondary) global measures affecting distributed networks. To further the implementation of these measures in drug development, it will be critical to not only to determine the relationship between individual structural imaging measures and behavioural performance, but also their sensitivity to detecting a clinical improvement with specific therapeutic interventions (Matthews and Wise 2006). This may pose a particular

challenge in the evaluation of novel therapies aimed at modifying disease processes and reversing pathological processes (e.g. hippocampal neurogenesis, see Newton and Duman 2007), as such relationships between clinical improvement and biologically meaningful measures of efficacy may take years to emerge. As the biology underlying specific CNS diseases becomes clearer, the application of structural measures must become increasingly innovative. Thus, while hippocampal volume change is of great interest in the current development of Alzheimer's disease drugs, such structural damage may occur at a stage where its measurement may no longer be relevant for disease-modifying agents.

In conclusion, volumetric analyses of anatomical imaging measures provide key insights into structural bases of disease symptoms. Careful assessments of pre-symptomatic and progressive pathological changes in CNS diseases have called into question the historical clusterings of certain symptoms as constituting distinct diseases. By informing the global impact of localised disease-related atrophy, volumetric analyses, combined with diffusion, spectroscopic, myelin-sensitive, genetic and PET measures may enable identification of novel therapeutic avenues for CNS conditions with overlapping symptoms. The integration of structural and functional measures of CNS disease will further enable better prediction of the optimal stage in a disease to introduce specific therapeutic intervention, and allow early assessment of drug efficacy/action. Similarly, with thorough understanding of the specific processes to which a given imaging measure is sensitive, biomarkers may provide early validation of specific drug targets by demonstrating its impact on precipitating processes, disease mechanisms or symptoms (Matthews and Wise 2006), and importantly, may begin to offer direct insight into the mechanisms underlying pharmacological resistance in conditions ranging from depression to temporal lobe epilepsy.

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Diffusion Tensor Imaging and Drug Development

Dominique L. Jennings and A. Gregory Sorensen

Introduction

Non-invasive clinical MRI relies on the intrinsic behavior of water protons to provide a contrast between tissues. Various techniques are used to sensitize images to different contrast types such as T1- and T2-weighting, but these parameters are not directly linked to the functional physiology of the tissue being examined. Movement of water within and between cellular compartments can provide valuable information about tissue structure and function, and therefore, diffusion MRI has evolved into a sophisticated technique. In addition to providing the scalar diffusion coefficient from a purely isotropic medium, a more advanced MR technique, DTI, is capable of providing a quantitative, directionally-estimated diffusion parameter that is useful in regions of high anisotropy (or the degree from which diffusion deviates from a spherical distribution), for example, in the white matter tracts of the brain. These tensors can be related to the brain architecture, sensitive to pathophysiological conditions such as white matter tract injury and edema (Leung et al. 2004; Price et al. 2006).

Diffusion Magnetic Resonance Imaging

Diffusion MRI is an important imaging method, designed to sensitize images to the random translation motion of water molecules. The rate of diffusion of protons on these water molecules depends on many physical parameters including membrane permeability, extracellular volume fraction and local water concentrations

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(Latour et al. 1994). Because of these physical components influencing the path of the proton, the distribution of movement allows for a unique insight into the geometrical structure and organization of tissues being examined. Unlike other MR methodologies that require the use of exogenous contrast agents or alteration of basic MRI parameters for assessment of tissue T1 or T2 relaxation, diffusion is completely independent of these physical parameters or magnetic field.

Physical Concepts

Pulsed field gradients are a useful way to measure the diffusion of water protons because they are non-destructive, and in most cases with MR modalities, it is a non-invasive measurement (i.e., no chemical tracers are introduced or are necessary for sensitization of these images to diffusion). The modification to the Hahn echo for water diffusion measurement resulted in the Stejskal–Tanner's derivation for the effect of anti-phased, amplitude-matched, time-dependent field gradients in the presence of spin diffusion (Stejskal and Tanner 1965). The two gradient pulses of duration δ and amplitude G are separated by a diffusion time Δ. Following the 90° pulse, the first diffusion gradient pulse introduces a phase shift in the nuclei in the direction of its application. After a finite time period, a 180° refocusing pulse inverts the phase shift and the second amplitude-matched gradient induces phase shifts identical to those induced by the first gradient pulse. Stationary spins will be phased back to their original phase following the 180° pulse. If a spin has diffused from its position at the moment of the first gradient pulse, the second gradient pulse will fail to refocus the spin to its initial phase. A measure of the extent of locomotion a spin or a group of spins will undertake is represented by the diffusion coefficient. Signal attenuation resulting from this incomplete refocusing, or diffusion of spins is represented as:

$$S = S_0 e^{-bD} \tag{1}$$

where $b = -\gamma^2 G^2 \delta^2(\Delta - \delta/3)$, γ is the gyromagnetic ratio of a hydrogen proton and S_0 is the net magnetization of the system when no diffusion weighting is applied (i.e., $G=0$). The diffusion coefficient, D, is often referred to as the 'apparent' diffusion coefficient (ADC) because each voxel in an image may represent numerous tissue types, and measured diffusion coefficients may reflect motion of water molecules from both the intra- and extra-cellular compartments in those tissues.

A simple diffusion MRI experiment is usually acquired with diffusion-weighting in three orthogonal directions. One image, required for an accurate estimation of S_0, is acquired with no diffusion-weighting. The images acquired with diffusion-weighting are geometrically averaged to provide an average diffusivity (van Gelderen et al. 1994). The diffusivity of a group of spins is typically reflective of the directionally averaged ADC.

Diffusion Tensor Imaging

Although diffusion MRI provides valuable information regarding the average mobility of tissue water, the values are relegated to a single scalar parameter that does not reveal the underlying *directional* motion of water that arises due to physical barriers (e.g., cellular membranes). This section reviews the physical basis of a more advanced diffusion MR acquisition technique that is capable of evaluating the more comprehensive three-dimensional motion of tissue water in space.

Physical Concepts

There exist some structures in which the diffusivity has a high directional dependence, or anisotropy. Anisotropy is a concept that was originally contemplated in vivo in the brain white matter and the spinal cord (Chenevert et al. 1990; Moseley et al. 1990). Diffusion anisotropy is quite high in asymmetric tissues like muscle fibers or white matter in the brain, which is primarily composed of collateral myelinated axonal fibers. This straightforward method of measuring diffusion may be considered a ground-level acquisition of the true geometrical and structural milieu of the system. A more sophisticated measure of diffusivity can be encoded into a diffusion tensor, or a three-dimensional representation of diffusion in the tissue:

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} \tag{2}$$

The diffusion tensor is derived from diffusivity measurements in at least six non-collinear directions. Since the tensor is positive and symmetric, with these six measurements, all the values within the matrix are known. Typically, many more directions are used to scan space more uniformly and avoid sampling direction biases, a paradigm that is necessary for more complex applications such as fiber orientation mapping (Jones et al. 1999; Papadakis et al. 1999).

Once the diffusion tensor components have been acquired, the tensor is diagonalized (off-diagonal terms of **D** are nulled), and the eigenvalues (λ), which correspond to diffusivity, and eigenvectors (ε), which correspond to the main diffusion directions, are provided. In an anisotropic system, the diffusion tensor can be graphically represented as an effective diffusion ellipsoid. The shape of the ellipsoid has a very useful physical interpretation. In a given system, very short diffusion times would result in a relatively isotropic tensor, and the ellipsoid would adopt a spherical geometry. Longer diffusion times might illuminate the true nature of the anisotropy in the system, allowing spins to reach their physical barriers and seek alternative

pathways, resulting in an ellipsoid with a more prolate geometry. Eigenvalues are the major, medium and minor axes of the ellipsoid, and the associated eigenvectors dictate the orientation with respect to the main magnetic field.

Several quantitative parameters can be derived from the tensor. The mean diffusivity (D) is the directionally averaged or mean-squared displacement of the molecule:

$$D = (\lambda_1 + \lambda_2 + \lambda_3)/3 \tag{3}$$

Another common parameter derived from the tensor is the fractional anisotropy, which is the fraction of the magnitude of the anisotropy in **D**:

$$\mathrm{FA} = \frac{\sqrt{3(\lambda_1 - \langle\lambda\rangle)^2 + (\lambda_2 - \langle\lambda\rangle)^2 + (\lambda_3 - \langle\lambda\rangle)^2}}{\sqrt{2(\lambda_1^{\,2} + \lambda_2^{\,2} + \lambda_3^{\,2})}} \tag{4}$$

where λ denotes the mean of the three eigenvalues. Fractional anisotropy ranges from 0 to 1, where 1 represents a completely anisotropic system.

The lattice index calculates the anisotropy in a voxel by incorporating the anisotropy value of its nearest neighbor. Though the index benefits from a relative reduction of noise in the measurement, it suffers from increased partial volume effects (Pierpaoli and Basser 1996).

The extent to which a tensor adopts a shape can be used in a quantitative manner, and the physical orientation of the lowest entropic path of water in the laboratory frame can be mapped to the RGB color vectors in order to display a qualitative visualization of the variation in anisotropy (Pajevic and Pierpaoli 1999). Fiber tracking or tractography is a technique that utilizes mathematical algorithms to identify neighboring voxels from seed regions that would likely be located within the same fiber tract (Conturo et al. 1999; Mori et al. 1999).

Diffusion Tensor Imaging in Human Brain Pathologies

Molecular motion of water is considered a direct reflection of physiology, as opposed to oxygenation (the BOLD effects), or contrast-enhanced MRI, where signal changes from exogenous contrast agent effects on the intrinsic T1 and T2 relaxation of tissue protons are indirectly correlated with concentration, and therefore, hemodynamic physiology. Changes in cell membrane permeability, active transport mechanisms, viscosity, tissue interstitial fluid pressure, and structural integrity of cellular networks may all contribute to alterations in the microscopic diffusion of water molecules, especially in the context of a therapeutic insult to normal and pathological tissue (Sugahara et al. 1999; Szafer et al. 1995).

Oncology

The clinical utility of diffusion MRI has been demonstrated quite extensively in brain tumors, where increased diffusion values were measured shortly after the initiation of treatment (Batchelor et al. 2007; Mardor et al. 2003) and in some cases, the magnitude of these changes corresponded to clinical outcomes (Chenevert et al. 2000; Hamstra et al. 2005). Diffusion MRI is thus being heralded as a surrogate marker of therapeutic response in human brain tumors. Diffusion tensor imaging is rapidly becoming a routine clinical imaging modality in neuro-oncology, partly because of its unprecedented insight into the white matter tract anatomy and the implications for treatment and surgical planning in the context of a tumor, and also because of its potential quantitative nature.

Field et al. has identified four distinct modes by which a cerebral neoplasm might alter the natural state of a white matter tract (Field et al. 2004; Jellison et al. 2004). The first pattern of alteration illustrates a mass that displaces the white matter tract as it develops and expands, but leaves the tract intact. The second pattern of alteration illustrates a mass that is not in contact with the fiber tract, but rather has a large edematous region that will not affect the tract location or orientation, but will induce a large reduction in fractional anisotropy. The third pattern, observed in only infiltrating gliomas, is characterized by a substantial reduction in the fractional anisotropy without a substantial bulk mass effect on displacement of the fiber tract. The fourth pattern, observed in both high and low grade malignant tumors, is characterized by a complete abrogation of anisotropy and directionality such that the fiber tract was no longer identifiable.

Head Trauma

Head trauma injuries can be defined as either focal or diffuse, with focal injuries resulting from an external force applied directly on the brain, while diffuse injuries result from shear strain due to a rapid acceleration and deceleration event followed by a sudden change in the momentum. The latter type of injury can be further identified with the extent of diffuse ischemia, histopathologic changes in edema with subsequent diffuse swelling or diffuse axonal injury (DAI). DAI manifests at the cellular level and is thus considered a microscopic injury (as opposed to focal injury, which is localized and can be identified macroscopically). A predictable exploratory evaluation of head injury is initiated with standard Computed Tomography (CT) imaging. This is followed in many cases by conventional MRI, which is typically invoked to identify diffuse lesions, though it is limited by low sensitivity for the extent of injury. Since DTI is capable of elucidating axonal white matter architecture, it outperforms both CT and conventional MRI for identifying DAI (Huisman et al. 2004; Lee et al. 2003; Ptak et al. 2003; Rugg-Gunn et al. 2001). This specificity can

have profound effects on the outcome of the injury because various categories of head trauma have different prognoses and treatment protocols. In general, most studies find that FA is significantly reduced in the posterior limb of the internal capsula, genu, stem and splenium of the corpus callosum, column of the fornix and the centrum semiovale in patients with head trauma compared to healthy, demographically-matched controls (Akpinar et al. 2007; Naganawa et al. 2004; Nakayama et al. 2006; Sidaros et al. 2008). In some cases, these values correlated with injury severity (Akpinar et al. 2007; Nakayama et al. 2006) or long-term outcome (Ptak et al. 2003; Sidaros et al. 2008), a finding that may ultimately lend FA the tenability to progress into a non-invasive biomarker for traumatic brain injury.

Huntington's Disease

Though few studies to date have been published, DTI has been shown to produce quantitative information, specifically, significant reductions of FA, in both white and gray matter in patients diagnosed with Huntington's Disease (Reading et al. 2005; Rosas et al. 2006), as well as in patients with suspected or known mutations in the Huntingtin gene (Kloppel et al. 2008). Pre-symptomatic (no cognitive impairment) carriers of the mutation in the Huntingtin gene are important components to this research because they provide a model for defining the pre-symptomatic versus early stage of pathophysiology when treatment is likely to be the most effective. DTI fiber tractography has been used to map the anatomical connections between the frontal cortex and the striatum, and significantly lower percentages of these connections were found in pre-symptomatic carriers. Furthermore, this particular metric was negatively correlated with the onset of symptoms. Thus, DTI indices may prove to be a valuable measure of the therapeutic outcome, identifying short to mid-term neurobiological changes.

Pain

DTI has promoted a greater understanding of pain processing and control in fibromyalgia (Sundgren et al. 2007) and migraines (DaSilva et al. 2007), and the functional connectivity of the pain processing network (Hadjipavlou et al. 2006). Fibromyalgia is a chronic pain disorder characterized by widespread tenderness and sensitivity. The most pronounced differences observed using DTI were significant reductions in FA found within the right thalamic region, the magnitude of which were statistically greater in individuals classified as experiencing more intense clinical pain (Sundgren et al. 2007). However, there were no significant differences in FA compared to controls in any other region of the pain matrix (i.e., the primary and secondary somatosensory cortices, the insula, the anterior cingulate, the dorsal lateral pre-frontal cortex and the basal ganglia). In chronic migraine sufferers, changes

in the structures involved in the trigeminal pain processing pathway have been observed (DaSilva et al. 2007). Significant reductions in FA were observed in two subtypes of migraine sufferers (migraineurs with aura and migraineurs without aura) compared to controls. Additionally, lower FA values were observed in migraineurs with aura in the ventral trigeminothalmic tract and in migraineurs without aura in the ventrolateral periaqueductal gray matter. The changes observed in this study could be attributed to the increase in the axonal diameter and decrease in the myelination, and are not necessarily linked to lesion consolidation.

Stroke

The temporal evolution of stroke is categorized into the following phases, relative to the onset of symptoms: hyperacute (<12 h), acute (1–7 days), subacute (1–16 weeks), chronic (>16 weeks) phase. The ADC decreases within minutes after the onset of stroke and can remain depressed well into the acute stage of infarction before pseudonormalization and subsequent increases occur (Huang et al. 2001). One utility of DTI is that it can reliably differentiate between the white and gray matter, and therefore, it can be used in the quantification of the ADC in the gray and white matter separately. In fact, this method has been used to evaluate these differences over time, from the acute to subacute stage (Mukherjee et al. 2000).

FA is another metric that can reliably differentiate between the gray and white matter of infracted tissue (Munoz Maniega et al. 2004; Sorensen et al. 1999). Indeed, a stronger utility for DTI lies in its ability to quantify anisotropy in acutely, subacutely and chronically ischemic WM. The use of DTI in evaluating hyperacute stroke, however, has not delivered diagnostically useful information to date (Harris et al. 2004; Ozsunar et al. 2004).

DTI is used in the evaluation of subacute stroke and in the elucidation of Wallerian degeneration in the corticospinal tract (CST), demonstrating a negative correlation between FA and motor function (Moller et al. 2007). Another group reported reduced FA values in the CST resulting from acute anterior choroidal artery (AchoA) infarcts, and these values correlated with the long-term motor outcome (Nelles et al. 2008). Results led investigators to conclude that long-term recovery, ergo positive clinical outcome is related to the preservation of the integrity (i.e., anisotropy) of CSTs. Additionally, reduced FA in the superior longitudinal fasciculus and arcuate fasciculus are correlated with pronounced difficulty to repeat spoken language (Breier et al. 2008). Directionally encoded color maps and 3D tractography have been used to relate localization of stroke lesions with WM tracts (Lee et al. 2005; Lie et al. 2004; Yamada et al. 2004) and estimate the level of disruption or distortion of these tracts (Gillard et al. 2001; Parmar et al. 2006), an index which can have a significant impact on the prognosis and treatment of the patient. DTI has also been useful in understanding CADASIL, an autosomal dominant vasculopathy that causes recurrent ischemic events in the subcortical WM (Chabriat et al. 1999). Decreases in FA have been shown to parallel disease progression.

In general, the use of DTI during the subacute to the chronic phases of stroke is better characterized. Along this continuum, FA becomes progressively more attenuated (Buffon et al. 2005; Yang et al. 1999; Zelaya et al. 1999). It has been suggested that sensitivity to these variable stages of ischemic proliferation may improve treatment and ultimately, provide clinicians with the ability to identify and potentially preserve viable tissue from irreversibly injured tissue (Ozsunar et al. 2004).

Multiple Sclerosis

Although conventional T1-weighted MR Imaging, Magnetic Resonance Spectroscopy and Magnetization Transfer imaging demonstrate a high sensitivity for identifying sclerotic lesions, DTI has gained momentum in this arena because it delivers structural information that can be correlated with disease outcome. DTI has been shown to differentiate the normal white matter in patients from MS compared to control subjects (attributed to axonal loss and/or gliosis) (Bammer et al. 2000; Ciccarelli et al., 2000; Filippi et al. 2001; Werring et al. 1999). It has also been shown to differentiate between acute and chronic lesions (Bammer et al. 2000; Ciccarelli et al., 2000; Filippi et al. 2001; Werring et al. 1999) and correlate with disability (Castriota Scanderbeg et al. 2000; Ciccarelli et al., 2000; Filippi et al. 2001).

Depression

DTI has also been used to correlate changes in FA with late-life depression. Specifically, reductions in FA have been observed in the frontal and temporal regions of the brain of patients with late-life depression compared to healthy, age-matched controls (Alexopoulos et al. 2002; Nobuhara et al. 2006; Taylor et al. 2004); furthermore, FA values were inversely correlated with severity of symptoms (Nobuhara et al. 2006).

Autism

DTI studies have shown decreased FA values in regions of the brain associated with social cognition, including areas important for face and gaze processing, (the fusiform gyrus and superior temporal sulcus) and areas important for emotional processing (anterior cingulate, amygdala, ventromedial prefrontal cortex) in subjects with autism compared to control subjects (Barnea-Goraly et al. 2004). Disruptions have also been observed in the superior temporal gyrus and temporal stem in autism, areas critical to language and social cognition (Lee et al. 2007). Such disruptions in the white matter tract organization have been attributed to abnormal levels of neurotrophic brain factors during brain development (Anderson et al. 1990; Vanhala et al. 2001).

Obsessive Compulsive Disorder

Some studies have observed reduced FA values in the rostrum of the corpus callosum in patients with OCD compared to normal subjects, and values were inversely correlated with symptom severity (Saito et al. 2008). Other studies have shown bilateral reduction in FA values in the anterior cingulate gyrus in patients with OCD compared to healthy controls. The same study found additional areas of altered FA that correlated with disease severity including the parietal lobes (supramarginal gyri), the right posterior cingulate gyrus and the lingual gyrus (Szeszko et al. 2005). One group showed that drug-naïve patients with OCD demonstrated a higher FA in the corpus callosum, and that following 12 weeks of pharmacotherapy, most of the areas investigated exhibited a "normalization" or pre- to post-therapy decrease in these FA values (Yoo et al. 2007). Such a discrepancy has been postulated to be related to drug-naïveté, treatment period or subject characteristic (e.g., age).

Schizophrenia

A very comprehensive review of 19 studies conducted between 1998 and 2004 has been published, covering mostly chronic, medicated patients with adult-onset schizophrenia demonstrating altered or abnormal FA, D and RA compared to healthy, demographically matched controls (Kanaan et al. 2005). The two most common types of analyses were ROI-based and voxel-based. The majority of studies employed the former, although the ROI-based methods are subjective, and it is imperative that repeatability/reproducibility of the method be demonstrated. Voxel-based analyses are more powerful when DTI is used as an exploratory device, and areas of abnormality are more diffuse or widespread than focal. Interestingly, there was a great diversity of anatomical regions studied, though the corpus callosum and cingulum tended to inspire the most interest. However, for as many studies that found significant decreases in FA in various regions, there were approximately an equal number that found no change or abnormality in FA in the same regions.

Since this review, between 2005 and 2008, at least 16 more studies emerged, with an emphasis on a first episode and early-onset (onset before the age of 18) demographic; the remaining 44% of the studies still focused on adult-onset schizophrenia. Although many regions were evaluated, the corpus callosum was a common focus. For the most part, these studies found significant or trend-level decreases in FA in the corpus callosum (or the study's particular region of focus, e.g., the optic radiation) in the first episode (Cheung et al. 2008; Friedman et al. 2008; Karlsgodt et al. 2008; Szeszko et al. 2005), early-onset (Ashtari et al. 2007; Kumra et al. 2005; Kyriakopoulos et al. 2008) and the adult onset schizophrenia (Buchsbaum et al. 2006; Butler et al. 2006; Hao et al. 2006; Kuroki et al. 2006; Shergill et al. 2007; Tang et al. 2007). Only one study from each demographic found no change in the regions studied (Jones et al. 2005; Kendi et al. 2008; Price et al. 2005) using both ROI- and voxel-based methods.

Alzheimer's Disease

DTI has been widely used to understand the pathophysiology of Alzheimer's disease (AD), and areas of abnormal white matter FA are consistently observed in the frontal (Bozzali et al. 2001; Head et al. 2004), parietal (Bozzali et al. 2001; Medina et al. 2006) and temporal (Bozzali et al. 2001; Takahashi et al. 2002; Xie et al. 2006) cortices. It has been shown that the corpus callosum is also affected with reductions in FA in both the genu (Head et al. 2004; Xie et al. 2006) and the splenium (Medina et al. 2006; Rose et al. 2000). The superior longitudinal fasciculus is commonly affected as well (Rose et al. 2000; Xie et al. 2006). Studies have also shown that the posterior cingulate gyrus, which is associated with episodic memory performance, may also contribute to neurodegeneration. Reduced anisotropy may result in decreases in acetylcholine since these fibers are an important component to the cholinergic system, providing a possible reason why procholinergic drugs may be so effective in the treatment of AD.

One study found that the lattice index was significantly reduced in the splenium of the corpus callosum and in the superior longitudinal fasciculus in patients with probable AD compared to healthy controls, and that these reductions correlated positively with the mini-mental state examination (MMSE) scores (Rose et al. 2000).

Mild cognitive impairment (MCI) rests within the continuum of cognitive decline from normal aging to AD. Similar regions are affected by losses in anisotropy between MCI and AD (Fellgiebel et al. 2004; Medina et al. 2006; Zhang et al. 2007), though attenuation of FA is more pronounced in AD (Fellgiebel et al. 2005; Zhang et al. 2007). Such sensitivity has engendered hope that DTI will be a useful methodology to aid in the early diagnosis of AD.

On the mechanism of WM inhomogeneity of AD, it is postulated that a reduced FA in combination with increased ADC values are derived from Wallerian degeneration or ischemic alterations that lead to axonal damage, gliosis and WM rarefaction (Stahl et al. 2007).

Recent efforts have been made to understand differences in the population of mutation carriers that increase the risk of developing AD. Lower FA values were observed in the mean whole brain WM, columns of the fornix, area of the perforant pathways bilaterally and the left orbitofrontal lobe in carriers of the familial Alzheimer's disease (FAD) mutation in the preclinical stages (some MCI) of the disease (Ringman et al. 2007). Additionally, lower FA values were observed in the columns of the fornix and in the left orbitofrontal lobe in carriers of the FAD mutation in the pre-symptomatic stages (no observable cognitive impairment) of the disease.

DTI and Drug Development

The path to FDA approval of a new pharmaceutical agent or device is made treacherous by various levels of research and development (R&D), with only a handful entering preclinical testing (5%). Only 0.1% of the original group will successfully survive

clinical trials and move on to a regulatory review. The time it takes to finalize the process can range from 10 to 12 years, and the cost is prohibitively expensive (> $500 million) (Bolten and DeGregorio 2002).

Biomarkers, or a biological marker of a normal or pathological process or indicator of response to therapy, can be employed in any stage of R&D, from the drug discovery to Phase IV clinical trials. DTI is considered a functional imaging method (as opposed to molecular imaging method) because it measures tissue water mobility and is related to cellular density. In order for some quantitative DTI parameter to be considered a useful biomarker for response to therapy, among other things, it must be sensitive and specific (i.e., have a high predictive value) and it must produce information about the best time to image post-therapy. Because DTI is such a new method, clinical experience is still accumulating, and thus its link to more established clinical outcomes such as improvements in patient symptoms or in survival is not yet well established. This has not prevented investigators from studying these relationships and even proposing DTI as a surrogate marker. However, until diffusion or DTI is qualified for use as a surrogate marker in a particular disease, its greatest application may be in testing biological hypotheses in humans, and in improving our understanding of how disease and intervention interact. Here we will describe in detail one application, brain oncology and briefly highlight other disease potentials as well.

Oncology

A key question in oncology is response rate, in the context of therapeutic trials. There is precedence, albeit very little, for the use of DTI in measuring tumor response to therapy. The most compelling example is from a group that attempted to correlate the mean diffusivity, D, and the fractional anisotropy in a low-grade glioma to metabolite concentrations obtained from chemical shift imaging, a multi-voxel spectroscopic acquisition method (Sijens et al. 2007). This particular study was a case report of a single, 65 year-old male subject with a low-grade glioma. The subject was treated with 200 mg/m^2/day temozolomide and imaged after every 6, 9 and 12 cycles of therapy. The highest levels of choline were localized in the center of the tumor and systematically decreased in the center and over the whole tumor volume with treatment. The lowest levels of NAA were also localized in the center of the tumor, though only moderate increases in NAA were observed throughout the treatment regimen. Nevertheless, a positive correlation between relative concentrations of NAA and the fractional anisotropy ($p < 0.001$) was demonstrated, suggesting a re-emergence of existing, functional axonal structures concomitant with response to therapy. Additionally, a negative correlation was demonstrated between relative choline concentration and D ($p < 0.001$), suggesting a decrease in tumor cellularity and altered membrane phospholipid metabolism.

Another important contribution to drug development in brain neoplasia is the ability of DTI to resolve vasogenic edema and infiltrative tumor (Lu et al. 2003; Lu et al.

2004). This is increasingly important because anti-angiogenic therapies (or therapies that target tumor-associated microvessels) rapidly alleviate edema. Resolution of this edema may result in a lack of tumor enhancement, possibly leading to a misinterpretation that the tumor is responding to therapy. Thus, in the light of the influx of antivascular and antiangiogenic therapies, it would be prudent to have a non-invasive imaging method that can reliably differentiate between edema and tumor tissue. One group hypothesized that the mean diffusivity and the fractional anisotropy are significantly altered in the peritumoral regions of both high-grade gliomas and metastatic brain lesions, and further that the magnitude of fractional anisotropy changes in the peritumoral region of high grade gliomas are greater due to tumor infiltration (Lu et al. 2003). Compared to normal, contralateral brain tissue, the mean diffusivity in the peritumoral region of both the glioma and the metastatic lesion increased ($p<0.005$), and the fractional anisotropy decreased ($p<0.005$). The mean diffusivity was significantly lower in the glioma than in the metastatic lesion ($p<0.005$), but there was no statistical difference between the two types of lesions in the fractional anisotropy. Nevertheless, statistical differences are apparent between the normal tissue and the peritumoral edema, and thus, it is conceivable that resolution of tumor infiltration from edema may soon be possible with more advanced diffusion approaches.

Another group investigated the use of DTI to detect changes in the mean diffusivity (D) and fractional anisotropy in the genu and splenium in the corpus collosum following 45 weeks of radiation therapy (Nagesh et al. 2008). Significant increases in D and decreases in fractional anisotropy were observed ($p<0.001$) over time, suggesting disruption of the white matter architecture. Furthermore, both the perpendicular ($\lambda_{\perp}$) and parallel ($\lambda_{\parallel***}$) diffusivities increased significantly ($p<0.002$ and $p<0.04$, respectively) in a dose-dependent manner, suggesting systematic white matter injury or demyelination. Although this study has not demonstrated the DTI utility in providing a means of measuring tumor response to therapy, such a study will aid in establishing a method by which the extent of radiation-induced white matter injury and axonal degradation will be measured.

One group capitalized on a new analytical method, capable of separating the tensor into its isotropic (p) and anisotropic (q) components,

$$p = \sqrt{3}D \tag{5}$$

$$q = \sqrt{(\lambda_1 - D)^2 + (\lambda_2 - D)^2 + (\lambda_3 - D)^2} \tag{6}$$

thereby providing a method to delineate between the whole tumor region and any surrounding normal tissue infiltrated by glioma cells (Field et al. 2004). Based on the relative size of each component, three different patterns of abnormal growth emerged: (1) diffuse – the extent of isotropic diffusion (p) was greater than the extent of anisotropic diffusion (q); (2) localized – the extent of the isotropic and anisotropic regions were predominantly comparable, and tumor infiltration progressed only in regions where this symmetry failed and; (3) minimal – both regions were comparable.

Other Applications

For other brain pathologies (e.g., depression, OCD, schizophrenia and stroke), the use of DTI as an alternative metric of response to therapy is notional and is still preliminary. In a late-life depression study, patients treated with citalopram achieved remission (or failure to meet DSM-IV criteria for a depressive disorder), a factor which was correlated with significantly higher fractional anisotropy values in the right frontal matter compared to patients that demonstrated an overall lower fractional anisotropy in the same region (Alexopoulos et al. 2002). In drug-naïve patients with OCD, 12 weeks of therapy with citalopram resulted in the "normalization" of FA in the corpus callosum and internal capsule (Yoo et al. 2007), corresponding very well with an improvement in all clinical measures of response employed. Though it has been suggested that dosage of antipsychotic drugs correlate with the degree of reduced FA in the WM of schizophrenic patients (Okugawa et al. 2004), to our knowledge, there are no studies that directly correlate therapy with changes in FA over time.

For stroke, currently the main focus is to establish a method for staging an ischemic lesion in the hope that an accurate diagnosis is made and consequently, an appropriate treatment can be applied. The status of the lesion throughout the various phases of damage is critical to establishing the most effective treatment program.

Regarding research on the remaining pathologies (Alzheimer's and Huntington's disease, MS, autism and pain), available treatments are unfortunately still not disease-modifying. For example, there is no cure for Alzheimer's disease; but a program of drugs that maintain or improve cognitive function and/or slow progression of the disease, in addition to some metric like FA capable of assessing the efficacy of those drugs can have a profound effect on the quality of life for the patient. In general, since the pathophysiology of these disorders consistently manifests as an attenuation of the FA derived from DTI, any changes following successful curative or palliative therapy of these pathologies would likely result in the "normalization" or return of FA to normal values (or values comparable to contralateral normal tissue or healthy control tissue), and thus, DTI will continue to simulate a natural curiosity for its therapeutics use.

Limitations

A DTI dataset is most typically acquired using single-shot echo-planar imaging (EPI) sequence because of the inherent sensitivity of diffusion MRI to small bulk motion (e.g., head motion). Unfortunately, the trade-off for motion insensitivity is sensitivity to field inhomogeneities (Jezzard and Balaban 1995) and eddy currents (Jezzard et al. 1998), both of which affect the diffusion measurement. Furthermore, diffusion is intrinsically signal-to-noise limited. Work continues to focus on improvement of these limitations.

Concluding Remarks

Most of the studies mentioned in this chapter are low-power and preliminary, and thus, the results must be reviewed with aggressive criticism. However, with the continued improvement in the technical limitations, the potential for diffusion remains strong.

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Functional Magnetic Resonance Imaging in Drug Development

Jaymin Upadhyay, David Borsook, and Lino Becerra

Introduction

The relationship between neuronal activity and the metabolic requirements necessary to support it have been investigated as early as the late nineteenth century (Roy and Sherrington 1890). It has been known that neuronal activity is coupled with alterations in hemodynamic properties such as increased local cerebral blood flow (CBF), local cerebral blood volume (CBV), and oxygenated to deoxygenated hemoglobin concentration ratios. With the advent of neuroimaging techniques of Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI), measurements of hemodynamic changes as a means to detect neuronal activity in humans would later be possible (Lauterbur 1973; Mansfield 1977; Ter-Pogossian et al. 1969; Ter-Pogossian and Herscovitch 1985). An early use of MRI was focused on the study of neuroanatomy in both health and disease. However, with initial observations of MR signal changes due to (1) variation in deoxyhemoglobin concentration (Ogawa et al. 1990a, b), (2) CBV changes observed during contrast enhanced MRI (Kwong et al. 1991), (3) visual stimulation (Belliveau et al. 1991), and (4) oxygenation changes observed with echo-planar MRI (Turner et al. 1991), the noninvasive brain mapping method of functional MRI (fMRI) was initiated. Subsequently, blood oxygenated level-dependent (BOLD) fMRI was first applied to investigate function in the human brain (Bandettini et al. 1992; Kwong et al. 1992; Ogawa et al. 1992). This chapter explores the utility of fMRI in drug discovery and introduces the field of pharmacological MRI (phMRI) (Jenkins et al. 2003; Leslie and James 2000). A focus is given to the known benefits and limitations of fMRI as applied to characterizing the central nervous system response of current therapeutics, particularly those prescribed to treat pain. Furthermore, the potential role of fMRI as a supplemental method in the development of new therapeutics is also discussed.

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Measuring the BOLD Response with fMRI

The conventional and predominant MRI experiment involves the manipulation of hydrogen nuclei of water molecules with radio frequency (RF) pulses and both static (B_0) and spatiotemporally varying (B_1) magnetic fields. The hydrogen nuclei of water molecules are specifically manipulated due to their abundance in tissue. When hydrogen nuclei are only subjected to the B_0, the nuclei are in a low-energy state. In an MRI experiment, an RF pulse is used to transiently transition hydrogen nuclei into a high-energy level; thus, energy is introduced into the system. The time it takes for hydrogen nuclei to naturally return to the low-energy state (pre-RF excitation state) and emit energy, is expressed by two exponential time constants, T_1 (Longitudinal Relaxation) and T_2 (Transverse Relaxation). During the relaxation period, distinct types of tissue will emit specific amounts of energy that is determined by intrinsic properties of the tissue itself and also, the nearby local environment. Thus, some tissue will have a shorter or longer T_1 and T_2 times or expel a smaller or larger amount of energy based on the magnetic properties of the tissue of interest and the magnetic properties of adjacent matter. The net amount of energy that is expelled by the transitioning hydrogen nuclei of water molecules is what is measured in MRI.

In tissue samples, such as brain tissue, the T_2 times is shortened due to inhomogeneities of the tissue. This more rapid decay of the transverse relaxation is expressed as T_2*. The oxygenated to deoxygenated hemoglobin ratio in blood vessels affects the degree of inhomogeneity of surrounding brain tissue and directly determines its T_2*. Thus, in BOLD fMRI experiments the measurement of T_2* is reflective of the metabolic state of the local brain tissue, which in turn reflects the level of activation and/or deactivation of a localized neuronal population. Generally speaking, in a majority of fMRI experiments, a longer T_2* correlates with a higher oxygenated to deoxygenated hemoglobin ratio. The connection between T_2* and neuronal activation as determined by the BOLD response is given below.

Pauling and Coryell first demonstrated that deoxygenated hemoglobin is paramagnetic and the magnetic properties of blood are dependent on its physiologic oxygenation state (Pauling and Coryell 1936). Specifically, the presence of bound oxygen to hemoglobin in erythrocytes, or lack thereof, determines the magnetic properties of a bolus of blood. In the oxygenated state of hemoglobin, an outer electron of the iron molecule is bonded to an oxygen molecule. In the presence of the externally applied static magnetic field B_0, no changes in the magnetic moments or changes to the external magnetic field will occur, thus oxygenated hemoglobin behaves like a diamagnetic material. On the other hand, in deoxygenated hemoglobin, unpaired outer electrons of the iron molecule cause a large intrinsic magnetic moment, and thus, the hemoglobin in this state has the properties of a paramagnetic material. In the presence of an external magnetic field, these magnetic dipole moments will perturb or distort the magnetic field. In other words, deoxygenated hemoglobin will produce local bulk magnetic susceptibility (χ) changes relative to the surrounding tissue and impart a spatially dependent variation in spin resonant frequencies

$$\omega(x,\ y,\ z) = \gamma B_0[1+\chi] \tag{1}$$

where γ is the gyromagnetic ratio of the nuclear spin, B_0 is the applied external magnetic field, χ is the local bulk magnetic susceptibility, and ω is the resultant spatially dependent angular resonance frequency. As spins evolve in the transverse plane, which lies perpendicular to B_0, the spatial variations in the resonant frequencies will lead to phase dispersion. In addition to phase dispersion, χ will also have an effect on the measured T_2* time; specifically, it will quicken T_2* relaxation in regions with increased deoxyhemoglobin concentration. Therefore, signal loss will not only be caused by phase dispersion, but also shortened T_2 relaxation. The combined effects of decreased T_2 relaxation and phase dispersion is commonly referred to as T_2*. Signal loss or signal intensity degradation with time (t) within the imaging volume can then be expressed as

$$S = e^{-t/T_2*} \tag{2}$$

This is what was initially observed by Ogawa et al. (Ogawa and Lee 1990; Ogawa et al. 1990a). MRI pulse sequences such as echo-planar imaging (EPI), which measure or exploit these hemodynamic and magnetic phenomena, are known as T_2-weighted pulse sequences.

The BOLD response following an increase in neuronal activity is a complex mechanism with multiple known components. Following an increase in neuronal activity due to sensory stimulation, cognitive task or low frequency fluctuations during a resting state condition, the following changes occur in the capillary bed: (1) an increase in oxygen consumption, (2) oxygen extraction from the arterial blood (3) increase in regional CBF, and (4) increase in regional CBV. Interestingly, the increase in the oxygen-rich cerebral blood supply is highly excessive and much greater than the extraction and consumption of oxygenated hemoglobin. This effect causes a decrease in concentration of deoxyhemoglobin in the localized capillary bed which supply and drain blood from the activated cortical region. Thus, there is a drop in the paramagnetic deoxyhemoglobin concentration and simultaneous increase in diamagnetic oxygenated hemoglobin. Keeping in mind the signal attenuation effects of deoxygenated hemoglobin, the local region experiences less signal loss due to T_2* effects; and therefore, a localized increase in MR signal intensity results.

The mechanisms of cerebral hemodynamics and their relevance to neuronal activity are not fully understood or are still under debate. For example, a very fundamental question is what does a positive BOLD fMRI signal represent? It is predominantly believed that a positive BOLD response most often reflects an increase in excitatory activity of a localized neuronal cluster, yet the positive BOLD signal can also result from repeated inhibition of a neuronal population (Logothetis 2008). Moreover, properties (shape, duration, amplitude, etc.) of the BOLD response could depend on factors such as the neuronal substrate of interest, cognitive task being performed, type of stimulus being processed by the brain, or intersubject

variability (Aguirre et al. 1998; Birn and Bandettini 2005; Buckner 1998; Friston et al. 1998; Kruggel and von Cramon 1999; Miezin et al. 2000; Thierry et al. 1999). With such unexplained fundamental phenomenon present regarding the BOLD fMRI signal, much emphasis has been given to justifying the implementation of fMRI to study brain function, particularly to further understand the BOLD response and its relation to neuronal activity. For example, groundbreaking works by Logothetis and colleagues have validated that the BOLD response is directly reflective of the neuronal response resulting from a stimulus (Logothetis et al. 2001). Logothetis et al. showed that the BOLD responses, as measured by fMRI, and neuronal responses, as measured by local field potentials, have a linear relationship. Multiple studies have also revealed a correlation of a negative BOLD response with neuronal inhibition or spiking activity in neuronal ensembles located in motor and/or visual brain regions (Shmuel et al. 2002, 2006; Stefanovic et al. 2004). To date, neurophysiological and fMRI studies have revealed key mechanisms underlying the BOLD mechanism and its relation to neuronal activity. This being said many fundamental mechanisms of the BOLD response from the level of the neuron to entire brain systems have yet to be properly described. It is believed that further insight into the BOLD response and its relationship to neuronal activity will likely be gained by implementing a multimodal approach [fMRI, electroencephalography (EEG), magnetoencephalography (MEG), intracranial recordings, etc.] such as that utilized by Logothetis and colleagues and to do so under various experimental conditions; be it cognitive tasks, sensory stimulations, or pharmacological challenges. For further review of underlying mechanisms of the BOLD fMRI signal and its relation to neuronal activity, see reviews by Logothetis and colleagues (Logothetis 2008; Logothetis and Pfeuffer 2004; Logothetis and Wandell 2004).

Previous and Common Uses of fMRI

Over the past several years, significant technological advancements have been made in multiple domains of fMRI research, and in turn, have led to the widespread and frequent implementation of fMRI. These technical domains range from enhanced data acquisition methods that minimize image distortions to sophisticated modeling approaches which enable interactions among active neuronal substrates to be quantified (Friston et al. 2003; Li et al. 2007; Roebroeck et al. 2005; Stevick et al. 2008). Initial utilization of fMRI revolved around a basic identification of the active neuronal substrates during various types of sensory stimulation, be it visual, auditory, somatosensory, olfactory, and also pain processing. Global brain activation arising during the performance of motor and complex cognitive tasks continues to be of equal interest. More recent fMRI studies, however, focus not only on which brain regions yield a significant BOLD response during a specific stimulation or task, but also the complex interactions that underlie neuronal processes throughout the brain. Two types of neuronal interactions or connectivity can occur on small and large network scales: functional connectivity and effective connectivity (Buchel and Friston 1997;

Friston 2002; Friston and Buchel 2000; Friston et al. 1993a, b, 2003; Horwitz 1990, 2003; McIntosh and Gonzales-Lima 1994; McIntosh et al. 1994). While functional connectivity refers to coherence of neuronal activity among multiple neuronal structures, effective connectivity identifies how the activity in one region of interest may drive the response in another brain region. Thus, modeling approaches such as Dynamic Causal Modeling (DCM), Granger Causality Mapping (GCM) or time-resolved fMRI enable functional and effective connectivity to be defined despite the fact that the timescale of BOLD signals are not at the same level as neuronal spiking (Friston et al. 2003; Roebroeck et al. 2005). It is noted that modeling approaches such as DCM and GCM have limitation such as an inability to concretely determine whether or not an interaction between two brain regions is direct or indirect. Thus, combining fMRI with other MRI methods such as diffusion tensor probabilistic tractography where structural connectivity can be characterized is extremely appealing (Aron et al. 2007; Kim and Kim 2005; Upadhyay et al. 2006, 2008).

A recent application of fMRI is the characterization of the brain during the resting-state condition, where no stimuli are given or cognitive tasks carried out (Biswal et al. 1995; Greicius et al. 2003; Gusnard et al. 2001; Raichle et al. 2001). The goal of resting-state fMRI studies is to determine whole brain default networks or multiple brain regions expressing a common spontaneous and continuous activation or deactivation pattern. Those structures identified as being part of a single network are assumed to portray functional connectivity among each other. Initially, default neuronal networks in humans were characterized by imaging methods such as MEG (Ribary et al. 1991). However, model-free analysis methods such as Independent Component Analysis (ICA) have been implemented to extract default connectivity patterns or networks from fMRI data throughout the brain, where multiple neuronal clusters of a network are observed to express the same low-frequency modulation as represented by the BOLD fMRI signal (Beckmann et al. 2005; Beckmann and Smith 2004; De Luca et al. 2006; Esposito et al. 2008; Formisano et al. 2004; Ma et al. 2007). Resting-state fMRI studies have not only been instrumental in defining default networks, but also, in determining how these networks are compromised in a diseased state (i.e., schizophrenia, Alzheimer's or autism spectrum disorder) or subsequent to structural alterations in gray or white matter (Bassett et al. 2008; Cherkassky et al. 2006; Johnston et al. 2008; Sorg et al. 2007; Supekar et al. 2008). Therefore, resting-state fMRI studies may yield an account of baseline activity for a healthy or specific patient population. A characterization of baseline activity could be vital, particularly when comparing the neuronal response to a sensory stimuli or cognitive task being carried out between healthy control and patient populations. Differences in the BOLD response observed between the two groups during sensory stimulation or performance of a task could be a result of underlying differences in the default network(s) of the brain that exist during baseline or resting-state. This is in contrast to the idea that differences in the BOLD response between control and patient cohorts stem solely from processing of stimuli or are task specific. Furthermore, fMRI analysis methods, particularly ICA, that identify whole brain functional and effective connectivity can also be extended to identify and filter widespread time series which are not of interest and hamper a more exact

characterization of relevant fMRI time series. These unwanted time series include, but are not limited to linear drift, head movement artifact, BOLD responses stemming from macrovasculature (veins and arteries) or noise originating from MR scanner hardware (Beckmann and Smith 2004).

Pharmacological fMRI: Applications in Drug Development

The field of pharmacological MRI (phMRI) is a relatively nascent application (~10 years) of MRI yet one that shows great promise in defining the neurophysiological effects of a drug of interest and more importantly, in the development of new therapeutic compounds (Jenkins et al. 2003; Leslie and James 2000). Depending on factors such as the developmental stage of the drug of interest, phMRI can be appropriately applied to investigate the brain response to a specific compound in both animals and humans. The ability to perform similar experiments in animals and humans would enable a qualification and quantification of translational aspects of a drug.

In a very basic sense, phMRI involves the measurement of changes in cerebral hemodynamic properties due to an administered pharmacological challenge, which can be of oral or intravenous form in human investigations and largely in intravenous or intrathecal form in animal studies. In human or animal phMRI investigations, a primary objective is to assess what brain regions does the drug target either directly or indirectly as a result of interconnectivity of brain regions. The cerebral metabolic changes that can in turn produce detectable changes in the MR signal include variations in CBF, CBV, and oxygenated hemoglobin concentration or BOLD contrast. CBF, CBV, and/or BOLD signals can be pharmacologically altered by, (1) systemic physiological changes (e.g., heart rate, end-tidal CO_2, or respiratory rate), (2) compound–receptor binding, or (3) direct elevation/suppression of neuronal spiking. While phMRI does not yield a direct quantitative or qualitative measure of receptor occupancy caused by the drug as is possible by PET, other robust and useful measures representative of drug action are possible. One such measure termed herein as the BOLD infusion response, is the change in BOLD fMRI signal arising from intravenous drug administration during the continuous collection of fMRI data (Becerra et al. 2006a; Borras et al. 2004; Wise et al. 2002). PhMRI paradigms that measure the BOLD infusion response contains three aspects; (1) Measurement of the BOLD fMRI signal during baseline and prior to drug administration. (2) Measurement of the BOLD response during drug administration. Depending on the compound of interest, the infusion can occur as a single or multiple injections of a bolus of drug plus vehicle, and also, as a continuous drip of drug plus vehicle. (3) Measurement of the BOLD fMRI signal upon completion of drug administration. Assessing the BOLD fMRI signal during and after compound administration enables an observation of which brain structures are affected by the drug and at which point in time do certain structures show an increase or decrease in BOLD activity. This temporal point is referred to as the inflection point. While some

structures may show an immediate response to the drug, other brain regions may show and inflection points a few minutes after drug administration. Furthermore, depending on the pharmacokinetics (half-life, Cmax, Cmin, Tmax, etc.) of the drug, structures showing a sustained elevated or suppressed response can also be identified. In phMRI investigations where multiple doses of the same drug are of interest, the amplitude of the sustained elevated or suppressed BOLD fMRI signal for each dose would likely reflect a dose response for each drug dose.

Characterizing the BOLD infusion response is particularly appealing given that both blood samples can be simultaneously collected and physiological signals monitored during phMRI data acquisition. This enables a very important and strong combination of conventional pharmacokinetic/pharmacodynamic assessment of drug concentration, measurement of systemic physiological changes, and characterization of global brain response to the drug. Conventionally, this infusion response to the drug is compared to infusion of solely saline or vehicle. PhMRI studies containing a single dose often implement a single-blinded experimental paradigm, while those interested in, for example, two doses would utilize a double-blinded cross over paradigm.

With the recent interest and development in functional and effective connectivity analysis methods, particularly during the resting-state condition, efforts in phMRI investigations have been made to extract distinct networks that represent neurotransmitter systems of interest (Schwarz et al. 2007a, b, c). It is plausible to probe the whole brain to extract a network of neuronal substrates that are all direct targets of the pharmacological challenge, while other identified networks consist of substrates that are downstream targets of a specific neurotransmitter circuitry. Thus, a spatial map representing the neurotransmitter systems of interest would consist of cortical and/or subcortical structures throughout the brain possessing similar temporal features in their BOLD fMRI time series. A second and distinct spatial map would correspond to a downstream network of substrates sharing a common and slightly delayed BOLD fMRI time series. With regards to measuring the BOLD infusion response, structures showing a very early inflection point (direct targets of the drug) in the fMRI signal at the start of drug administration may indicate one network, whereas structures showing a significantly later inflection point may represent a downstream network.

A common methodological difference between human and animal phMRI studies is the specific MR data acquisition method implemented to measure cerebral hemodynamic changes as a result of the pharmacological challenge. In animal phMRI, alterations in CBV are most commonly measured with an administration of MRI contrast agents such as Endorem (Guerbet, France), ultrasmall superparamagnetic iron (USPIO), monocrystalline iron oxide nanocompound (MION). Human phMRI studies avoid the use of contrast agents when possible and utilize endogenous markers of cerebral hemodynamic changes. Thus, human phMRI investigations predominantly exploit changes in blood oxygenation or CBF. Changes in oxygenation are measured with BOLD fMRI, while CBF changes are probed with arterial spin labeling (ASL) (Detre and Alsop 1999; Detre et al. 1992; Detre and Wang 2002; Williams et al. 1992). There are technical limitations of using BOLD fMRI to extract CNS structures

targeted by a particular drug. For example, in phMRI studies where the compound has long blood–brain barrier penetration times or long times to reach Cmax, long duration fMRI scans (>10 min) are needed to capture the increase or decrease in the BOLD signal off of baseline. This is particularly true in intravenous drug infusion studies. Such long fMRI scans are prone to the presence of an increasing or decreasing signal drift, where the drift could hinder the ability to accurately quantify a positive or negative CNS response to the drug. It is also plausible that a compound directly targets a specific brain structure; however, the change in hemodynamics resulting from the drug does not subsequently produce a strong enough BOLD response.

Alternatively to BOLD fMRI, ASL is an MR technique yielding very similar capabilities in measuring CBF as PET imaging (Detre and Alsop 1999; Detre et al. 1992; Detre and Wang 2002; Williams et al. 1992). Specifically, ASL offers a direct measure of tissue perfusion of blood occurring within the capillaries and arterioles. The ASL method involves magnetically tagging arterial blood just prior to its entry into a volume of brain which is of interest, and altering the longitudinal relaxation or T_1 times within that volume. A second brain volume, the control volume, is also collected where arterial blood is not magnetically tagged. The tagged and untagged volumes are always collected at adjacent timepoints and in a pair-wise fashion. If there is a localized increase in metabolic demand, the local CBF will increase and yield an increased amount of magnetically tagged blood in the perfused brain tissue. Thus, the longitudinal relaxation or signal change is proportional to the amount of perfusion of magnetically labeled arterial blood. It is noted, however, that the specific ASL data acquisition techniques implemented would determine whether an increase or decrease in signal change correlates with an increase in CBF.

In a very basic sense, the perfusion or signal changes in ASL experiments are obtained by a pair-wise subtraction of magnetically tagged and nontagged brain volumes that are collected at adjacent timepoints. This method of obtaining contrast leads to the ASL technique to not be hampered by signal drift or motion artifact. This is in contrast to BOLD fMRI where image artifacts such as signal drift are problematic, particularly in experimental paradigms that utilize long fMRI scans. Furthermore, the BOLD response is reflective of changes in concentration of oxygenated blood, blood flow, and blood volume occurring in venules and veins. Given that ASL solely measures changes in CBF, a more specific metabolic process related to neuronal activation or deactivation can be probed. Despite some of the benefits of ASL over BOLD fMRI, this particular MR technique is less frequently implemented in comparison to BOLD fMRI for five fundamental reasons: (1) low spatiotemporal resolution, (2) lack of whole brain coverage, (3) small signal changes (~1% or less), (4) need for better modeling of CBF as measured with various ASL techniques such as that proposed by Gallichan and Jezzard and Parkes (Gallichan and Jezzard 2008; Parkes 2005) ,and (5) need for better MR pulse sequence design for ASL experiments such as that proposed by Holm and Sidaros or Garcia et al. (Garcia et al. 2005; Holm and Sidaros 2006). Once some of the technical challenges relevant to ASL data acquisition and analysis are addressed, the ASL technique is likely to be comparable MR technique for fMRI investigations in general. To date, BOLD fMRI is currently the most common means to robustly measure cerebral hemodynamic changes resulting from a drug.

A conventional nonneuroimaging means by which drug action is assessed in humans is pharmacokinetic (PK) and pharmacodynamics (PD) modeling of a drug in conjunction with an evaluation of its side effects. The PK/PD modeling method primarily yields an indication that the compound is present within the subjects system, and at what concentrations the compound is present throughout time or receptor association/dissociation kinetics. Many compounds under investigation have dose-dependent side effects such as cognitive or motor impairment, physiological changes (e.g. increases or decreases in heart rate or respiration), or nausea and vomiting. The majority of side effects are easily evaluated and monitored during or after drug administration and subsequently compared to baseline. For example, cognitive and motor dysfunction can be examined via performance of cognitive or motor tasks or self-reported hedonic evaluation by the subject, while physiological signals are easily monitored. Combining PK/PD modeling with simultaneous evaluation of side effects play an important role in clinical studies where the optimal dose for a drug is sought. In such clinical studies, it is possible to obtain the dose at which there is a balance between treatment and relief of an ailment and the side effects a patient experiences.

Clinical investigations as those described above are extremely important in the drug discovery process. However, very large subject populations are often required to determine if a drug is effective or to have a statistically powerful result. In these clinical investigations, the subject population can be easily on the scale of hundreds of patients and healthy controls. Thus, performing clinical investigations with large numbers of patients and controls indefinitely results in a significantly large amount of time and money needed to complete the study. PhMRI is not meant to be a method that completely replaces PK/PD modeling or neuropsychological assessment, but rather a method that can be used in conjunction with these conventional and accepted methods that evaluate drug action. Given that such large subject populations are often not needed in clinical fMRI studies, it is possible that phMRI can be used to test drug efficacy and to do so in a more efficient manner.

fMRI of Drug Effects: Interactions Between Drug and Processing of Stimulus

PhMRI has been implemented to examine several neurotransmitter systems in healthy controls and patient populations. Past investigations have observed the impact of a drug on a specific neurotransmitter pathway, and in addition, how that drug positively or negatively affects brain function. For example, Mattay et al. and Furey et al. pharmacologically modulated the dopaminergic and cholinergic system circuitry in healthy controls, respectively (Furey et al. 2000; Mattay et al. 2003). In both studies, the performance of working memory was tested upon modulation of either neurotransmitter system. Similar types of studies have been carried out in patients suffering from psychiatric illnesses, neurological disorders, and drug addictions (Honey et al. 1999; Pariente et al. 2001; Sell et al. 1997). A review of past applications of phMRI in healthy and diseased states is available elsewhere

(Honey and Bullmore 2004). In this section, a specific focus is given to the application of fMRI in pain processing and how phMRI has become a pharmacological tool for assessing therapeutics for pain treatment and the development of new drugs. Although functional imaging of pain using PET was applied in the early 1990s (Di Piero et al. 1991; Jones et al. 1991), Davis and colleagues first initiated the utilization of fMRI to characterize the whole brain response to pain in 1995; only a few years after the discovery of fMRI (Davis et al. 1995). However, during the past 5–10 years the potential of utilizing fMRI to understand basic aspects of somatosensory and pain processing has gained a great deal of impetus in academia and the pharmaceutical industry (Borsook et al. 2006; Borsook and Becerra 2006; Schweinhardt et al. 2006; Tracey 2008; Wise and Tracey 2006).

Understanding pain processing is extremely complex as a result of the numerous brain processes that directly determine how an individual perceives pain. Pain is a phenomenon that involves large-scale neuronal networks that process sensation, emotion, anticipation, fear, evaluation, and expectation. To complicate matters, the same brain structures may modulate any one or more of these brain functions. Thus, the emotional and cognitive state along with the behavioral reaction to pain is just as if not more significant than the basic sensation of pain. FMRI has been implemented to elucidate the functional role of neuronal networks relevant to pain processing, be it transmission of pain to cortical and subcortical structures or evaluating how painful a stimulus is. These studies, simply termed "pain imaging" studies, have used fMRI to comprehend how healthy controls perceive acute (experimental) pain, and how patients perceive acute pain while coping with chronic pain symptoms. Acute pain is commonly given in the form of noxious mechanical (von Frey, pin prick, pressure), thermal (cold, heat, laser), or chemical (capsaicin) stimuli. The responses to these painful stimuli are often compared to innocuous modes of the painful stimuli, which then allows for a comparison of somatosensory processing with pain processing. For example, the BOLD response to a noxious heat stimulus at 49°C could be compared to an innocuous heat stimulus at 42°C or innocuous brush stimuli.

The good majority of fMRI studies focusing on pain and somatosensory processing involve healthy subjects and their reaction to acute pain. Such studies have proven invaluable in further defining the functional properties of peripheral and central neuronal substrates and pathways of pain circuitry. Functional properties include intra-structural somatotopic organization, inter-structural functional, and effective connectivity or the unique temporal features of the BOLD response to a noxious stimulus across brain regions (Baliki et al. 2006; Becerra et al. 2001; Bingel et al. 2004a, b; Borsook, et al. 2003; Brooks et al. 2005; Chen et al. 2002; DaSilva et al. 2002; Labus et al. 2008; Ohara et al. 2008). Some findings such as somatotopic organization in the trigeminal ganglion were known prior to the functional imaging results using invasive anatomic or physiological experimental methods (Williams et al. 2003; Ziyal et al. 2004). However, if known functional properties of structures relevant for pain processing can be shown noninvasively with fMRI, then these same structures can be further examined using the same fMRI methodology during the diseased state (Becerra et al. 2006b), upon influence

of a drug (Wise et al. 2004) or possibly in a postsurgical state. BOLD fMRI has also revealed unknown functional properties of pain processing. One notable observation initially demonstrated with fMRI is that of a single peak BOLD response to an innocuous stimulus (soft brush or low temperature heat stimuli), while dual peak BOLD responses were detected in response to a painful heat stimuli in regions such as primary somatosensory cortex (S1) (Becerra et al. 2001; Chen et al. 2002). These studies have suggested that the dual peak in the BOLD responses arise from interaction of multiple neuronal circuits (e.g., sensory and reward circuits) or input from multiple circuits into a common structure (i.e., S1). In the case of a phMRI study of pain processing, it would be of interest to determine if a particular drug affects the temporal or physical features of the dual peak BOLD response, and would in turn be an indication of a marker of drug action.

To a lesser degree, pain imaging studies have included patient populations suffering from physical pain symptoms. Physical pain can be subdivided into two classes: nociceptive pain and neuropathic pain. While nociceptive pain results from harm to tissue, neuropathic pain arises from abnormalities in the central or peripheral nervous systems. These abnormalities of neuropathic pain include structural lesions in gray or white matter, local or network level chemical imbalance or even cortical thinning. According to the International Association for the Study of Pain, neuropathic pain is "inherited or caused by a primary lesion or dysfunction of the nervous system."

It is noted that the abnormalities causing neuropathic pain are also likely to be associated with or can cause dysfunction in behavior, cognition, and overall mental health. Long-term exposure to pain cannot only lead to psychiatric problems (depression, anxiety, substance abuse), but these same psychiatric illnesses can cause chronic pain or change pain and somatosensory processing (Borsook et al. 2007; Clark et al. 2008; Fava 2003; Ploghaus et al. 2001; Smith et al. 2002). For example, neurological disorders such as autism spectrum disorder are known to be associated with hyper- or hyposensitivity to touch and pain (Miyazaki et al. 2007; Nader et al. 2004). These correlations between pain and psychiatric illnesses or neurological disorders are not surprising given that there are neuronal structures or networks that are relevant for pain processing that overlap with those that are essential for mediating a frame of mind. Structures such as anterior cingulate cortex, insula, amygdala, and nucleus accumbens are often observed to be active as a result of the emotional response to pain (Aharon et al. 2006; Becerra et al. 2001, 2004; Craig et al. 2000; Schweinhardt et al. 2008), yet are structures that generally are important for processing mood, emotion, pleasantness, etc. (Lane et al. 1997; Murphy et al. 2003; Phan et al. 2002; Wager et al. 2003). Thus, it is likely that diseases such as chronic pain are neurological disorders with a psychological disturbance as a result of the interconnectivity between specific structures and networks (i.e., sensory, emotional, reward/aversion). This being said, the implementation of fMRI is very important to studying chronic pain given that the whole brain or multiple neuronal networks can be simultaneously probed for function and dysfunction.

FMRI has been applied to a number of clinical pain populations. These pain diseases include neuropathic pain (Becerra et al. 2006b; Endo et al. 2008), chronic

pain (Baliki et al. 2008; Giesecke et al. 2004; Schweinhardt et al. 2008), complex regional pain syndrome (CRPS) (Lebel et al. 2008; Maihofner et al. 2005), and fibromyalgia (Cook et al. 2004; Gracely et al. 2004). To develop therapeutics it is extremely important to study these patient populations directly considering that patients are very likely to be in a different emotional and cognitive state in comparison to healthy controls that experience acute or experimental pain during an fMRI study. This is not to say that important conclusions and findings cannot be made about pain imaging studies solely consisting of healthy controls, but difference in processing of acute pain stimuli are likely present in patients suffering from clinical pain. There are a few ways to better characterize pain processing in general and to also evaluate the efficacy of pain therapeutics. For example, it is suggested that resting-state fMRI be performed in the control and clinical pain populations to quantify what difference are present between the two groups at baseline and then characterize the response to acute pain in control and clinical pain populations. With respect to the testing and development of therapeutics for pain treatment, the same fMRI experimental paradigms should be performed both in healthy and then perhaps in patient populations.

A further application of fMRI is the combination of fMRI and phMRI in healthy and clinical pain populations. In these studies, when a pharmacological challenge is introduced, a quantification of how the drug modulates the central nervous system response to an acute noxious stimulus is obtained by a simple comparison of the same stimulus during a placebo condition. Wise et al. performed the first combined pain fMRI and phMRI study in healthy controls, where the impact of remifentanil (μ opioid receptor agonist) on global brain activation was assessed during processing of noxious heat stimuli. In their investigation, remifentanil was observed to reduce pain-related activation in insular and anterior cingulate cortices (Wise et al. 2002). Subsequently, others have carried out similar studies using drugs such as naloxone (μ opioid receptor antagonist) (Borras et al. 2004) and gabapentin (voltage-gated N-type calcium ion channels) (Iannetti et al. 2005). Studies implementing fMRI and phMRI in clinical pain populations have not been as frequent as those utilizing healthy volunteers. Morgan et al. administered amitriptyline (serotonin and noradrenaline reuptake inhibitor) to patient suffering from clinical pain and demonstrated a relationship between activation in reduced perception of pain and activation in the anterior cingulate cortex (Morgan et al. 2005). Similarly, Baliki et al. found that cyclooxygenase-2 enzyme (COX-2) inhibitor (anti-inflammatory) prescribed to arthritic patients reduced both the self-reported pain intensity and brain activation in region such as the anterior insula and secondary somatosensory cortex (Baliki et al. 2005). Moreover, similar studies in rat models of neurological disorders related to pain (i.e., neuropathic pain) have also been recently carried out (Millecamps et al. 2007). The results of these types of combined fMRI and phMRI studies in patient populations can be difficult. In these investigations, the brain of the patient is likely to be permanently altered in an unknown manner by the long-term effects of the disease and therapeutics taken. In addition, brain activation is temporarily affected by the compound that is under investigation and the noxious or innocuous stimuli that is given during the fMRI study. Thus, it can become difficult to determine

to what degree each factor (i.e., disease, drug, stimuli) contributes to the observed BOLD signal or activation patterns.

When performing fMRI and/or phMRI studies in clinical pain patients some possibly confounding factors should be taken into consideration. The patient group is likely to consist of individuals who have taken different types of drugs for pain treatment and for different amounts of time. Also, each patient is also likely to experience symptoms of his or her disease for a different duration. To what extent drugs and disease structurally and functionally alter the brain is in part determined by how long the patient has been on drug treatment or how long the disease has persisted. These confounding issues are likely to add variance to the data, thus making group-level conclusion and comparison to control datasets slightly more difficult in comparison to a healthy population. Furthermore, the experimental paradigm implemented in the control cohort may not be easily applicable in the patient group. In pain diseases such as CRPS, some subjects may not be able to sustain certain stimuli even if they are innocuous in consequence to a hypersensitivity to touch and pain. Some clinical pain patients (migraine sufferers, chronic back pain, CRPS) may simply not be able to simply withstand the acoustic noise or strong vibrations of the MRI.

Both fMRI and phMRI data can be combined with behavioral measures such as a self report of perceived pain at the time of stimulation by the subject and during the fMRI acquisition. It is not concretely and concisely defined as to what extent the measured brain response to pain is telling of the reported pain rating or vice versa. It has also yet to be determined if the level of variance known to be present in neuroimaging data is analogous to solely behavioral results. Nonetheless, the ability to observe how brain activity, be it the response localized to a specific structure or the global brain response, correlates to a behavioral measure is highly useful. In a combined fMRI and phMRI study where noxious heat stimuli are presented, if subjects report lower pain ratings in conjunction with reduced BOLD activity upon receiving a therapeutic instead of placebo, it would be possible to assess drug action from two distinct but highly relevant perspectives. Furthermore, inclusion of PK/PD results can also be relatively easy to incorporate to determine to what extent drug concentration has on analgesic effects.

As mentioned above, pain processing can elicit activity in neuronal substrates or even specific segments of substrates that are part of sensory, pain, attention, emotional or reward networks. The substrates of these networks are likely to overlap with neurotransmitter circuitry targeted by the pharmacological challenge of interest. Thus, concisely characterizing drug action or the cause of analgesia in pain fMRI experiments can be challenging. Consider a drug such as buprenorphine (mixed agonist and antagonist for μ opioid receptors), which is often used to treat various types of clinical pain. Given that μ opioid receptors are found in a wide range of neuronal structures modulating pain sensation or the emotional aspects of pain perception, it can become difficult to determine the underlying cause for an analgesic experience. Do subjects experience analgesic effects to painful stimuli due to the effect of the drug on somatosensory structures (i.e., primary or secondary somatosensory cortex), or is the effect more on emotional structures (i.e., amygdala or anterior cingulate cortex)? With fMRI it is possible to compare properties such

as functional and effective connectivity or temporal features of the BOLD fMRI signal in regions of interests between drug and placebo runs. Such comparisons in addition to the characterization of the infusion response to the brain may make it possible to better determine which neuronal networks or structures are directly targeted by the pharmacological challenge and result in analgesic effects. It is believed that fMRI, BOLD or ASL, is the best currently available tool to assess drug action on functional brain activity and no other currently available noninvasive clinical methodology has such capabilities.

Standardization and Reproducibility

Compared to many of the electrophysiological based methods (i.e., single and multi-unit recordings or local field potential recordings) that measure brain activity, BOLD fMRI is relatively in an earlier developmental stage. Currently, there is widespread use of fMRI internationally, and the profound usefulness of fMRI in understanding the function and organization of the brain at the level of a single neuronal structure to the level of a neuronal network is recognized beyond the neuroimaging community. This having been said, the present mindset within and outside of the neuroimaging community is that fundamental aspects of the fMRI technique relevant to the reproducibility of fMRI data have yet to be concretely defined and accepted. The reproducibility of fMRI data, either within a study or across multiple studies examining a similar hypothesis or theory, can be compromised by factors in two major domains: nonphysiological and physiological or cognitive. In this section, we aim to describe how variances in these two domains can compromise the reproducibility of fMRI data.

Nonphysiological

The fMRI technique is widely implemented and the MR scanner systems across neuroimaging facilities can differ. MR scanner variations include which vendor is used (Siemens, Philips, GE, Varian, etc.), static field of the MR system (1.5, 3.0, 4, or 7 Tesla), receiver head coil (quadrature or phased-array), or MR gradient systems. Moreover, the above MR scanner hardware factors cannot only vary between MR imaging facilities, but within site instabilities of the MR scanner can also exist across time. For example, it is quite possible that inhomogeneities in the magnetic field or shimming can vary across time. However, such within site variability is easier to control, given that routine quality assurance (QA) checks are commonly performed to assure that scanner stability, signal-to-noise ratio, and contrast-to-noise ratio, are within the limits of manufacture's specifications. Routine QA checks are particularly vital for the validity of longitudinal fMRI studies. Given the complexity of the fMRI technique, from acquisition to analysis to interpretation of data, a number of factors outside of the physiologic domain exist that can contribute variance to the measured fMRI signal.

A fundamental question is how reproducible is fMRI data? Reproducibility of fMRI can be hampered by variance introduced by factors such as: (1) differentiation in MR scanners and scanner hardware, (2) pre- and post-processing analysis methods, (3) statistical test (*t*-test, Pearson's correlation coefficient, etc.) used to represent results, (4) subject population, or (5) method used to define region of interest or neuronal substrates. Recently, a number of multi-site and test–retest studies were performed by a number of different groups to address issues related to reproducibility and reliability of fMRI data (Friedman and Glover 2006; Friedman et al. 2008; Loubinoux et al. 2001; Smith et al. 2005; van Gelderen et al. 2005; Zou et al. 2005). Most recently, a series of multi-site and test–retest or scan-to-scan fMRI studies were performed by Friedman and colleagues and Zou et al., (Functional Biomedical Informatics Research Network (www.nbirn.net)), where the same set of healthy male subjects were scanned multiple times to assess test–retest reliability (Friedman and Glover 2006; Friedman et al. 2008; Zou et al. 2005). The same subjects and the exact same fMRI experimental paradigms (sensory-motor tasks) were carried at ten distinct MRI facilities to quantify inter-site variability. While within site test–retest reliability of fMRI results such as percentage signal change or contrast-to-noise ratio were found to be high, high variance was detected when performing site-by-site comparisons, thus causing variance in fMRI results to be high. Nonetheless, in subsequent analysis, simple analysis modifications (i.e., redefining regions of interest) did yield statistically significant results. Other studies have also shown that test–retest reliability of fMRI data is high, yet the implementation of slightly different analysis methods (i.e., spatial filtering, temporal filtering, intensity normalization, functional volume registration method to standardized space, etc.) can lead to significantly different results (Smith et al. 2005). Reproducibility studies have been performed under a number of different experimental paradigms and have reported good reproducibility of data. The fMRI paradigms include: (1) visual stimulation, motor task, and cognitive tasks (Smith et al. 2005; van Gelderen et al. 2005); (2) working memory tasks (Casey et al. 1998; Manoach et al. 2001); (3) sensorimotor tasks (Friedman et al. 2008; Loubinoux et al. 2001); or (4) learning tasks (Aron et al. 2006). However, as suggested by Friedman et al., when performing multi-site or multi-scanning session scans, it may be best to initially carry out reproducibility studies prior to the fMRI study involving the main and original scientific hypothesis. Doing so may reveal sources (i.e., scanner hardware or analysis procedure) of instability that would introduce a significant variance into the data, and also define if certain statistical benchmarks are met relevant to reproducibility and reliability of data.

Physiologic or Cognitive

The variance in the data could stem from the subjects' level of attention, or even the subject becoming accustomed to the MRI environment. This is true whether it is within a single subject's scanning session, across scanning sessions of the same subject or across scanning sessions of different subjects. For example, if a subject

is naïve to receiving an MRI, that subject may be under a certain amount of anxiety in comparison to a subject that has had an MRI or is completely at ease while being inside the scanner. Such subject-dependent cognitive factors would likely affect how well the task is performed or how much attention is focused on the stimuli being presented. In turn, the brain response elicited by the task or stimulus may be unstable across time. Another means by which the BOLD response may be altered or become unstable in consequence of cognitive factors is related to the subjects' habituation to a stimulus or their performance of a task. It is often the case that multiple runs of the same stimulation paradigm or performance of a task are necessary to achieve adequate signal-to-noise ratio. Depending on the specific paradigm, the brain response to a stimulus in the beginning of the fMRI scanning session may have a slightly higher percentage signal change in comparison to the response toward the end. In combined fMRI and phMRI studies, it could be possible that an increase or decrease in the subjects' BOLD response is incorrectly attributed to drug action. To decrease the occurrences of subject- or cognitive-dependent affects and improve time-dependent BOLD response stability, simple design procedures can be implemented such as practicing of a task outside of the scanner or scanning subject who are unperturbed by the MRI environment.

Conclusion

The techniques of fMRI and phMRI have gained a great deal of momentum in the drug discovery community. The ability to combine fMRI and phMRI with other MRI techniques (MR spectroscopy or diffusion tensor imaging) as well as other currently implemented experimental methods (behavioral measures or PK/PD analysis) make fMRI and phMRI even more attractive to implement in determining the efficacy of a pharmacological challenge of interest. Fundamental aspects of fMRI reproducibility, signal stability, and reliability must be determined to meet statistical benchmarks in order for further validation of fMRI implementation in drug discovery and development. Once these benchmarks are met in conjunction with further development of fMRI data acquisition and analysis methods, ASL and BOLD fMRI are likely to be the optimal methods to assess drug action on the brain for both current and new therapeutics.

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Magnetic Resonance Imaging of Pharmacological Systems

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What Is Pharmacological MRI

Pharmacological MRI (phMRI) is a relatively novel application of MRI and is broadly defined as the use of a pharmacological agent (either therapeutically or as a neurochemical probe) in MRI experiments. Such phMRI studies hold great promise to better understand intrinsic neurobiological mechanisms and may provide an important new window into disease states and their treatment. The drug (usually a receptor agonist or antagonist) is typically used as a direct probe of a neurotransmitter system or receptor type/subtype or as a modulator of a particular cognitive or affective system in healthy individuals or applied therapeutically in a disease state (or in preclinical models).

One can identify at least four general classes of imaging experiments using pharmacological agents: (1) acute drug challenge to determine the sites and properties of drug action, including such pharmacokinetic parameters as dose-response properties, location of CNS effect, time to peak, half return and effect magnitude; (2) to understand the mechanisms of a cognitive or emotional construct or process, for example, nicotine and working memory or sustained attention, marijuana and implicit memory; (3) to study basic neurochemical processing mechanisms, for example, role of DRD1 in working memory, cholinergic basis of time perception, memory or attention; and (4) to better understand the pathobiology of or treatment for a disease (e.g., drug abuse, Parkinson's and Schizophrenia).

Finally, when designing any phMRI study, one must always consider the pharmacodynamic and pharmacokinetic properties of the drug to be administered including: route of administration, rate of entry into the CNS, duration and termination of action, drug metabolism versus redistribution, tolerance – either tachyphylaxis or as a consequence of chronic use, peripheral versus central properties, drug-induced

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cardiovascular or respiratory effects that could impinge on the hemodynamic signal and, finally, the safety profile of the drug, always keeping in mind the hostile MRI environment if the need arises to intervene medically. While a pharmacologist would find these entries rather trivial, when combined with, for example, the technical demands and limitations of cognitive, affective or pathological conditions, they can be quite daunting. For example, the need rises to perform a task over multiple events or blocks to enhance statistical power needs to be considered in the face of a drug whose action is shorter than the task being studied. Further, unlike studies in animals, drugs are most often given systemically in humans and thus available to the entire brain based on blood flow distribution. Thus, interpretation of primary from secondary sites of action may be difficult to dissociate (for reviews of phMRI studies, see [Honey and Bullmore 2004; Salmeron and Stein 2002]).

What Can Be Measured

Hemodynamic Signals

It has long been recognized that healthy brain function depends upon the continuous provision and regional distribution of oxygen and glucose. Decades of experiments in humans and laboratory animals have established the tight coupling between regional cerebral blood flow (CBF) and neuronal activity, as first hypothesized by Roy and Sherrington (1890). With the advent of the 2-deoxyglucose technique (Sokoloff 1976), this notion was extended to neuronal activity – metabolism coupling. Such tight coupling has formed the theoretical basis for employing various hemodynamic indices, including CBF, cerebral blood volume (CBV) and blood oxygenation as surrogates of neuronal activity. Functional MRI techniques exist that permit imaging with contrast determined by each of these hemodynamic parameters, which are named perfusion imaging, CBV-weighted fMRI and blood oxygen level dependent (BOLD) fMRI.

BOLD contrast (Ogawa et al. 1993) provides the highest sensitivity, can provide whole brain coverage in about 2 s, and the pulse sequence is provided on modern MR scanners. Perfusion imaging using arterial spin labeling (ASL) can achieve better capillary specificity and long-term temporal stability. However, this technique remains less useful for typical neuroimaging studies due to lower sensitivity and less brain coverage with a single RF “tag” pulse. Nevertheless, unlike the BOLD signal, which is measured in arbitrary units, ASL can quantitatively measure CBF both at baseline and during activation, which are important considerations for both normal and pathophysiological studies. ASL can be particularly useful for studying the long-term effect of pharmacological interventions, for example, in the case of slowly developing therapeutic effects of antidepressants or the effects of withdrawal from chronic drug use.

A potential nonhemodynamic, albeit based upon hemodynamic measures, MRI measure is regional cerebral metabolic rate of oxygen ($CMRO_2$). At rest, oxidative

metabolism of glucose is the major source of ATP generation in the brain. By measuring CBF, CBV and BOLD signal, and following biophysical modeling (Hoge et al. 1999), $CMRO_2$ can be calculated. The key limitations lie in the fidelity of the measurements and the fidelity of the models that address the nature of neurovascular responses that are highly dynamic and heterogeneous. Nevertheless, $CMRO_2$ directly reflects metabolic demand, which is presumably virtually entirely of neuronal origin.

Finally, a recent noninvasive technique called "vascular space occupancy" (VASO) has been put forward as sensitive to CBV changes (Lu et al. 2003). This technique, which remains to be fully established, nulls the blood signal at the time of data acquisition, thus reducing signal intensity in active voxels. VASO has the potential for greater spatial specificity at comparable sensitivity to the BOLD signal. Noninvasive simultaneous measurements of CBF, CBV and BOLD signal in human subjects has been proposed (Yang et al. 2004).

Animal phMRI-Specific Contrast Mechanisms

Many preclinical fMRI studies employ a technique called CBV-weighted fMRI using an iron oxide contrast agent. This technique is based on the superparamagnetic property of iron oxide nanoparticles, which shortens blood T_2 substantially (Mandeville et al. 1998). During neuronal activation, the susceptibility effect caused by the contrast agent overwhelms that caused by the BOLD effect, yielding a CBV weighted signal. Advantages of CBV-weighted fMRI includes increased sensitivity of the signal by a factor of 3~5 at 3T and ~2 at 9.4T, together with improved spatial specificity, in contrast to BOLD CBV-weighted fMRI responses are seen localized in deep cortical layers, achieving laminar resolution in rat whisker barrel cortex (Lu et al. 2004) and columnar resolution in cat visual cortex (Zhao et al. 2005). In general, contrast agents with long half-life in blood are preferable in phMRI experiments. Iron dose and BOLD contamination are important considerations for optimizing contrast-to-noise ratio and accurate CBV quantification (Lu et al. 2007a).

It should be emphasized that while more invasive types of experiments are possible using animal models (e.g., drugs that are not appropriate or approved in humans, direct administration into specific brain regions), these studies come with their own inherent complications. To minimize motion, either anesthesia must be used with the concomitant problems of interfering with the targeted drug effect or animals must be forcibly restrained while remaining awake, resulting, even after training, in difficulty isolating stress factors from the pharmacological response. Further, most anesthetic agents alter various peripheral and/or central autonomic processes, requiring animals to be invasively instrumented in order to monitor/regulate such parameters as blood pressure, heart rate, respiration rate, blood chemistry, etc; any one of which could alter the measured hemodynamic signal independent of, or in combination with, the pharmacological probe. On the plus side of animal

experiments, is the ability to tightly control subject history and environmental factors including prior drug exposure, genetic homogeneity, direct CNS manipulations, including lesions and central drug administration, and importantly, with the use of various mouse knock out models, the ability to more directly infer mechanisms and sites of action.

Nonhemodynamic-Based Animal phMRI Methods

Manganese-Enhanced MRI (MEMRI) is an alternative approach to the above hemodynamic based signals to map neuronal activity (Lin and Koretsky 1997) and is based on the ability of manganese (Mn^{2+}) to serve as a calcium (Ca^{2+}) analog. Once translocated, Mn^{2+} remains intracellular for a prolonged period (Cotzias et al. 1968). Critically, since Mn^{2+} is a potent MRI relaxation agent (Mendonca-Dias et al. 1983), the accumulation of Mn^{2+} into activated neurons leads to signal enhancement in T_1-weighted images. Thus, MEMRI directly maps only those cells involved in electrical transmission. Further, in the absence of hemodynamic transduction, it may be especially useful to monitor drug-induced neuronal activation independent of potentially confounding vasculature effects. This technique has been successfully applied to investigate cocaine-induced brain activation (Lu et al. 2007b), as shown in Fig. 1. Once Mn^{2+} is translocated into cells, it can be transported anterogradely along axonal tracts. Upon reaching the presynaptic membrane, Mn^{2+} is released into the presynaptic cleft along with the neurotransmitter glutamate (Takeda et al. 1998). This property has been employed to trace neural tracts in rodents and monkeys in vivo (Saleem et al. 2002), allowing longitudinal examination of an entire circuit in the same subject. A drawback of MEMRI, however, is that

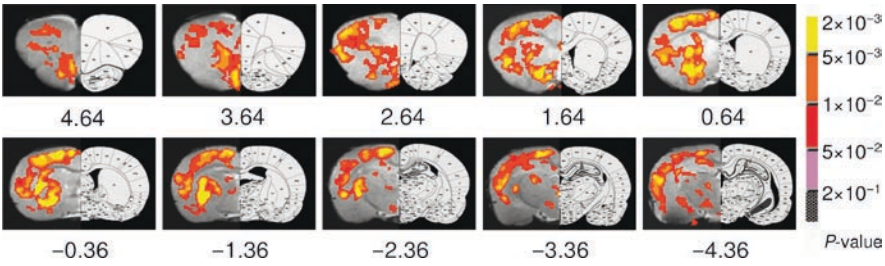

Fig. 1 Acute cocaine-induced brain activation detected by manganese-enhanced MRI. Activation maps were superimposed onto T_2-weighted structural images with corresponding rat brain atlas section shown on the right. Activated voxels (based on a one way ANOVA, thresholded at $p<0.05$) are clustered in the hemisphere with the BBB disrupted by hyperosmolar mannitol. The contralateral hemisphere had an intact BBB and did not show activation. Activated structures include: olfactory cortex, medial, ventral and lateral orbital cortex, prelimbic cortex, cingulate cortex, NAc, caudate putamen, ventral pallidus, external globus pallidus, agranular insular cortex, thalamus, hypothalamus, retrosplenial dysgranular cortex, hippocampus, primary and secondary somatosensory and motor cortex [Lu et al. (2007b), Copyright 2007 National Academy of Sciences, USA]

Mn^{2+} permeability through the blood brain barrier (BBB) is very low. Disrupting the BBB such that a sufficient amount of Mn^{2+} can enter the extracellular space appears to be required for acute MEMRI experiment. Further, Mn^{2+} is highly toxic, especially to cardiac tissue. These issues have been recently reviewed (Silva and Bock 2008).

Resting-State fMRI

It has long been recognized that spontaneous, low-frequency fluctuations can be measured in regional blood flow, oxygen partial pressure and oxidative metabolism in human and animal brains that are independent of the cardiac and respiratory cycles (Hudetz et al. 1998). Renewed interest in these spontaneous fluctuations have revealed that in the absence of overt task performance or explicit attentional demands, spatially coherent low-frequency (i.e., <0.1 Hz) fluctuations exist in the fMRI time course. Several spatial patterns have been identified, including a sensory-motor network and the so-called default mode network (Biswal et al. 1995; Fox and Raichle 2007). Such precisely patterned spontaneous activity has also been reported in anesthetized nonhuman primates and rats (Lu et al. 2007c; Vincent et al. 2007). The neurobiological bases of such coherent spontaneous fluctuations remain to be elucidated. A recent study from our laboratory (Lu et al. 2007c) suggests that coherent EEG power oscillations in the low-frequency delta band correlates with the resting-state fMRI signal observed in bilateral sensorimotor cortex of anesthetized rats.

Recent studies suggest that resting-state fMRI could be a useful tool for evaluating the effects of pharmacological agents, either acutely, chronically or during withdrawal from chronic use, by examining the alterations in coherence strength of known functionally connected neuroanatomical pathways (Hong et al. 2009). Such an approach could reveal not only how a drug acts to strengthen or weaken an existing functional pathway, but also to potentially follow the systems-level consequences of neuronal plasticity as a function of the development of drug-induced tolerance, sensitization or withdrawal.

Imaging Direct Effect of Drugs

The simplest phMRI experiments one can do, conceptually, are those to measure the drug's direct pharmacokinetic/pharmacodynamic effects in the brain by imaging during the administration of the agent. Numerous examples of these types of studies exist in both the preclinical and clinical literature for substances such as nicotine, cocaine and heroin; see Honey and Bullmore (2004) and Salmeron and Stein (2002) for a review. Subject to the numerous caveats outlined below, an acute administration experiment can answer basic questions like: "where is the drug acting in the brain?" and "what are the pharmacokinetic parameters such as time-to-peak effect

and how do these vary by region?" By combining behavioral measures into the phMRI analysis, such as self-reported mood states, one can ask more pharmacodynamic questions such as "where is a craving response mediated in the brain?" (Breiter et al. 1997; Risinger et al. 2005).

Of critical importance for direct studies is the context of the administration. Response to an acute administration in a naïve animal may be different than that in a drug-experienced animal. Similarly, one might expect a markedly different response when a drug is administered in a withdrawn human participant than in someone with acute drug on board. Even when equating drug load, it is entirely possible that, for example, a person with chronic refractory pain would show different phMRI responses depending upon their current level of pain, that is, a state-dependent effect. Thus it seems critical to control drug load, individual state, prior drug experience, etc., in group experiments. However, how variability in, for example, pharmacological state, emotion or drug use history affects the response to an acute administration may well be an interesting experimental manipulation, at the cost of a larger cohort.

Imaging Indirect Effects of Drugs

Frequently it is not the direct action of the agent that is of interest but rather the effect this agent has on the organism's behavior. For example, when imaging the opiate system, the brain regions activated concomitant with drug administration may not be of as much interest as how the drug mediates its analgesic or mood altering properties. Thus it may be useful to use a pain manipulation as a probe of opiate-mediated analgesia. Wise et al. (2002) did just this, by periodically applying painful thermal stimuli during the administration of the short-acting opiate remifentanil. The duration of action was such that they were able to map the drug's pharmacokinetic properties through the use of the brief painful stimuli. As a counter example, nicotine levels from a transdermal patch application are relatively steady over a long period compared to that of an imaging session. One can then use a relatively long probe, such as an attention task, to examine regions of the brain where nicotine exerts its attention-enhancing properties (Hahn et al. 2007; Lawrence et al. 2002).

Biomarkers

With the realization that imaging is a more sensitive measure of brain than any behavioral measure, one of the most exciting potential new uses of MRI for imaging pharmacological systems is the identification of potential biomarkers. These biomarkers could be identified by imaging either direct or indirect effects of drugs, although the latter is more likely. It is not uncommon that neuronal changes precede behavioral changes. Biomarker examples include measuring dopamine (DA) function

in Parkinson's patients or the therapeutic action of antipsychotic medications in schizophrenia. Clinical depression provides an interesting hypothetical example of using imaging as a biomarker. The ability to perform repeated studies in a given individual allows for the powerful ability to follow a drug's action over time and to understand how its effects change (or not) therein. It is known that neurogenesis in the hippocampus is halted in depressive disorders. Animal models show that all efficacious treatments for depression restore the neurogenesis (Sahay and Hen 2007). In humans, it takes weeks to determine if a given antidepressant is working in an individual. However, imaging the hippocampus during the treatment regime, possibly probing hippocampal function with a memory task or resting state functional connectivity, may be useful biomarker(s) for drug efficacy even in the absence of overt, drug-induced behavioral changes.

Interpretation of the phMRI Signal

In contrast to direct electrochemical measures of brain activity, most fMRI techniques use some type of hemodynamic signal as a surrogate of neuronal activity. Recent work has established that the BOLD signal and CBF changes are more correlated with synaptic activity (local field potentials) than to cellular spiking (Logothetis et al. 2001). Given the complex nature of the signal transduction processes from neuronal activity to hemodynamics (i.e., neurovascular coupling) to MR signal, the interpretation of brain "activation" maps as a function of neuronal activity is inherently difficult. This is of fundamental importance for extending fMRI applications to the clinical environment where various pathophysiological conditions could affect one or more of the transduction steps independent of changes in the underlying brain activity (e.g., a disease could alter the ability of blood vessels to dilate or the ability of one or more vasoactive factors to be synthesized or released).

Functional hyperemic mechanisms, thought to involve such vasoactive agents as hydrogen and potassium ions, CO_2, oxygen, adenosine and nitric oxide, increase local blood supply as a function of regional changes in neuronal activity (Iadecola and Nedergaard 2007). Various neurotransmitters and neuropeptides have also been implicated in the regulation of CBF (see [Iadecola 2004] for a review). Recent experimental evidence suggests that neuron-to-astrocyte signaling is also an important mechanism for functional hyperemia (Zonta et al. 2003).

It should be noted that most functional hyperemia studies use sensory stimulation to investigate neurovascular coupling. Pharmacological stimuli could induce functional hyperemia through multiple different neurovascular coupling pathways. For example, DA has been shown to produce direct vasomotor responses in cortical microvessels (Krimer et al. 1998). Recent studies (Choi et al. 2006) suggest that D1 receptor agonists induce large positive changes in CBV that are not sensitive to nitric oxide synthase inhibition. D1/D5 receptor antagonists prevent vascular responses without concomitant effect on DA release. D2/D3 receptor agonists, in contrast, induced negative changes in rCBV in brain regions that contain these

receptors. Thus, the neurovascular response induced by DA altering agents could be the result of at least three factors: the direct vasoconstrictive effects of enhanced extracellular DA on D2/D3 receptors, direct vasodilatory effects via D1/D5 receptors, and/or the neurovascular coupled response induced by DA's neuronal effects. As cortical vessels are known to be innervated by axons containing norepinephrine (Raichle et al. 1975), acetylcholine (Sato and Sato 1992), serotonin (Reinhard et al. 1979) and several peptides (Dacey et al. 1988), the same arguments apply when these receptor systems are activated by pharmacological agents. Studies demonstrating that electrical stimulation of the basal forebrain cholinergic fibers elicited robust increases in cortical CBF without concomitant increases in local glucose metabolism support this view (Sato and Sato 1992).

Imaging studies have identified several cases where pharmacological agents modulate neurovascular coupling. For example, caffeine and theophylline increase glucose utilization while at the same time decreasing CBF in both humans and animals (Nehlig et al. 1992). It was postulated that this was because methylxanthines compete with adenosine, an important CBF modulator in the CNS. Likewise, amphetamine has been shown to induce a similar percentage increase in glucose utilization in both the globus pallidus and the striatum, while the rCBV changes in the striatum are very prominent, they are minimal in the globus pallidus (Choi et al. 2006; Jenkins et al. 2004).

Thus, caution must be executed in explaining phMRI data. The sites of drug action may differ from receptor localization (Herkenham 1987); the hemodynamic response may be affected by a direct vascular effect of neurotransmitters released presynaptically, or be the result of neuronal activation through such pathways as neural–glial–vascular coupling; indirectly via changes in autonomic physiology (Wise and Tracey 2006), or finally it may reflect increased neuronal activity (of the local neuronal assembles and/or upstream of the measured network).

Control Procedures

Compared to conventional imaging experiments, phMRI studies require special consideration when planning control procedures. Drug studies frequently have limits on their scan duration and number of administrations that lead to lower statistical power than nonadministration counterparts. Furthermore, clinical studies may be confounded by expectancy effects – if the subject knows what particular manipulation might occur. For these reasons it is highly desirable to have a vehicle-only scan as part of the imaging protocol, most usefully in a cross over, double blind design. Analysis of this scan, which should be identical to that of the drug administration scan with equal statistical power, will provide a measure of nondrug-specific effects [e.g., direct action on the cerebral vasculature, alterations in autonomic parameters or coupling agent(s)] and is tantamount to a measure of the false-positive rate.

Another recommended control procedure involves a CO_2 challenge. CO_2 is a powerful vasodilator, with actions directly on vasculature smooth muscle, a mechanism of

action distinct from those thought to mediate the hyperemic response. Breathing room air with 5% CO_2 will generate a 47–58% increase in blood flow independent of any changes due to increased neuronal firing (Kim et al. 1999; Rostrup et al. 1994). Thus an acute CO_2 challenge, possibly coupled with ASL, is a powerful way to probe the vascular state independent of changes mediated via coupling agents.

A final control procedure is to use simple, low-level, sensory/motor tasks as a probe to verify a normal hyperemic response. The underlying assumptions are that the cerebral vascular and the coupling agents are reasonably homogenous throughout the brain and that the sites of action of the administered pharmacological agent are heterogeneous and not localized to regions activated by the probe task. Thus equivalent activation in the drug and vehicle conditions provides strong, albeit indirect evidence against direct vascular action of the agent. However, care must be taken in choosing a task whose performance is not affected by the drug per se as, for example, attention can modulate sensory/motor activation levels and rate, amplitude and force can all modulate finger-tapping response.

Analysis Issues

Analysis of data from pharmacological experiments can be complicated for several reasons. Naturally the issues depend upon the system being probed and the nature of the experiment. Here we address some of the more common pitfalls and strategies.

Direct imaging of neuronal systems, that is, scanning during the administration of a drug with the intent of discerning the locations of its central actions, is analogous to event-related imaging paradigms. In traditional event-related experiments the stimulation occurs frequently, typically 15–30 times, in order to accurately measure the response. Analysis proceeds under the assumptions of linearity and time-invariance. However, both of these assumptions are suspect during pharmacological experiments. Subsequent drug administrations often result in reduced or enhanced neuronal and/or behavioral responses compared to the initial administration due to tolerance or sensitization, respectively, thus invalidating time-invariance. It would therefore seem that a single-administration paradigm is necessary unless tolerance and sensitization can be ruled out completely, or can be incorporated in the analysis model by scaling the expected responses by the change in behavior.

Direct imaging experiments can be long in duration, as the scan time must be long enough to capture changes in the signal, for example, one half-life to capture much of the signal return to baseline. This can be problematic for BOLD imaging, as there are frequently drifts in the BOLD signal. Regardless of the source of these drifts, it can be shown that noise in BOLD imaging is approximately 1/f in nature (Aguirre et al. 2002). Thus, for a slow response like that expected from a drug administration with a long half-life, the expected signal is in the noise peak. One would be advised to attempt removal of as many possible sources of noise prior to and/or as part of the analysis by including terms such as the motion correction curves (Friston et al. 1996) and average global, white matter and CSF time courses

and performing physiological corrections using techniques such as RETROICOR (Glover et al. 2000). A potential analysis alternative, independent component analysis (ICA) is discussed below. However, an alternative signal acquisition approach may also be appropriate. Although ASL is much less sensitive than BOLD (Aguirre et al. 2002), since it is usually implemented as the difference of two sequentially acquired images, it has a flat noise profile. Additionally, it provides a quantitative rather than relative measure and thus direct comparisons can be made between separate imaging runs, whereas for BOLD between-run comparisons are always made by comparing within-run changes.

Actual analysis can proceed down any number of analytic pathways. The earliest phMRI studies were analyzed, for example, using a nonparametric Kolmogorov-Smirnov test (Breiter et al. 1997) or a binary-decision model (Bloom et al. 1999; Stein et al. 1998), which determines if a response has the characteristics expected and thus classifies it as a drug response or not. Many current analyses rely on some form of a general linear model. If a response shape is known, then it may simply be used as a linear regressor, possibly with its first (and higher) temporal derivatives to help account for response variability. If only a general response shape is known, one can synthetically generate many possible response curves and subject them to principle components analysis (PCA) to generate a set of orthogonal reference curves. Alternatively one can model an unknown response as a series of boxcar waveforms (McKie et al. 2005).

Linear analyses are fast, can be mostly model free and are easily extended to group analysis. However, it can be difficult to interpret the results when multiple regressors are included to help account for variability. Specifically, it may be difficult to determine parameters of interest beside the amplitude of response, for example, the time to peak or the half-life of elimination. For these reasons it can be desirable to fit the imaging data to a drug model using nonlinear regression. For example, drug plasma concentration can be well modeled as the difference of two exponentials. This would therefore be a reasonable choice for analyzing phMRI data if one assumes that the neuronal response would be linearly driven by drug plasma concentration. Nonlinear modeling is flexible, extensible and can give meaningful parameter estimates, at the expense of speed, the danger of reaching a local minimum instead of a global minimum due to poor initial parameter estimates and possibly no obvious model choice.

Given potential drug effects such as tolerance and sensitization and the complex interactions between pharmacological agents and receptors and vascular systems, it may be difficult to generate a reasonable hypothesis of the expected imaging signal. Recently, there has been considerable interest in data-driven approaches to analyzing fMRI data. Examples of data-driven approaches include fuzzy clustering, canonical correlation and ICA (Comon 1994; McKeown et al. 1998), with the latter of these being the most popular. ICA is completely data driven, that is, requires no model hypothesis, and, as typically used in fMRI analysis, partitions data into independent spatial maps and nonindependent component time courses. Figure 2 demonstrates the power of ICA in detecting phMRI responses. Since the time courses are not independent, and indeed are frequently fairly similar, it is possible for this technique

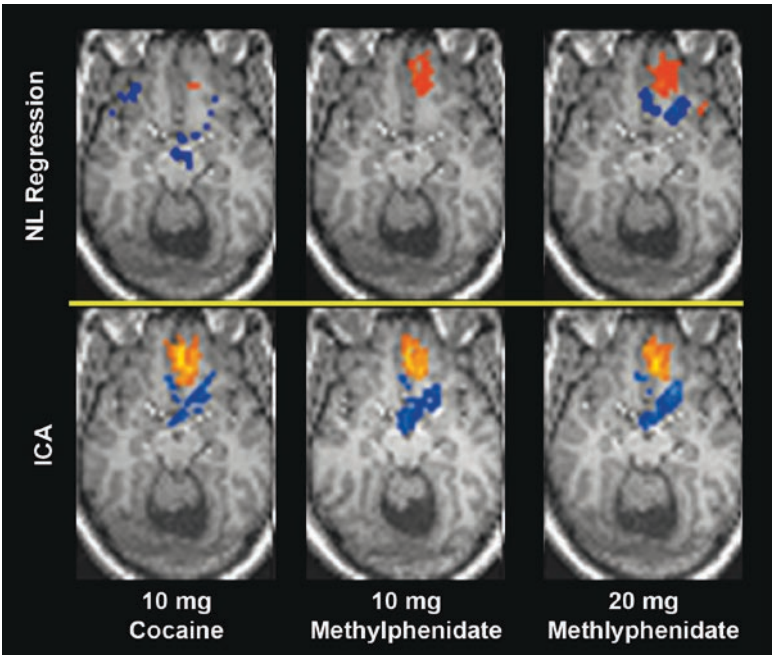

Fig. 2 Activation detected by nonlinear (NL) regression compared to those detected by independent components analysis (ICA) for three different drug/dose combinations (*all dopamine transport blockers*). Note that ICA detects a consistent pattern of activation, whereas NL regression seems to be influenced by the differing magnitude of responses

to remove confounding effects such as stimulus-correlated motion and physiological signals leading to low-frequency drift as these noise sources should have independent spatial maps from those driven by responses to the pharmacological manipulation. Furthermore, it is possible for this technique to tease out multiple response shapes, as seen, for example, in Marota et al. (2000), as they would fall into different components. Group analysis using these techniques is not well standardized and is an area of active research (Calhoun et al. 2001).

Analysis of the indirect effects of pharmacological agents, for example, how these agents affect behavioral responses to pain, memory, cognition, etc, is generally much more straightforward, as these experiments are usually based on more "typical" experimental designs, just in the presence or absence of a pharmacological agent. Analysis then proceeds with that agent (or its dose) as a within or between subject factor as appropriate; see Lawrence et al. (2002) as an example. However, care must be taken to insure that the effect of the drug is constant, or nearly so, throughout the experiment. Failing that, the data must be analyzed allowing for the task response to change as a function of the drugs effect; see Wise et al. (2002) for an example. Finally, as discussed above, control tasks should be undertaken to insure that the response changes seen are not due to the drug's effects on neurovascular coupling (Gollub et al. 1998).

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Molecular Imaging: Basic Approaches

Elisenda Rodriguez and John W. Chen

Introduction

Molecular imaging is an emerging research tool and clinical discipline that aims to discover and apply novel molecules (probes) and methods to image normal and pathological biological processes on a cellular and molecular level in vivo (Weissleder and Mahmood 2001; Wang et al. 2006; Rudin 2005; Pomper 2001; Hoffman and Gambhir 2007; Herschman 2003). One might think of molecular imaging as performing histology and pathology in vivo, without harming the subject. The molecular imaging probes that target specific cells, molecules, or biological events are equivalent to the stains and antibodies used in histology and pathology. The imaging technologies and methods, much like the microscope, provide the means to visualize these probes and report on the in vivo process.

Molecular imaging research is broadly multidisciplinary, integrating many areas of basic and clinical sciences (biology, physics, imaging sciences, chemistry, bioinformatics, engineering, to name a few). It has applications across nearly all clinical disciplines, including radiology, neurology, oncology, cardiology, and rheumatology. Molecular imaging has started to change the clinical practice, especially in the use of ^{18}F-2-fluoro-deoxy-D-glucose (FDG) as a probe for positron emission tomography (PET), in oncological and neurodegeneration imaging. In addition, of particular interest and relevance to drug discovery is the development of rodent imaging systems, which when combined with novel animal models of diseases and molecular imaging probes, would allow efficient assessment of the effectiveness of novel therapeutic drugs.

The current goals of molecular imaging are: (a) to synthesize highly sensitive and specific probes, targeting cells and molecules of interest to biological and pathological processes, (b) to develop new imaging technologies with high probe

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detection sensitivity, high image resolution, and fast acquisition time, (c) to detect disease in the early stage of development, and (d) to facilitate drug development and therapeutic monitoring.

In this chapter, we will introduce the imaging modalities commonly used in molecular imaging, describe relevant applications, and illustrate strategies for designing molecular imaging probes. The readers are encouraged to consult the references included in this chapter as a foundation for a more detailed understanding of the topics discussed.

Imaging Modalities and Imaging Agents

The imaging modalities used in molecular imaging include nuclear imaging (positron emission tomography (PET) and single photon emission computed tomography (SPECT)), magnetic resonance (MR) imaging, optical imaging (fluorescence and bioluminescence), and ultrasound (US) imaging. In Table 1, advantages and disadvantages based on sensitivity, spatial and temporal resolution, depth of signal penetration and cost are summarized. The selection of the imaging system will depend on the problem to be addressed.

Nuclear Imaging (Phelps 2004; Beekman and Vastenhouw 2004)

The main advantages of nuclear imaging are the high intrinsic sensitivity in the nano- to pico-molar concentration scale (only a very small dose of the molecular imaging probe will be needed) and the high penetration depth. Nuclear imaging modalities detect radioactive emission from decaying radionuclides injected into the subject. Nuclear imaging includes PET and SPECT. PET detects the simultaneous arrival of two photons emitted in opposite directions, resulting from the destructive collision between a positron and an electron within the tissue (dual photon detection). SPECT detects single gamma ray photons emitted from the tissue after gamma isotope emission (e.g., ^{99m}Tc, ^{111}In, ^{201}Tl, ^{123}I, and ^{131}I).

The main disadvantage of nuclear imaging is the low spatial resolution of the images. However, this limitation has been partially addressed with the development of integrated systems such as PET-CT (clinical and research) and SPECT-CT (research) scanners. The integration of computed tomography to the nuclear imaging modalities allows one to correlate the active regions with anatomical positions. Despite the low doses of the radiopharmaceuticals needed, radiation risk to both the subject and the staff performing the synthesis, handling, and injection of radiopharmaceuticals is a real concern. Another concern, especially in the FDG PET imaging, is the need for a nearby cyclotron to synthesize the radionuclides because of the short half-lives of some of the agents.

PET radioisotopes: the most common PET isotopes are ^{18}F, ^{11}C, ^{13}N, ^{15}O which can be incorporated within the tracer without a chelation chemistry pathway. ^{11}C is

Table 1 Imaging modalities commonly used in molecular imaging and their characteristics

Modality	Electromagnetic spectrum	Spatial resolution	Temporal resolution	Penetration depth	Imaging agent	Cost
PET	γ-Rays	1–2 mm	Low	High	^{11}C, ^{18}F, ^{124}I, ^{68}Ga, ^{64}Cu	High
SPECT	γ-Rays	<1 mm	Low	High	^{99}mTc, ^{123}I, ^{125}I, ^{111}In	Medium
MRI	Radio-frequency	100 μm	High	High	Paramagnetic and superparamagnetic agents	High
Fluorescence	UV–vis and near infrared	Low	High	Low	Fluorescent protein and fluorescent dyes	Low
Bioluminiscence	Visible	Low	High	Low	Luciferases	Low
Ultrasound	N/A	0.5 mm	Very high	Medium	Microbubbles and liposomes	Low

the most versatile isotope because it can label a large number of functional groups and then be incorporated into organic molecules. The main advantage of using these radioisotopes is that their incorporation will not modify the molecular properties of the final compound, thereby making them good candidates for studying the pharmacological biodistribution of new drugs as well as for targeting neurotransmitter and neuroreceptor systems. The limitation of ^{11}C is its short half-life (20 min). Fluorine-18 overcomes this problem because its half-life is 109 min, allowing more time for image acquisition. But in contrast to ^{11}C, fluorine incorporation may produce slight chemical changes that alter the biodistribution or binding properties of the initial molecule, hence fluorinated probes must be carefully evaluated before they can be used to substitute for the unlabeled compounds. The most common PET probe used is FDG (Sokoloff et al. 1977). The accumulation of the FDG in cells is directly related to high glucose uptake. Other nuclei being employed in clinical research include ^{124}I and ^{64}Cu.

SPECT radioisotopes: radioisotopes used for SPECT present longer half-lives than PET isotopes, from hours to several days. In order to target biological probes, radioisotopes such as ^{99m}Tc can be chelated with DTPA or DOTA, which themselves can be conjugated to different moieties for targeting to specific molecules and tissues. On the other hand, iodine-123 isotope, due to its similarities with endogenous iodine, can be used to monitor gene expression (Tjuvajev et al. 1996).

MR Imaging (Merbach et al. 2001)

More than 60 million clinical imaging procedures are now performed worldwide each year with magnetic resonance imaging (MRI), and emerging preclinical and clinical fusion technologies are becoming available. The main advantage of MRI is that no ionizing radiation or radiopharmaceutical agent is used. Instead, MRI detects the magnetic signals from nuclei (mainly protons). Unlike other tomographic modalities, MRI allows any arbitrary plane of imaging. High-resolution images with excellent soft tissue contrast between different tissues can be used to assess the anatomy with excellent spatial resolution. Despite these attractive parameters, the main obstacle in using MRI as a molecular imaging modality is its relatively low sensitivity to molecular probes compared to nuclear methods such as PET and SPECT.

The use of imaging agents has become an integral part of MR imaging for many applications because the use of exogenous imaging agents allows for better image contrast to be obtained between pathological and healthy tissues. About 35% of the MRI examinations make use of imaging agents, but this percentage is likely to increase further following the development of more effective and specific molecular imaging probes than are currently available. Unlike contrast agents used in X-ray computed tomography and in nuclear medicine, MR imaging agents are not directly visualized in the image. Only their effects are observed: increased contrast is brought about by the effect the imaging agent causes on (usually shortening of) the proton relaxation times, which consequently alters the intensity of the MR signal.

MR imaging agents can be divided into two main groups: positive or negative agents, depending on whether the contrast mechanism predominately affects the T_1- or T_2-weighted signal of the protons.

Positive imaging agents increase the T_1-weighted signal on the images by shortening the T_1 (as well as T_2, although the effect of T_2 shortening is usually not apparent on imaging unless very high local concentration is achieved, such as in the kidneys), and are predominately paramagnetic complexes. The choice of Gd(III) and Mn(II) as metals from MR applications stem from their optimal electronic properties (Lauffer 1987). Of the six clinically approved imaging agents used worldwide for intravenous administration, four of them are based on Gd(III). The first imaging agent approved for clinical use was the anionic $Gd(DTPA)^{2-}$ (Magnevist®, Schering AG, Germany) that, in more than 10 years of clinical experience, has been administered to more than 20 million patients. Other Gd(III) based imaging agents similar to Magnevist® are: $Gd(DOTA)^{-}$ (Dotarem®, GE Health, USA) and Gd(HPDO3A) (Prohance®, Bracco Imaging, Italy). These first generation imaging agents have been very useful, and have contributed to the rise of MRI utilization in clinical and preclinical imaging. They distribute mainly into the intravascular and interstitial space, and therefore are non-specific (Weinmann et al. 2003). As such these agents cannot cross intact blood–brain barrier, and have become useful in identifying lesions that cause disruption of the blood–brain barrier (e.g., tumors).

Negative imaging agents decrease the signal on T_2-weighted imaging by causing a large shortening of the proton T_2, and are exemplified by superparamagnetic iron oxide nanoparticles (SPIO) (Weissleder et al. 1990; Jung and Jacobs 1995). Iron oxide nanoparticles without explicit molecular specificity are well known in the literature to be used as reporters for several physiological processes and diseases. These include non-targeted cellular uptake, enhanced retention in tumors, macrophage phagocytosis, as well as accumulation in the liver, spleen, and lymph nodes. T_2- and T_2*-weighted sequences allow the detection of nanoparticle accumulation through T_2 shortening effect to provide dark contrast in the areas with the iron oxide uptake. Biodistribution of these particles and their efficient delivery depends mostly on their hydrodynamic radius and surface characteristics, as these parameters control the circulation time of the nanoparticles, accessibility to tissues, opsonization, and rate of cell-type uptake.

Ultrasound Imaging (Foster et al. 2000)

Ultrasonography in clinical imaging is highly useful because of its relatively low cost, the small size of the equipment, as well as its safety. Ultrasound (US) is based on the emission and detection of sound waves that are absorbed, transmitted, reflected, or refracted as the waves travel through tissues.

While US has low detection sensitivity compared to nuclear imaging, US imaging can provide real time imaging (high temporal resolution). The use of US imaging agents to increase the sensitivity is becoming more common. US agents include encapsulated

microbubbles, liposomes, and perfluorocarbon emulsions. Microbubbles contrast relies on differences in the acoustic impedance (differences in the backscattered wave) due to the higher compressibility of gases, making them more reflective than normal tissues (Lanza and Wickline 2001). To obtain higher scattering, bigger bubbles are necessary. The main drawback of these probes is the unusually large size (6–8 μm), which prevents these probes from crossing the intravascular space, confining their applications to vascular anomalies. Microbubbles with smaller size (~3 μm) that can cross into the intravascular spaces are more amenable for non-vascular in vivo applications (Rudin 2005). To increase the lifetime in circulation, some microbubbles incorporate perfluorocarbon (PFC) (Schutt et al. 2003). PFC is an inert liquid that forms relatively good solvents for gases because of the very low intermolecular forces. In contrast to microbubbles filled with air that dissolve quickly in the blood, PFC microbubbles increase the lifetime of the air bubble from seconds to minutes. Another type of US imaging agents is liposome formulations, mainly constituted by phospholipids, glycerol, and cholesterol. Because these particles are of similar density as most tissues, they do not cause backscatter as much as the microbubbles. Another option is to fill the liposomes with PFC; the resultant nanoparticles have increased reflectivity (Hall et al. 1977). Functional groups that target cells or molecules can be added to these bubbles to include molecular imaging capabilities (Villanueva et al. 2007; Rychak et al. 2007; Behm and Lindner 2006).

Optical Imaging

While the abovementioned modalities have been translated to human applications, the optical imaging modalities are currently predominately used in the research settings. The optical imaging modalities are fluorescence molecular (mediated) tomography (FMT) and bioluminescence (Ntziachristos 2006; Negrin and Contag 2006).

Physical principles of fluorescence rely on the initial absorption of photons in the optical region of the electromagnetic spectrum by a specific molecule. A fluorophore or fluorescent protein can be excited by an external energy source, resulting in a transition from the electronic ground state to an excited state. The transition from the excited state to a lower energy state results in the emission of light photons which will be detected with highly sensitive charge-coupled device (CCD) camera using appropriate filters (in fluorescence spectroscopy, the emission is at longer wavelength compared to that of the excitation).

Bioluminescence means production of light by a living organism. This technique is based on the observation that organisms such as fireflies and some deep sea fishes can produce light as a result of a chemical reaction, usually through enzymatic cleavage, during which chemical energy is converted to light energy.

One of the drawbacks of the optical imaging is that both hemoglobin and water are the major absorbers in the visible and infrared light regions, resulting in a strong signal attenuation (loss of imaging resolution). FMT overcomes this by utilizing the near infrared (NIR) region of the spectrum (650–900 nm), which has the lowest absorption coefficient in the UV–vis region. Fluorescence imaging has received particular

attention in recent years because of the increasing number of NIR fluorochromes. Bioluminescence presents low background noises since the enzyme (luciferase) as well the substrates are usually not endogenous to the animal being imaged, resulting in an increase of the sensitivity. However, bioluminescence requires genetic manipulation in order to insert the genes necessary for the enzyme expression.

Another disadvantage of the optical techniques is the very low spatial resolution and low penetration depth. Optical tomographic techniques employ techniques to overcome some of these limitations. Currently, FMT can achieve a penetration depth as great as 1–2 cm.

Fluorescence probes: the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*, is widely used in biological applications (Contag and Bachmann 2002). GFP has an excitation maximum at 395 nm and emits green light at 509 nm, but due to its lower wavelength, autofluorescence of the endogenous tissues is a concern. To solve the problem of autofluorescence, agents based on the cyanine scaffold to increase the wavelength of absorption and emissions are being explored (Lin et al. 2002). Several modifications to the π-system, such as elongation of the conjugated bonds, have a direct influence on the spectroscopic properties of the compounds, and also affects the solubility properties (Bouffard et al. 2008).

Quantum dots consisting of semiconductor nanostructures that were originally developed for optoelectronic applications with high quantum yield can be used as fluorescent probes (Yoffe 2001). Quantum dots, for instance, consists of a CdSe core with a ZnS shell. They have to be coated by an organic matrix in order to become biocompatible (Bruchez et al. 1998; Ballou et al. 2004). The main disadvantage of quantum dots for in vivo applications is the toxicity from released cadmium, though efforts are underway to minimize this toxicity.

Bioluminescent probes: the best-studied bioluminescent enzymes are luciferases where light is produced by the oxidation of a luciferin (pigment) sometimes involving ATP. The reaction is energy efficient. Luciferase can be produced in the laboratory through genetic engineering to label molecules and cells.

Design and Examples of Molecular Imaging Probes

There are several important considerations in the development of new molecular imaging probes: (a) high specificity for the target and reasonable pharmacodynamics; (b) the ability of these probes to overcome biological delivery barriers (vascular, interstitial, cell membrane); and (c) suitable amplification strategies to enhance the signal (chemical or biological).

Design of Target-Specific Probes

Target identification and validation with high-affinity probes can be performed using two different strategies of interaction between the probe and the target: direct and indirect (or activatable, also called “smart” probes) (see Fig. 1).

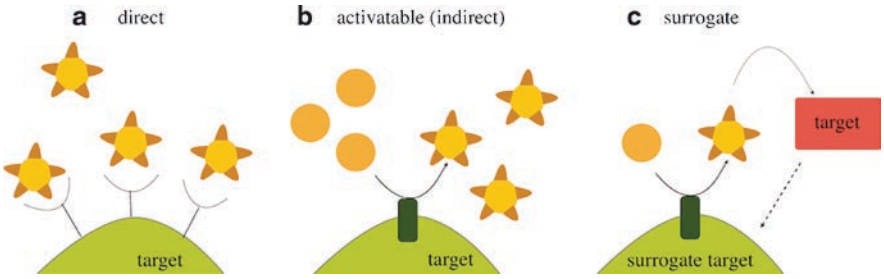

Fig. 1 Mechanisms of target–probe interaction: (**a**) direct mechanism, (**b**) activatable (indirect, smart) mechanism, and (**c**) surrogate mechanism. The figure illustrates a surrogate probe with an activatable mechanism, but a direct probe can also be used

Probes based on the "direct" strategy (Fig. 1a) can be attached to the molecule of interest or are taken up by the cell of interest. The image intensity will be relative to (but also limited by) the amount of the target present. Because of the lack of signal amplification, this kind of strategy is predominately used in nuclear imaging. Antibodies can be conjugated to the probe to increase the affinity and simultaneously, the biocompatibility. However, the use of antibodies can still result in a high background noise due to non-specific interactions. Moreover, due to their size (200–400 kDa) only endovascular and extracellular receptors can be targeted.

"Indirect" or "activatable" probes (Fig. 1b) possess the ability to alter their structure in response to changes in the local environment, often resulting in signal amplification. The most common method to accomplish this is by enzymatic cleavage. This is most often used in fluorescent probes. For example, several activation-sensitive peptide–fluorochrome conjugates have been recently introduced, described with specificity for cathepsins, matrix metalloproteinases, and other enzymes (Law et al. 2007; Ho et al. 2007).

Moats and co-workers were the first to introduce this cleavage strategy for MRI probes (Moats et al. 1997). They designed a gadolinium-based complex consisting of a galactopyranose moiety positioned in the ninth coordination position of the gadolinium complex. In the presence of β-galactosidase, this blocking sugar group is removed by enzymatic cleavage and the T_1 relaxivity of the imaging agent increased 20% because of the improved access of the water molecules to the metal after enzymatic cleavage.

Another strategy to design activatable probes is to design a molecule that can be chemically modified by the target enzyme into a different molecule. This class of enzymatic activation is best illustrated by peroxidase sensitive MRI probes. The endogenous peroxidase (such as myeloperoxidase, which is a key enzyme secreted in active inflammation) amplifies the T_1-weighted signal by oxidizing and radicalizing the parent probe, causing it to polymerize. The activated probe is significantly larger in size and has slower rotational dynamics compared to the parent compound, resulting in higher signal (200% higher) and prolonged pharmacokinetics in vivo (Chen et al. 2004, 2006; Bogdanov et al. 2002).

A third probe type, called the surrogate probe, is sometimes discussed. This is a probe that targets a downstream entity remote from the molecule/event of interest (Fig. 1c). This probe can be either a direct or an indirect probe. The myeloperoxidase probe discussed above is an indirect probe for myeloperoxidase but is also a surrogate probe for active inflammation.

Probe Delivery

Another important issue is the ability of the probes to reach the intended target with high enough concentration and persist in that location long enough to be detectable in vivo. Probe delivery is one of the most challenging issues, in particular, when targeting intracellular receptors and enzymes. One method to overcome this problem is by designing peptide sequences that facilitate the intracellular incorporation of the target.

One example is the use of the HIV-tat peptide because it allows uptake across cell membranes. HIV-tat peptides have been conjugated to CLIO (cross-linked iron oxide nanoparticles) to visualize and track stem and progenitor cells by MR imaging (Josephson et al. 1999; Lewin et al. 2000). Cell specific uptake can also be achieved via receptor mediated endocytosis. Asialoglycoprotein (ASG) receptors are present on normal hepatocytes but are missing in primary malignant or metastatic tumors. Weissleder et al. described an ultrasmall superparamagnetic iron oxide (USPIO) probe conjugated to asialoglycoprotein (ASG) to differentiate hepatic tumors from normal tissues (Reimer et al. 1990). As ASG receptors on normal cells have a high affinity for the terminal galactose groups, AG-USPIO would accumulate in normal tissues but not in tumor cells, thus improving the tumor-liver contrast.

Suitable Amplification Strategies (Chemical or Biological)

In addition to the amplification strategies described in "Design of Target-Specific Probes," another approach to facilitate the differentiation of target and background fluorochromes is to use "molecular switches or beacons". The probes are optically silent in their native (quenched) state and become highly fluorescent after enzyme-mediated release of the fluorochrome (Tung et al. 2000; Ntziachristos et al. 2002). In addition, this highly specific approach has the advantage that one target (e.g., an enzyme) can convert many individual beacons, resulting in several levels of amplifications (10–1,000-fold) over simple tagging.

Another strategy is magnetic relaxation switching (MRS). The MRS technology is based on the conversion of the assembly of iron oxide nanoparticles into highly stable nanoassemblies (clusters), with a concomitant decrease in the spin–spin relaxation time (T_2) of adjacent water protons. Recently, Weissleder et al. have developed MRS biosensors to detect molecular interactions (DNA–DNA,

protein–protein, protein–small molecule, and enzyme reactions) and analyte levels (Perez et al. 2002; Sun et al. 2006). In the low analyte state, the nanoparticles are microaggregated, resulting in a decrease in T_2 (low T_2 state). While in the high analyte state, the nanoswitch is dispersed, causing the nanoswitch to move to a high T_2 state. The degree of T_2 change is proportional to the amount of analyte (e.g., glucose) present.

Applications in Neurological Diseases

Unlike other organs, the brain presents a special challenge with the blood–brain barrier. Only small and lipophilic agents could go through it. However, in pathological states, the blood–brain barrier may be compromised allowing agent access. Molecular imaging in the central nervous system (CNS) can be performed with nuclear imaging, MR imaging, or optical imaging modalities. An interesting application for each modality to illustrate the power of molecular imaging is highlighted below.

One of the disadvantages of using FDG PET in the CNS is the intrinsic high glucose metabolism of gray matter. New molecular imaging agents, designed to target the amino acid transport or incorporated into DNA, have been described (Chen et al. 2005; Jacobs et al. 2005). Unlike FDG, the main advantage of using these agents for PET imaging is that in the normal brain the uptake is low. An example of these agents is the compound L-[methyl-^{11}C]methionine or [^{11}C]MET (Kim et al. 2005), which is responsive to the degree of protein synthesis, providing significantly higher sensitivity for detecting brain tumors compared to FDG PET (Fig. 2a).

Optical molecular neuroimaging is useful to monitor cell trafficking events. Recently, Shah et al. designed a bioluminescence method to validate and track the use of neural progenitor cells (NPCs) in therapy for brain tumors. NPCs were transfected with luciferase gene and implanted intracranially and intraventricularly into nude mice. After 2–3 weeks, there was clear NPCs migration toward the tumor (Fig. 2b) (Tang et al. 2003), which can be labeled with selective chemotherapeutic agents to perform focused therapy (Corsten et al. 2007; Kock et al. 2007; Shah et al. 2005a, b).

It is well known that enhancement in the CNS from conventional, non-specific MRI contrast agents such as Gd-DTPA reflects breakdown in the blood–brain barrier rather than active inflammation. In multiple sclerosis imaging, this poses a particular problem because lesions at all stages demonstrate some breakdown in the blood–brain barrier, hence breakdown in the blood–brain barrier and active inflammation may not always correspond (Cotton et al. 2003; Bruck et al. 1997). Chen and co-workers have recently reported an MR imaging agent (Chen et al. 2006) that is highly specific and sensitive to the enzyme myeloperoxidase, a key enzyme secreted in inflammation and in multiple sclerosis plaques (Nagra et al. 1997). They demonstrated that this myeloperoxidase imaging sensor can detect and confirm more smaller, and earlier active inflammatory lesions in living mice by in vivo MR imaging, and that MPO expression corresponded with areas of inflammatory cell infiltration and demyelination

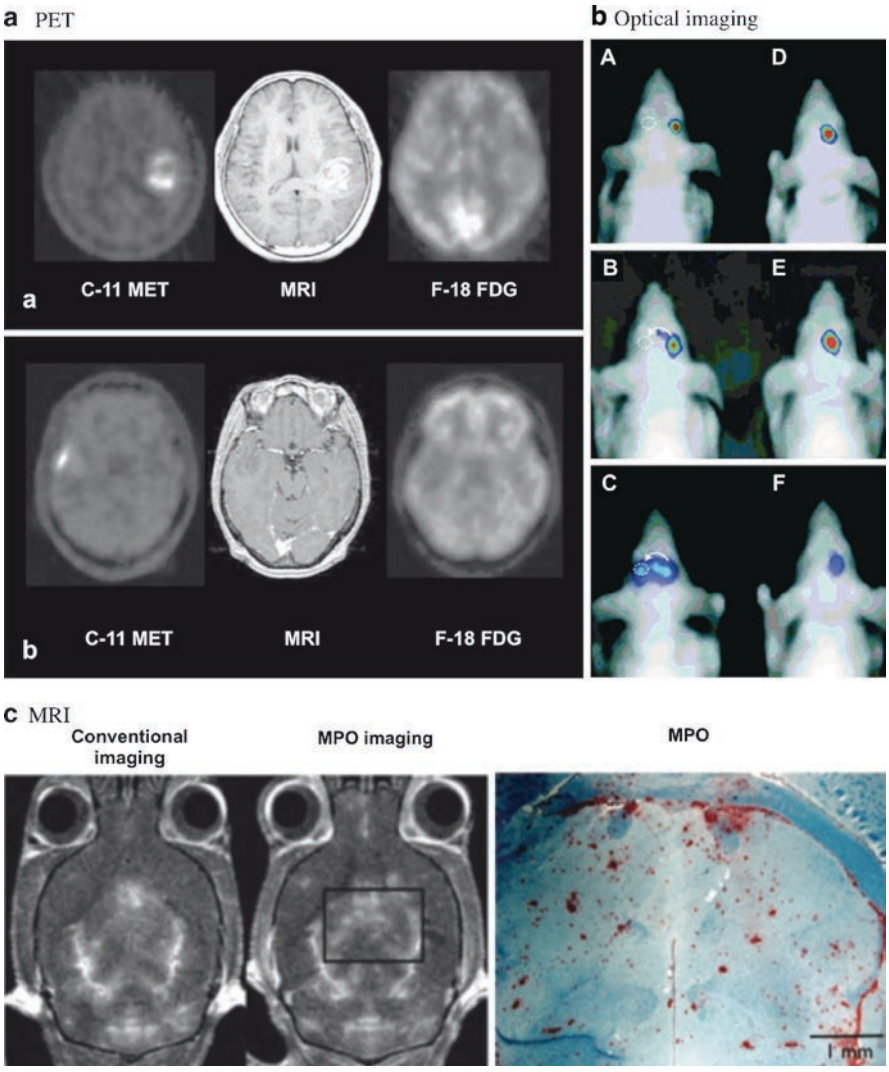

Fig. 2 Examples of molecular neuroimaging: (**a**) [^{11}C]MET PET, transverse enhanced MR, and FDG PET images of two glioma patients (top and bottom). [^{11}C]MET PET has a higher sensitivity for the detection of brain tumors. (Reprinted with permission from Kim et al. (2005).) (**b**) Optical images showing neural progenitor cells migrating towards the tumor obtained at (A) 0 day, (B) 1-week follow-up, and (C) 2-week follow-up. (D), (E) and (F) represent the time series for an animal without glioma and no migration of the NPCs was observed. (Reprinted with permission from Shah et al. (2005b).) (**c**) In a mouse experimental autoimmune encephalomyelitis model for multiple sclerosis, myeloperoxidase MR imaging revealed more and smaller lesions at an earlier time than imaging with the conventional Gd-DTPA. (Reprinted with permission from Chen et al. (2008).)

(Fig. 2c) (Chen et al. 2008). They also found that higher MPO activity, as detected by MPO imaging, biochemical assays, and histopathological analyses, correlated with increased clinical disease severity. This approach could be used in longitudinal studies to identify active demyelinating plaques as well as to more accurately track disease course following treatment in clinical trials.

Drug Development

Molecular imaging has and will continue to play an important role in drug development. An important example is in characterizing the biodistribution of a new drug to assess unfavorable pharmacokinetic properties (e.g., poor absorption or rapid excretion) (Rudin and Weissleder 2003). Classical biodistribution studies in animals have been performed using autoradiography. The drug is labeled with a long-lived radioisotope (^{3}H or ^{14}C) and then the biodistribution is quantified postmortem in histological sections. However, despite the excellent sensitivity and spatial resolution, no drug metabolism information is obtained. Nuclear imaging provides the best way to evaluate these parameters as the incorporation of ^{11}C and ^{18}F to the drug does not affect the properties of the molecule.

Molecular imaging can be a very efficient means to monitor the therapeutic efficacy by imaging the functional repercussions of drug–target interactions. To accomplish this, the probe should be highly specific to the chosen target, and depending on the imaging modality used, the resultant response may need to be amplified. Even in the event that no specific probe can be designed for a particular target, a surrogate strategy may be used, in which a probe may be designed that is specific to downstream molecules or cells that are produced or affected by the target of interest (Fig. 1c).

Molecular imaging can also be helpful to discover new drugs by providing imaging end points instead of invasive and often fatal animal manipulations (e.g., invasive surgical dissection and non-survival histology). Longitudinal and serial studies can be performed where the effect of a drug is followed non-invasively over time in the same animals. As a result, statistical significance of a study can be achieved with fewer animals, and the effect of a drug can be more easily identified.

Conclusions

In this chapter, we have covered the basic concepts of molecular imaging and showed both the advantages and disadvantages of different imaging approaches. It is important to keep in mind that many more molecular probes and applications are in development and in clinical trials. While most are not yet available for clinical use, many techniques and probes can be readily used in preclinical trials to study the efficacy of a drug.

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Chemical Imaging. Magnetic Resonance Spectroscopy: The Basics

Andrew P. Prescot and Perry F. Renshaw

Introduction

Magnetic resonance spectroscopy (MRS) is a noninvasive medical imaging modality that holds great promise for assisting the central nervous system (CNS) drug discovery process. MRS measurements yield spectral data that ultimately can be analyzed and interpreted to provide unique information regarding disease processes, pathophysiology and drug response in vivo. ^{1}H-MRS investigations are performed at many institutions although an attractive aspect of in vivo MRS is the potential range of other nuclei that can be exploited. The specific MR-active nucleus under investigation depends on the instrumental hardware available as well as the biological application and questions to be addressed. Table 1 summarizes the biologically important MRS-active nuclei that are of particular interest to drug discovery, and gives nucleus-specific MR properties such as the gyromagnetic ratio (γ) and natural abundance. The MR-sensitivity of a nucleus is directly proportional to both of these variables. The table also provides examples of "biological relevance" for each nuclide and several biochemical compounds are listed for each potential application.

The proton (^{1}H) is by far the most widely studied nucleus for both preclinical and clinical MRS investigations in vivo. The ^{1}H nucleus has the highest sensitivity owing to its high γ and high natural abundance, and ^{1}H-MRS can be performed using the hardware typically supplied with preclinical and clinical MRI systems such as ^{1}H-tuned radiofrequency (RF) amplifiers and coils. In addition, the ^{1}H nucleus is ubiquitous in nature and many biologically relevant organic compounds contain proton sub-groups that are detectable *via* ^{1}H-MRS. Molecular species that are pertinent to CNS drug discovery and are that are detectable using ^{1}H-MRS include the amino acid neurotransmitters Glu, Gln, GABA and Gly (see table 1 for metabolite acronyms). The neuroscience product pipelines of major pharmaceutical

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Table 1 Summary of MR-active nuclei relevant to biological investigations in vivo. Biological relevance and molecular examples are provided for each isoptope

Isotope (abbreviation)	Gyromagnetic ratio(γ; MHz/T)	Natural abundance (%)	Biological relevance	Molecular examples
Proton (^{1}H) and Carbon (^{13}C)	42.5810.71	99.991.11	Amino acids/neurotransmission Antioxidants Osmolytes Cellular Energetics Membrane and lipid metabolism	Aspartate (Asp)γ-aminobutyric acid (GABA) Glutamate (Glu) Glutamine (Gln) Glycine (Gly) *N*-acetylaspartyl glutamate (NAAG) Ascorbic acid (Asc) Glutathione (GSH) *N*-acetyl aspartate *Myo*-inositol Adenosine-triphosphate (ATP) Creatine/Phosphocreatine (Cr/PCr) Lactate (Lac) Glucose (Glc) Pyruvate (Pyr) Succinate (Suc) Choline (Cho) Glycerophoshocholine (GPC) Phosphocholine (PCho) Ethanolamine (Etn) Phosphoethanolamine (PEtn) Lipids (Lip)
Phosphorous (^{31}P)	17.25	100.0	Cellular Energetics Membrane and lipid metabolism	ATP, PCr, PCho,Inorganic phosphate (Pi) Glycerophosphoethanolamine (GPEtn), PEtn, Glycerophosphocholine (GPC) Membrane phospholipid (MP)
Flourine(^{19}F)	40.07	100.0	Drug-detection and metabolism	^{19}F-containing agents(e.g., fluoxetine, fluvoxamine)
Lithium(^{7}Li)	16.55	92.58	Drug quantification	^{7}Li-containing agents(e.g., lithium carbonate)

companies currently include glutamatergic, glutaminergic, GABAergic and glycinergic agents, which are under evaluation for treating a wide variety of neurologic disease and psychiatric disorders including affective disorders, Alzheimer's disease, anxiety, pain, schizophrenia, stroke and substance abuse.

The importance of MRS studies involving other types of nuclei should not be discounted. ^{31}P-MRS, for example, can provide unique information regarding NTP concentration and cellular energetics. Membrane phospholipids and their precursors such as PCho and PEtn can also be measured using ^{31}P-MRS and these types of studies hold great potential for monitoring tumor phospholipid metabolism in response to therapeutic intervention. Although hindered by low natural abundance and low sensitivity, ^{13}C-MRS lends itself nicely to labeled substrate infusion studies, which are performed at a handful of sites worldwide. Administration of ^{13}C-enriched glucose or acetate leads to a downstream ^{13}C-labeling of glutamate and glutamine at specific carbon positions, and tricarboxylic acid enzymatic rate constants can be extracted. Several CNS drug compounds, such as the selective serotonin reuptake inhibitors (SSRIs) paroxetine and fluoxetine, are fluorinated and thus detectable using ^{19}F-MRS techniques. Localized ^{19}F-MRS measurements could enable the in vivo monitoring of drug localization and catabolism and help elucidate drug pharmacokinetic parameters including half life and clearance.

All medical imaging modalities suffer from drawbacks and limitations and MRS is no exception. MRS is an insensitive measurement technique and a brain metabolite must be present at a sufficient level, typically in the millimolar (mM) concentration range, to be detected and quantified. Low-sensitivity measurements suffer from a low signal-to-noise ratio (SNR) and signal enhancement is crucial for accurate quantification. For MRS measurements SNR is typically enhanced by averaging packets of data recorded in discrete periods over time (signal averaging) and/or increasing the size of the MRS volume element (voxel). Unfortunately, these two potential methods compromise spatial and temporal resolution, respectively. As outlined later in this chapter, quantification of ^{1}H MRS data is hampered by a very low spectral resolution particularly at magnetic field strengths (B_0) associated with clinical MR systems. Another disadvantage of MRS is that resulting data must be processed offline and specialized software often is required for reliable quantification. Also, purchase of additional hardware such as dedicated radiofrequency (RF) coils and RF amplifiers is essential for MRS measurements involving nuclei other than the proton. These types of hardware along with the more useful (and complex) MRS techniques are not generally provided as standard on MRI/MRS scanners and their correct implementation requires some degree of local MRS expertise. This expertise is also critical for establishing reliable quantification protocols and ensuring meaningful interpretation of spectral data.

Nevertheless, MRS benefits from a range of advantages unparalleled by many other forms of medical imaging. MRS measurements do not involve delivery of ionizing radiation to biological tissue in contrast to other chemical imaging methods such as positron emission tomography (PET) and single photon emission computed tomography (SPECT). Therefore, MRS measurements can be incorporated into repeated measures study designs and are safe for investigations involving pediatric

populations. A primary advantage of ^{1}H MRS as applied to CNS drug discovery is its ability to quantify several amino acid neurotransmitters, coagonists and their derivatives. Therefore, MRS has the potential to establish the mechanism(s) of novel drug candidates at the level of individual neurotransmitter systems. Such pharmacodynamic responses might serve as important biomarkers of functional effects on amino acid neurotransmitter systems and provide crucial data for determining the biological mechanisms underlying treatment response. A large amount of literature has emerged demonstrating that MRS-detectable between-group differences do exist for many CNS disorders and MRS-detectable drug effects are beginning to emerge for several pathophysiologies.

Proton Magnetic Resonance Spectroscopy

In vivo ^{1}H-MRS can be implemented and performed at most MRI centers and the types of metabolites that can be detected through ^{1}H-MRS will be most applicable to CNS drug discovery. The main objective of this chapter is to give a brief and highly conceptual introduction to some of the fundamental aspects of in vivo ^{1}H MRS. The reader is directed to other theoretical sources that provide more complete descriptions of MRS concepts (Salibi and Brown 1998; de Graaf 2007). This chapter initially describes the basic steps that are required for the execution of 3D localized water solvent-suppressed ^{1}H-MRS experiments, and these sections are intended to introduce the uninitiated reader to some of the technical jargon often encountered in the MRS literature. The basic physical features of a ^{1}H-MRS spectrum including chemical shift, signal amplitude and scalar spin–spin coupling is then characterized using real data acquired from human brain in vivo. The same dataset is used to highlight some of the key metabolite proton resonances that can be detected using standard ^{1}H-MRS methods and examples of MRS spectral fitting algorithms are briefly described. Importantly, this chapter will outline several alternative ^{1}H-MRS methods that can be used for resolving amino acid neurotransmitter resonances that are difficult to isolate using standard approaches.

In Vivo ^{1}H-MRS Measurement

MRI and Shimming. The acquisition of low-resolution MR images is an important first step to any ^{1}H-MRS examination, as these can be used to assess and ensure optimal subject positioning within the RF coil and MRI scanner. The main magnetic field (B_0) homogeneity over the sample (subject) then can be optimized through a process known as B_0 shimming, which involves passing relatively small current through small electromagnets or “shim coils” that are carefully positioned within the magnet bore at the scanner installation. The shim coils provide small-amplitude auxiliary magnetic fields, which are iteratively adjusted using computer

controlled algorithms in order to compensate for B_0 inhomogeneities. A high degree of B_0 homogeneity across the sample is particularly crucial for MRS measurements as this resonance line width and enhances spectral resolution.

Spatial Localization. In vivo ^{1}H-MRS measurements require a form of spatial localization such that spectral data can be recorded from a preselected and well defined region-of-interest (ROI). Historically, localization was crude and achieved by positioning the so-called "surface" RF coils superficially to the ROI. Nowadays it is much more common to utilize B_0 field gradients for spatial localization and the approaches can be broadly classified into two common types: single-voxel and multiple voxel ^{1}H-MRS. Single-voxel ^{1}H-MRS methods achieve 3D localization through the sequential execution of three RF "pulses" of finite bandwidth, each of which is executed in the presence of a linear B_0 gradient applied along one of the three orthogonal axes. Each RF pulse – gradient combination selects a slice with the thickness being proportional to the RF pulse bandwidth/B_0 gradient strength. The interpulse timings together with the RF flip angles constitute what are referred to as "pulse sequences" and determine whether a spatially localized stimulated echo or a double spin echo originates from the intersection of the three selected slices. The two sequences routinely used for ^{1}H-MRS applications are the stimulated echo acquisition mode (STEAM; (Frahm et al. 1989)) and point-resolved spectroscopy (PRESS; (Bottomley 1987)) methods. The advantage of the STEAM sequence is that a low echo time can be achieved potentially giving access to increased metabolic information. The PRESS sequence on the other hand benefits from increased inherent measurement sensitivity due to the acquisition of a spin echo. Whether STEAM or PRESS is used, the echo signals are detected and digitized in the form of free-induction decays (FIDs), which yield spectral data following Fourier transformation (FT).

Multiple voxel ^{1}H-MRS methods such as magnetic resonance spectroscopic imaging (MRSI) are usually examples of B_0 gradient-based localization methods but combine spectroscopic and imaging concepts to yield multiple localized spectra. In MRSI a single slice is usually selected along one of the orthogonal axes through the combination of a RF pulse applied with a linear B_0 gradient. Pulsed B_0 gradients subsequently applied along the two other orthogonal axes act to phase encode the nuclear spins along those dimensions. The phase encode process is repeated in successive acquisitions until the desired spatial resolution has been achieved and a grid of multiple spectra are afforded following the necessary spatial and spectroscopic FTs.

RF Pulse Power Calibration. Once the single-voxel element or a MRSI slice has been positioned within the ROI the RF pulse power is automatically optimized, which ultimately ensures reliable peak quantification. "Local" B_0 shimming then should be performed over the ROI until the unsuppressed water signal line width is minimized. Finally, a method for solvent water suppression is activated and calibrated until the water resonance is reduced to a level where metabolite resonances can be readily quantified. Water suppression schemes typically employ a train of frequency-selective RF pulses that are applied immediately prior to the spatial localization scheme. The RF pulses are tuned to excite the water resonance

with minimal excitation of surrounding metabolite resonances, and each pulse is followed by a strong B_0 gradient that acts to dephase or "crush" the generated transverse water magnetization.

MRS Sequence Parameters. MRS is an insensitive measurement and the detected signal is weak. For in vivo measurements this problem is made worse by the low metabolite concentrations to be measured (mM) and the electronic thermal noise associated with MRI scanner hardware. It can be shown that signal-to-noise ratio (SNR) can be increased by *averaging* FIDs from a number of excitations (NEX). This is because the genuine MR signals coverage linearly whereas the noise increases by √NEX. Therefore, quadrupling NEX increases the observed SNR by a factor of two. The scanner operator is responsible for setting the NEX together with the time between NEX (repetition time, TR) and the time between excitation and onset of FID detection (echo time, TE). The product of TR and NEX gives the total MRS measurement, which should be kept relatively short for in vivo applications. In general, short TE's give access to increased metabolite information at the expense of increased spectral complexity (see below).

The In Vivo Brain ^{1}H-MR Spectrum

Figure 1a shows an axial T_1-weighted MR image recorded at 4.0 T from a healthy adult human subject and the red box corresponds to a section of a $2 \times 2 \times 2$ cm^3 MRS voxel positioned predominantly within the predominantly gray matter of the parietal occipital cortex. Figure b shows a ^{1}H-MRS spectrum that was obtained from the same MRS voxel without solvent water suppression. The resonance line observed in Fig. 1b corresponds to a single resonance frequency and is solely attributable to tissue water protons. If the proton nuclei within different molecular species (e.g., cerebral metabolites) exhibited the same identical resonance frequency as water, then a single MR peak would be observed and little information could be extracted from the resulting spectral data.

Chemical Shift and the Chemical Shift Scale. Fortunately, the resonance frequency of nuclear spins is highly sensitive to the local electronic environment. In some situations, electrons present within the local molecular structure act to shield the nuclear spins from the main B_0 field and the effective field experienced by the nucleus under investigation is effectively reduced. If a nuclear spin experiences a reduced B_0 field then it will exhibit a slightly lower MR frequency. Electronic 'deshielding' can also occur where electronegative atoms or functional groups within a given molecular species attract electron clouds away from a nuclear spin. In that situation, the local B_0 will be enhanced leading to a slightly higher MR frequency. These effects act to produce a spread of MR frequencies for a given nuclide and the shift effect due to chemical environment is known as the chemical shift. The x-axis scale used for displaying MRS spectra is termed the chemical shift or ppm scale and is convenient for several reasons. The resonance frequency, ω_0, of the proton nucleus is directly proportional to the static B_0 field as governed by the

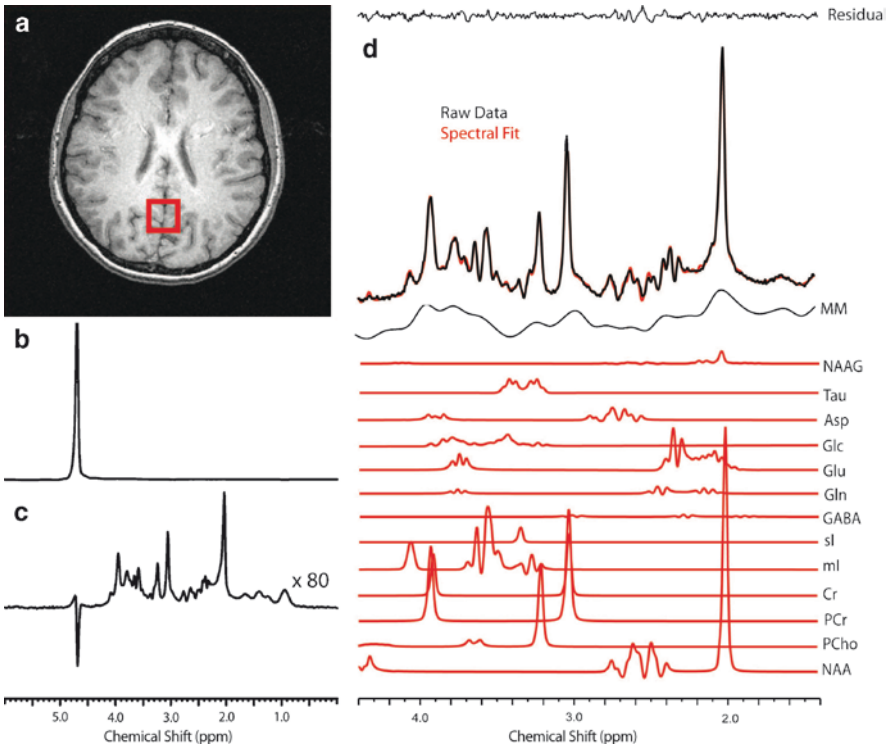

Fig. 1 (**a**) An axial high-resolution T_1-weighted MR image recorded from a healthy adult human head at a field strength of 4.0 T. The MR image shows a section of a 2×2×2 cm MRS voxel (*red box*) positioned within predominantly gray matter of the parietal-occipital cortex. (**b**) A water unsuppressed ^{1}H-MRS spectrum recorded from the voxel shown in Fig. 1a where only a single water proton resonance is seen at 4.7 ppm. (**c**) A water-suppressed ^{1}H-MRS spectrum recorded from the same voxel (PRESS; TR = 2,000 ms, TE = 30 ms, NEX = 256). The vertical scaling was increased 80-fold compared to the corresponding water unsuppressed dataset and metabolite resonances are observable between the 0.5 and 4.2 ppm chemical shift range. **(d)** LCModel analysis of the water-suppressed ^{1}H-MRS dataset presented in Fig. 1c. See text for details

fundamental Larmor equation, $\omega_0 = \gamma B_0$ (units: rad.s^{-1}). For example, at static B_0 field strengths of 1.5 and 4.0 T, the proton resonance frequency would be approximately 64 and 170 MHz, respectively. By convention the chemical shift scale utilizes the same idea for a basic reference, tetramethylsilane (TMS) or related analogs, whose four methyl proton groups would give rise to a single resonance frequency (ω_{ref}) referenced to 0 ppm. The chemical shift (σ) of other proton nuclei is then calculated using a simple formula given by: $\sigma = 10^6(\omega - \omega_{ref}/\omega_{ref})$. For example, water has a higher MR frequency to TMS and the difference is approximately 300 and 800 Hz at B_0 field strengths of 1.5 and 4.0 T, respectively. Hence, the water proton resonance resides at a chemical shift of 4.7 ppm regardless of the applied B_0. Note that when presenting MRS data the chemical shift scale (and frequency) increases from left-to-right.

Cerebral Metabolite ^{1}H-MRS Resonances. A water-suppressed ^{1}H-MR spectrum recorded from the voxel displayed in Fig. 1a is then displayed in Fig. 1c. Note the increased spectral complexity owing to the additional multiple metabolite resonances observed between the 0.5 and 4.2 ppm chemical shift range. The water resonance has been attenuated to a level where access to neurochemical information becomes available and the figure clearly demonstrates the problem of severe spectral overlap associated with in vivo ^{1}H-MRS applications. The spectrum presented in Fig. 1c is dominated by large-amplitude metabolite resonances positioned at 2.0, 3.0, and 3.2 ppm, and those peaks correspond to methyl (CH_3) protons of NAA, the sum of PCr and Cr (total Cr) and Cho-containing compounds, respectively. The peak centered at 3.9 ppm is attributable to the methylene (CH_2) proton nuclei of total Cr. Being enriched in viable neurons, NAA is generally accepted as a marker of neuronal viability and a growing body of evidence also supports NAA's role as a molecular water pump. ^{1}H-MRS NAA decreases have been observed in multiple sclerosis and degenerative diseases such as Alzhemier's disease (AD), possibly relating to depleted neuronal density and/or neuronal dysfunction. Total Cr on the other hand is related to energy metabolism and PCr serves as a precursor for ATP synthesis. The total Cr peak is relatively stable across age and disease types such that it is often used as a normalization reference for expressing metabolite levels. Individual contributions from Cho, GPC and PC make up the 3.2 ppm CH_3 peak with changes generally inferring impaired membrane metabolism. Increased total Cho signal has been reported in tumors, AD and multiple sclerosis. Note that the area underneath each MR resonance is directly proportional to the metabolite concentration and NAA, which is the highest concentrated metabolite in brain matter, produces the most dominant single resonance observed in ^{1}H-MR spectra recorded from healthy brain. Metabolite peak integral is also proportional to the number of proton nuclei giving rise to that specific resonance. Hence, owing to their unique local electronic environments, the CH_2 and CH_3 protons of Cr compounds produce resonances at discrete chemical shift positions with a peak integral ratio of 2:3.

Scalar Spin–Spin (J) Coupling. The three individual proton nuclei comprising the CH_3 group of Cho, Cr or NAA are both chemically and magnetically equivalent. The CH_3 proton nuclei of Cho, Cr and NAA therefore co-resonate at their respective chemical shift positions yielding simple line shape structures that are typically referred to as singlet resonances. In contrast, other types of proton nuclei are both chemically and magnetically inequivalent. Quantum mechanical selection rules, which are outside the scope and aim of this chapter, dictate that magnetically inequivalent nuclei within a given chemical structure are able to interact and "sense" one another's effective spin state through a process known as scalar spin–spin (*J*)-coupling. *J*-coupling is influenced and propagated by the electrons within covalent bonds and leads to a splitting of resonances into more complex lines distributed about a specific chemical shift position. *J*-coupling operates over relatively small covalent bond distances and its strength decreases with distance. We are generally concerned with two- and three-bond *J*-couplings that are usually denoted $^2J_{HH}$ and $^3J_{HH}$, respectively, where the subscript "HH" refers to homonuclear proton–proton coupling. Metabolite resonance splitting through *J*-coupling

effects leads to the observation of multiplet patterns for many proton groups in many of the metabolites detectable *via* ^{1}H-MRS. For example, the ^{1}H-MRS 2.0-3.0 ppm chemical shift region includes *J*-coupled resonances arising from Asp, GABA, Gln, and Glu. For an excellent source of proton chemical shift and *J*-coupling information see Govindaraju et al. (2000). *J*-coupling effects clearly increase the severity of spectral overlap and is a major problem for the quantification of metabolites of interest in CNS disorders including GABA, Gln, Glu and Gly. This necessitates the requirement for (i) the acquisition of high quality well-shimmed ^{1}H-MRS datasets with sufficiently low signal line width and (ii) rigorous spectral quantification procedures and/or alternative MRS measurement strategies.

Analyzing In Vivo ^{1}H-MRS Data

Spectral Fitting and Quantification. MRS-derived metabolite levels are expressed as metabolite ratios (e.g., metabolite:creatine) or absolute concentrations using tissue water as an internal reference standard. The complexity of ^{1}H-MRS data dictates that simple peak integration is a grossly insufficient approach for extracting the peak area. Instead, an unbiased and automated method for spectral fitting that uses a priori information should be used.

The ^{1}H-MRS spectral data presented at the top of Fig. 1d shows an expanded chemical shift region of the same spectrum presented in Fig. 1b. The black spectrum is the raw spectral data as obtained without additional signal processing prior to FT whereas the red spectrum that is overlaid represents as an estimated spectral fit that was carried out using a commercially available MRS fitting package (Linear Combination (LC)-Model; (Provencher 1993)). The spectral fit can be qualitatively evaluated by the residual (raw data minus spectral fit) shown at the top of Fig. 1d. This software performs its analyses in the frequency (spectral) domain and fits a series of 'basis' functions to in vivo MRS data. The basis functions are the individual metabolite spectra recorded from concentrated chemical solutions or "phantoms" or individual metabolite spectra that are simulated using a prior knowledge of chemical shift and *J*-coupling information from a molecular model. Note that it is crucial to employ the same choice of MRS pulse sequence and TE for acquiring or simulating individual metabolite spectra in order to closely match the resulting in vivo data. Furthermore, it is entirely up to the user as to how many basis functions should be included in the analysis and a total of thirteen simulated metabolite spectra were included in the basis set with the relative contributions of each to the final composite fit shown at the bottom of Fig. 1d. The macromolecule (MM) is a baseline function estimated by LCModel and accounts for resonances that are primarily attributable to the side chains of low molecular weight cytosolic proteins. The CH_3 peaks of NAA and Cho are clearly well-fitted and the dual peak nature of the 3.0 and 3.9 ppm resonances due to Cr and PCr components should be noted. The remaining metabolite resonances show complex line shapes and are distributed over several resonance frequencies including the *J*-coupled metabolite peaks

arising from Asp, Glu, Gln, and GABA. Note that NAA and Cho also contained *J*-coupled metabolite peaks (for an excellent source of ^{1}H-MRS chemical shift and J-coupling data for common brain metabolites see (Govindaraju et al. 2000)).

For each metabolite species, spectral fitting algorithms should provide a measure that provides some statistical insight into the relative reliability of the individual fit. In general, these measures often infer a high degree of reliability for fitting NAA, Cr and Cho CH_3 peaks although metabolite ratios and/or absolute concentrations returned for low-concentration *J*-coupled metabolite species such as GABA should be treated with extreme caution. For these metabolites, different ^{1}H-MRS acquisition strategies might be utilized for improved peak resolution and quantification.

^{1}*H-MRS Metabolite Spectral Editing*

A major area of ^{1}H-MRS research at many institutions worldwide has involved the development of novel acquisition strategies that aid the robust quantification of amino acid neurotransmitter species in vivo. CNS drugs may be designed to increase synaptic availability of a given amino acid neurotransmitter by inhibiting catabolic enzymes or through blockade of transporter proteins whereas other CNS drug candidates might be potent agonists or antagonists at postsynaptic receptors. Regardless of their specific mechanism of action, monitoring amino acid modulation via ^{1}H-MRS could provide a biomarker for drug response and deliver unique insights into the mechanism of drug action and efficacy following the administration of a pharmacotherapy.

Notably a handful of ^{1}H-MRS techniques have evolved for measuring GABA, for example, which is the major inhibitory neurotransmitter within the mammalian brain. *J*-difference editing ^{1}H-MRS has become a popular approach for quantifying GABA (Rothman et al. 1993; Mescher et al. 1998) and makes use of the *J*-coupling constant operating between the coupled GABA protons. The *J*-difference edited MRS pulse sequences introduce additional RF pulses whose frequency bands are selectively tuned to a specific GABA resonance. Using otherwise identical MRS sequence parameters (e.g., TR, TE, NEX) a series of signal averages are acquired with and without the application of the additional RF pulses. Posthoc subtraction of the "with" from the "without" spectra affords a *difference* spectrum that retains a GABA 3.0 ppm signal with the cancelation of most other metabolite peaks including the large creatine 3.0 ppm singlet peak. Figure 2a shows an example of a "*J*-difference edited" ^{1}H-MR spectrum recorded from the same voxel shown in Fig. 1a. The data shows that additional Gln, Glu, MM and NAA resonances are "co-edited" in the final *J*-difference spectrum and there is some MM-contamination of the GABA 3.0 ppm resonance. Nevertheless, this type of approach provides a more robust and reliable GABA quantification procedure particularly when compared to data acquired using conventional ^{1}H-MRS methods as shown in Fig. 1b. These editing techniques have successfully demonstrated significant increases of brain GABA levels in epileptic subjects and depressed patient populations following

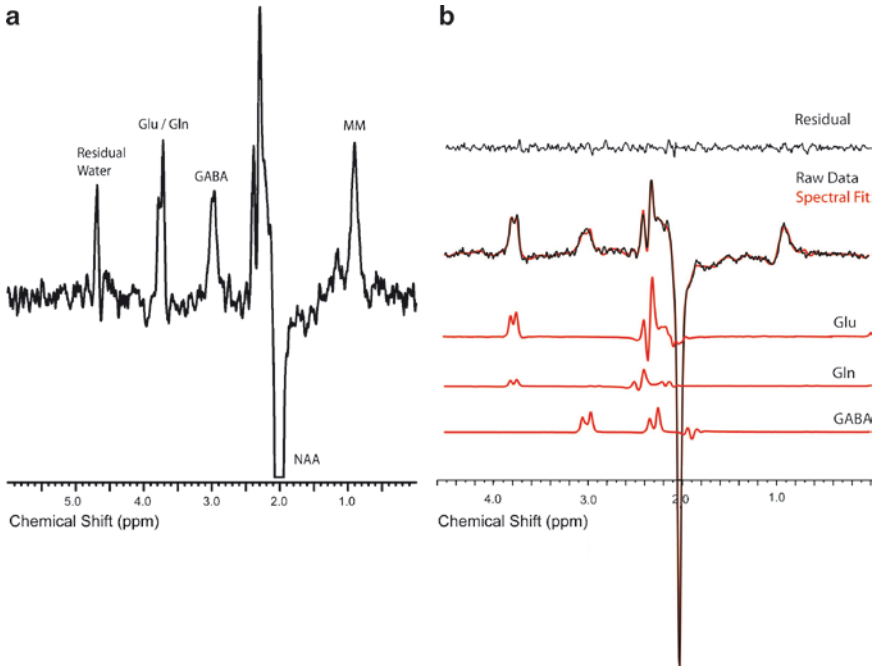

Fig. 2 (**a**) GABA *J*-difference edited ^{1}H-MRS spectrum recorded from the voxel shown in Fig. 1a. In addition to the GABA resonance that is clearly resolved at 3.0 ppm, co-edited proton resonances from Gln, Glu, MM and NAA have been labeled. The spectrum was recorded using a PRESS-based sequence implementation (TR = 2,000 ms, TE = 68 ms, NEX = 256) as described by Mescher and Garwood (Rothman et al. 1993). (**b**) LCModel analysis of a GABA *J*-difference edited ^{1}H-MRS dataset. Only the Gln, Glu and GABA basis function contributions to the composite fit are displayed for presentation purposes

administration of GABAergic anticonvulsants (Petroff et al. 1999) and antidepressants (Sanacora et al. 2002), respectively.

A second method of metabolite-editing known as multiple-quantum coherence (MQC) filtration also takes advantage of the *J*-coupling phenomenon. MQC filtration methods use specific MRS sequences that act to promote quantum mechanical energy level transitions, which are permitted for nuclei within *J*-coupled spin systems but are forbidden for uncoupled nuclei. As such, a specific *J*-coupled metabolite resonance can be targeted and resolved whereas the undesired and uncoupled proton spins are simultaneously suppressed. The "single-shot" nature of MQC-editing is a major advantage of this approach compared to *J*-difference editing and MQC-editing methods have been used to quantify GABA (Keltner et al. 1997) and GSH (Zhao et al. 2006) in vivo. However, the total signal yield and sensitivity associated with MQC filters is relatively low.

The ^{1}H-MRS data presented and methods discussed thus far have been one-dimensional in nature and characterized by a single frequency axis (i.e., the chemical shift axis). Another potential way to access increased metabolite information is

through the use of two-dimensional (2D) ^{1}H-MRS methods and, for these methods, the relevant MRS sequences introduce a timing variable that is systematically incremented between blocks of signal averages and following a 2D FT yield spectral data characterized by two frequency axes (e.g., chemical shift *vs.* *J*-coupling, chemical shift *vs.* chemical shift). The spectral resolution is effectively increased as the metabolite proton resonances are spread over a 2D surface, and a number of spatially localized variants of 2D ^{1}H-MRS have been implemented for in vivo applications with GABA (Ke et al. 2000), Gln and Glu (Schulte and Boesiger 2006; Hurd et al. 2004) being examples of the resolvable metabolites. Specifically, 2D ^{1}H-MRS methods have demonstrated significant GABA elevations in the rat brain following anticonvulsant treatment (Welch et al. 2003). Altered glutamatergic systems have also been linked to psychiatric illness including anxiety and depression and 2D ^{1}H-MRS might be useful for monitoring the efficacy of novel drug candidates that target metabotrophic glutamatergic receptors (Kugaya and Sanacora 2005).

2D ^{1}H-MRS variants also have been proposed for improving the detection reliability of the 3.55 ppm Gly singlet resonance, a peak whose resolution is compromised by the large overlapping and strongly *J*-coupled resonances of mI (Prescot et al. 2006). Single-shot 1D ^{1}H-MRS Gly-detection methods were recently introduced, which attenuate the mI resonances through optimized multiple-refocusing strategies (Choi et al. 2008). Gly is a coagonist at the *N*-methyl D-aspartate (NMDA) receptor subtype, and NMDA receptor dysfunction is associated with psychiatric disorders including schizophrenia. Novel drug species are emerging that enhance NMDA receptor function by blocking the type-1 Gly transporter protein and increasing synaptic Gly levels. Hence, Gly-detection ^{1}H-MRS strategies will serve as invaluable tools for monitoring glycinergic drug response.

Finally, the overall performance of metabolite editing procedures, whether *J*-difference editing, MQC-filtration methods or 2D ^{1}H-MRS, might be enhanced by combining the data acquisition method with an automated and unbiased spectral fitting algorithm. For example, GABA *J*-difference edited ^{1}H-MRS data could be analyzed with LCModel, where phantom-derived or simulated *J*-difference edited basis functions could be used for fitting the Gln, Glu, GABA and NAA contributions (see Fig. 2b). It is also worth adding that many of the spectral editing procedures described are not provided as stock sequences by scanner manufacturers and that local research physicists generally are responsible for implementation and development of editing methods. This often means that the exact choice of metabolite-editing methods at a given facility is institution and research group-specific.

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Part III
Imaging CNS Drug Action

Animal Imaging

Donald Williams, Alexandre Coimbra, and Fuqiang Zhao

Introduction

Animal models have long played an important role in pharmaceutical research, and their use is well embedded in many critical steps in the drug discovery process. The highly structured toxicology testing regimens and measurements of absorption, distribution, metabolism and excretion (ADME) in multiple animal species are required by regulatory agencies for approval of an experimental drug candidate to be administered to humans. Drug discovery also utilizes a wide range of animal studies geared towards risk management of expensive clinical trials by improving and predicting the probability of success in the clinic. Examples of the latter include target validation, lead optimization, and development of biomarkers of disease, compound efficacy and toxicology; some biomarkers can readily be used in the clinic allowing response in the human to be tested with the same metrics used in preclinical development, with minimal or no change in experimental design. The sequencing of human and mouse genome has opened yet another rich area of research using genetically engineered mice with new models of disease for studying disease progression and response to therapy, for providing proof of biology, and providing the possibility of new tests for the safety assessment of compounds (Mouse Genome Sequencing Consortium 2002; The International Human Genome Mapping Consortium 2001).

Imaging offers several advantages over traditional research readouts in animals. While noninvasive imaging of live animals allows longitudinal monitoring, and can significantly reduce the number of animals needed to address a biological question, imaging readouts are also believed to be more closely related to the disease phenotype, thus providing a more direct correlation between therapeutic effect and the measurement (Lawrence & Mackey 2008; Rudin 2008; Wang & Yan 2008; Badea et al. 2008; Henkelman et al. 2005; Beckmann & Rudin 2006). This is especially true

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when the disease phenotype has a spatial characteristic. No single imaging modality will answer all possible questions, with each modality's strengths lying in different domains with respect to the type of information it provides. A full discussion of the use of each imaging modality in drug discovery is beyond the scope of this chapter and the reader is referred to many excellent reviews in the literature (Beckmann & Rudin 2006; Hargreaves 2008; Gwyther & Schwartz 2008; Ripoll et al. 2008). This chapter will focus on Functional Magnetic Resonance Imaging (fMRI) with selected examples to illustrate its application to drug discovery using animal models.

The term fMRI is used here in a broad sense, representing all MRI procedures that provide information about brain function. In this sense, the term refers to techniques such as the now popular BOLD (Ogawa et al. 1990) or cerebral blood volume mapping (CBV) functional MRI techniques (Kennan et al. 1998), which measure regional hemodynamic phenomena associated with neuronal activation, in response to external stimuli or task execution, or Arterial Spin Labeling (ASL) techniques (Williams et al. 1992), which can be used to quantitatively measure regional Cerebral Blood Flow (rCBF) both at rest, and in response to external stimuli.

Exploiting the full potential of fMRI in animal studies requires special consideration. While controlling motion of the subject is important in any MRI experiment, fMRI places stricter standards because of very small signal changes observed, and the need for the signals to be quantified. Animal motion can be controlled either through anesthesia or using a neuromuscular blocker as a muscle relaxant, or in conscious animals, with a combination of restraint and animal acclimation to scanning conditions. While fMRI applications, particularly in rodents have predominantly been carried out on anesthetized animals, the value of conscious animal studies for functional readouts is increasingly being recognized (Lahti et al. 1998; Borsook et al. 2007). Even when the animal is completely immobilized, occasionally, physiological motion related to cardiac or respiratory processes needs to be accounted for; and their effects may be minimized by synchronization of signal acquisition to the motion's periodicity (Zhao et al. 2009), or by post-processing of data (Brooks et al. 2008).

The quality of fMRI signals, particularly with regard to sensitivity, spatial representation, and temporal stability are influenced by the MR measurement technique and other instrumental factors, and the scanning protocol needs to be tailored so as to optimize the desired output characteristics. Furthermore, robustness of the fMRI signal needs to be demonstrated through test-retest data so that studies can be designed with adequate statistical power (Zhao et al. 2008a).

In all fMRI experiments, it is essential to ensure that the signals measured are the direct result of the specific biological function being studied, and not due to any unrelated physiological changes; therefore maintaining a stable state of physiology represents a significant effort in animal fMRI. Key physiological parameters such as heart rate, arterial blood pressure, body temperature and blood gases are usually monitored throughout the experiment. Careful attention needs to be given to the choice of anesthetic, or any possible influence of restraint, such as stress to ensure that these factors do not interfere with the biological function being measured or the measurement itself.

To answer specific questions relating to CNS, a measure of neuronal function is desired, and one of the most challenging tasks is to confirm that the fMRI signal or signal change observed is a true reflection of the neuronal function being interrogated. This is because fMRI does not directly measure neuronal activity. Rather, it provides either a measure of hemodynamic change in response to this neuronal activity, or a resting physiological measure such as CBF or CBV, that is reflective of steady state neuronal activity. Confirming that the fMRI signal change is a direct result of neuronal activity is non trivial; validity of this hypothesis is often sought through additional experiments involving pharmacological tools, or by matching the spatial and temporal characteristics of fMRI data with those that provide direct information on neuronal activity, e.g., electrophysiological recordings (Logothetis et al. 2001; Shmuel et al. 2006). Supporting experiments may involve the use of pharmacological agents known to modulate the neuronal activity either by acting directly on the target neurons (at the synaptic level) or at some point upstream or downstream in a cascade of interconnected events.

The neural activity-induced hemodynamic changes can be measured by fMRI as a BOLD effect, representing the net change in deoxyhemoglobin due to cerebral metabolic rate of oxygen ($CMRO_2$) and CBF, as a change in cerebral blood volume (denoted CBV for simplicity, but includes brain and spinal cord), or as a pure CBF change. The most appropriate choice of fMRI readout will depend on several factors. For example, BOLD is the simplest to implement and is the most widely used, while blood volume measurements with a blood volume contrast agent provide enhanced sensitivity and better spatial specificity (Zhao et al. 2006). BOLD and CBV fMRI rely on a change in signal due to a stimulus, and requires measurement of response to an acute stimulus (i.e., in the same scanning session), and is not suitable for monitoring resting hemodynamic effects reflective of disease or therapy. Measurement of CBF using a quantitative technique such as ASL allows measuring resting blood flow as a marker of neuronal activity, but is also amenable to measuring stimulus-induced changes. In humans, while BOLD and ASL are commonly used, the need for an exogenous contrast agent makes CBV fMRI less common.

Despite the challenges, fMRI in animals can provide a wealth of valuable information often not obtainable by other modalities. It allows adequate flexibility to tailor experiments to obtain information critical to drug discovery process, such as target validation, lead optimization, dose selection, and to provide proof of biology and mechanism of action for novel agents.

Technical Approaches to Animal fMRI

Due to the sensitivity of the fMRI techniques to subject motion, it is imperative that every precaution be taken to maintain subjects as immobile as possible. In any brain MRI experiment, motion artifacts can be greatly reduced by mechanical restraint of the head. In fMRI of conscious animals, anxiety and stress can significantly affect brain function (Takamatsu et al. 2003), and animals need to be acclimated to the

restraint condition in order to minimize stress. The acclimation process typically consists of daily conditioning of the animals to simulations of the conditions observed by the animal while in the scanner, i.e., long periods of restraint and high noise level. It has been shown that through the acclimation process animal stress-related physiology and stress hormone levels gradually normalize and motion is reduced (Fig. 1, (Welsh et al. 2008a; King et al. 2005)). Even when physical movement of the animal is constrained by restraint or with the use of pharmacological agents, motion can also arise from physiological processes such as respiration and heart beat. Respiration can either directly induce motion in the brain or spinal cord, or cause signal instabilities as a result of periodic alterations in field homogeneity. In an fMRI study of the cervical spinal cord, it was demonstrated that respiration caused an MRI signal fluctuation large enough to override the fMRI signal change, completely obliterating any statistical significance of the stimulus-induced signal (Zhao et al. 2009). Synchronization of the data acquisition to the respiratory cycle (Zhao et al. 2009) and/or post-processing of the data to filter out the respiration-induced MRI signal fluctuation has allowed these motion artifacts to be removed effectively (Brooks et al. 2008).

Very significant challenges posed in maintaining conscious animals completely still and devoid of stress, particularly during administration of stimuli, have resulted in the majority of animal fMRI studies being carried out predominantly under anesthesia. However, as anesthetics are pharmacological agents, their choice and use in drug development application need to be carefully considered because of the potential confound effects such as drug–drug interaction (Tsukada et al. 1999) physiological changes (CBF, CBV, and blood pressure, induced by the anesthetic) (Welsh et al. 2008a; Sicard et al. 2003). In studies of CNS function, knowledge of

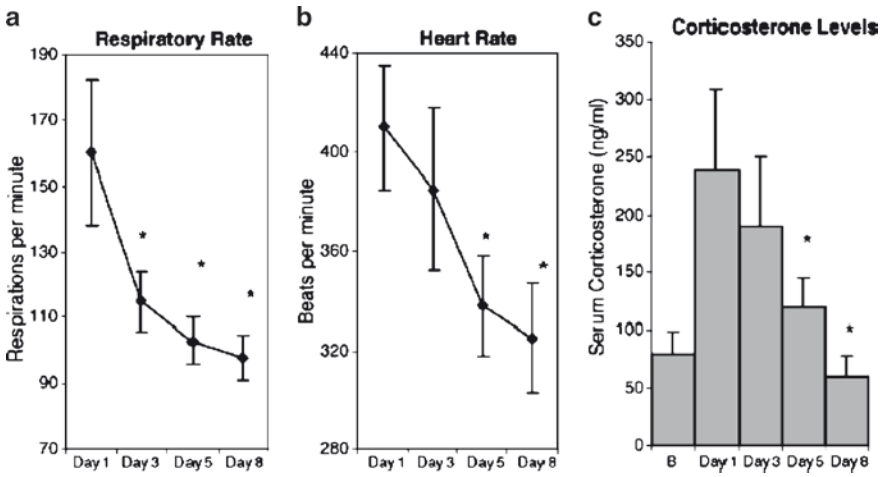

Fig. 1 Reproduced from King et al. 2005. Time evolution of stress-related physiological parameters, heart and respiratory rate, and stress hormone cortisol, indicating normalization of stress in acclimated rats with length of acclimation

the effects of the anesthetic on brain metabolism and hemodynamic function (CBF, CBV), and of interactions between the anesthetic and the specific system under investigation needs to be considered. Some anesthetics are known to affect specific neuro-receptor systems and therefore should be avoided in studies tackling these respective systems. For example, ketamine, is a noncompetitive antagonist of NMDA receptors and also affects the dopamine system (Tsukada et al. 2000). Propofol, was shown to interfere with processing of vibrotactile information in humans (Bonhomme et al. 2001). Isoflurane has also been shown to affect the dopamine system (Tsukada et al. 1999) and vascular responsivity to CO_2 challenge (Sicard et al. 2003) and the vasodilator Acetazolamide (Welsh et al. 2008a).

The ability to scan awake animals creates an opportunity to investigate neurologic processes under true physiologic conditions, and should provide more clinically relevant information during the drug development process. However, careful choice of anesthetic has allowed useful information to be obtained in several key experiments, and fMRI in anesthetized animals remains a viable platform for studying CNS function.

To date, most animal fMRI studies have been performed under anesthesia with α-chloralose or isoflurane (Lee et al. 1999; Masamoto et al. 2007). Alpha-chloralose is a chlorinated acetal derivative of glucose with anesthetic and sedative properties. Its advantages for fMRI studies are that it: (1) preserves metabolic coupling for somatosensory stimulation (Ueki et al. 1992), (2) provides good stability of baseline blood flow (Lindauber et al. 1993), and (3) preserves cerebrovascular reactivity (Bonvento et al. 1994). A drawback with α-chloralose is that its use is often limited to "terminal" experiments due to invasive intubation and catheterization, preventing its use in animal fMRI to investigate longitudinal changes in brain function. Isoflurane, however can be used as an anesthetic for "survival" experiments (Masamoto et al. 2007), but it causes hypotension and respiratory depression, and is a potent cerebral vasodilator, all of which lead to a hemodynamic response confounding the true fMRI signals (Sicard et al. 2003).

Medetomidine (domitor) offers a viable alternative for "survival" studies and an anesthesia protocol has been developed and validated recently (Weber et al. 2006; Zhao et al. 2008b). Medetomidine is an α_2–adrenoreceptor agonist which can provide sedation and anxiolysis, analgesia and some muscle relaxation (Lukasik & Gillies 2003). It is generally used as an adjuvant to reduce anesthetic requirements to tracheal intubation and surgical stimuli (Bol et al. 1999). It was first used in rat fMRI as an independent anesthesia by Weber et al. (2006). Since it is administered subcutaneously requiring no catheterization, the animal can be maintained in a free breathing state requiring no intubation, allowing longitudinal (survival) fMRI studies. With medetomidine anesthesia, well-localized activations in the somatosensory pathway (contralateral primary and secondary somatosensory cortex (SI & SII), contralateral thalamus) have been detected (Zhao et al. 2008b). Also, the incidence of activations in animals with Domitor anesthesia in thalamus and SII are higher than those under α-chloralose anesthesia (Keilholz et al. 2004). To determine reproducibility of long term fMRI signals under Domitor anesthesia, fMRI activations induced by electrical stimulation of bilateral rat forepaws were measured at three

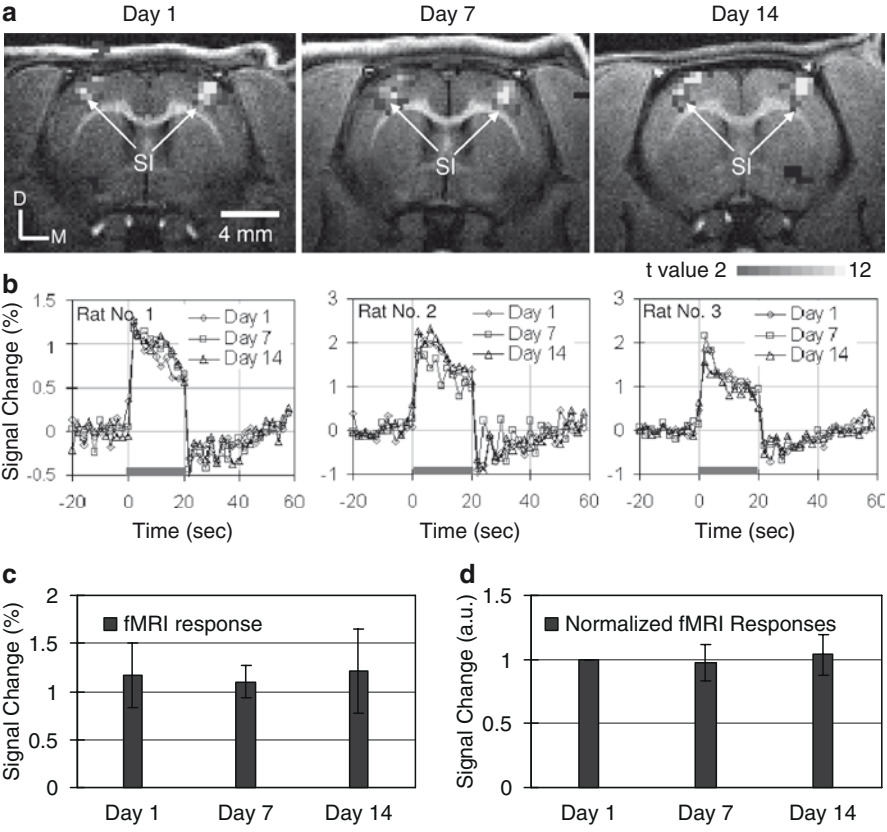

Fig. 2 Reproducibility of BOLD fMRI signals in rats under domitor-anesthesia. Rats ($n=3$) were subjected to bilateral electrical stimulation and fMRI activity maps were obtained using an EPI based BOLD technique at 7T. The same animals were scanned three times, at day 1, day 7 and day 14. (**a**) fMRI activation maps from a coronal slice through S1 of brain from one rat scanned on three different days. The pattern of activation at the three different time points are quite similar. (**b**) Time courses of fMRI responses from an ROI covering S1 of three different rats at the three different times. The temporal patterns of fMRI responses for the same rat at the three different times are also similar. Small variations exist in the temporal patterns of different rats. Bars under the time courses are the stimulation periods. (**c**) Averaged fMRI responses in S1 over three rats at the three different times (mean ± standard deviation). Stability of fMRI signals was examined by one-way repeated measures ANOVA: $F2,6=0.08$, $p=0.92$, indicating that the fMRI signals are stable over different measuring time. (**d**) Normalized fMRI responses (normalized to the response on day 1) at three different times (mean ± standard deviation). The variation of the average normalized fMRI signal change over different sessions is less than 4%

time points 1 week apart (personal communication from authors – unpublished). Figure 2a shows activation maps in a coronal slice through S1 for a representative rat at three time points 1 week apart. Figure 2b shows the time courses for three animals at the three time points. Figure 2c shows the maximum fMRI response, averaged over three animals at the three time points, and Fig. 2d shows the

same data after normalization, to the response on day 1. Excellent reproducibility was observed between the three longitudinal measurements. Most importantly, as indicated in (Zhao et al. 2008b), with the low dose suggested by Weber et al. (2006), rats are in "a sedation level adequate for reliable fMRI experiments" rather than in an anesthetized state, and robust functional connectivity maps were detected between large cortical regions in two hemispheres and in the caudate putamen (CPu) of two hemispheres, consistent with the results from awake humans (Zhao et al. 2008b).

Application of fMRI to CNS Drug Discovery

In the following sections, we illustrate through selected examples, the use of animal fMRI to address specific questions in CNS drug discovery. fMRI of pain is an area of active clinical research to understand how the CNS processes pain, to provide an objective measure of pain, and to evaluate the analgesic efficacy of experimental drug candidates. An fMRI assay to test the analgesic effect will involve measuring the pharmacological modulation of a noxious stimulation-induced activation in CNS, and should ideally have the following characteristics (Wise et al. 2002; Wise & Tracey 2006): (1) the stimulation-induced fMRI signal is repeatable in a time span of hours to acquire sufficient data for a baseline condition and during analgesic application; (2) the analgesics can be administered intravenously to achieve a fast onset; and (3) fMRI signals should have high sensitivity (high contrast to noise ratio) and a wide dynamic range. A pain fMRI assay that satisfies the criteria above has been developed in a rat model and validated with the known analgesic lidocaine as a positive control. The assay uses noxious electrical stimulation as a pain source. Subcutaneously applied noxious stimulus (electrical pulse with 2 ms width, 5 mA amplitude) has been proved to robustly evoke the C-fiber response based on electrophysiology studies (Le Bars et al. 1979) and it has been used as a surrogate of neuropathic pain in human studies (Klein et al. 2005). The reproducibility of such noxious electrical stimulation-induced fMRI signals has also been well established (Zhao et al. 2008a). To achieve a fast onset of the analgesia effect, an intravenous delivery of the therapeutic agent is used rather than oral or other delivery methods with a slower absorption. A fast onset makes it possible for pharmacological effect to be assessed with the data acquired in the same experimental session, so the additional between-session sources of variance (i.e., differences in animal physiology, variations in electrical stimulation efficiency due to needle positions, magnet field homogeneity and its effect on T2*, signal-to-noise ratios, etc.) can be avoided. The sensitivity of the fMRI signal is increased using a blood volume contrast agent (blood volume (BV)-weighted fMRI) rather than BOLD fMRI. A commonly used BV contrast agent is superparamagnetic iron oxide particles, which remain in the vasculature and produce a susceptibility gradient in surrounding tissue (Kennan et al. 1998). BV-weighted fMRI in the spinal cord using noxious electrical stimulation of rat hindpaws gave excellent sensitivity and reproducibility (Zhao et al. 2008a).

In that study, reproducibility of the signals was rigorously tested by examining the correlation of the pixel-wise fMRI activation signals between odd and even runs (Zhao et al. 2008a). Confirmation that the detected fMRI activity in the spinal cord was due to neuronal activity elicited by the electrical stimulation was provided by the fact that fMRI signal activity was located in the dorsal horn ipsilateral to the stimulated paw in the L3–L5 spinal cord segments, matching synapse location of the somatic peripheral fibers. In the cross-sectional direction, the highest noxious stimulation-induced neuronal activations are located in the middle of the ipsilateral dorsal horn, which is in agreement with the data from electrophysiological techniques, 2DG autoradiography, and C-FOS expression (Le Bars et al. 1979; Menetrey et al. 1977; Porro & Cavazzuti 1993), lending further support to the fact that we are indeed detecting a hemodynamic change directly related to noxious stimulation-induced neuronal activity.

Testing this spinal cord fMRI technique as an assay for pain is now presented with the known analgesic Lidocaine as a benchmark. Lidocaine, a sodium channel blocker, is widely used as a local anesthetic. When it is injected locally, it blocks peripheral nerve transmission, thereby attenuating the neural activity transmitted to CNS. To determine if BV-weighted fMRI of spinal cord (Zhao et al. 2008a, 2009) with noxious electrical stimulation in domitor anesthetized-rats can serve as a pain assay, lidocaine was locally injected around the stimulating electrodes in one hindpaw, while the same amount of saline was similarly injected around the stimulating electrodes in the other hindpaw as a control (both paws were subjected to electrical stimulation simultaneously). Figure 3 shows the activation maps obtained before and after injection of saline and lidocaine, respectively. Before local injection of lidocaine and saline, fMRI signals (left column in Fig. 3) show similar patterns of activation. After local subcutaneous injection of lidocaine, fMRI signals were ablated within 2 h and slowly recovered with time (the bottom row in Fig. 3). However, the fMRI signals after saline injection were relatively constant (the top row in Fig. 3). These results indicate that the BV-weighted fMRI in spinal cord can detect the blockage effect of locally injected lidocaine on the transmission of neural activity in peripheral nerves, verifying that BV-weighted fMRI can be used as a pain assay to test the analgesia effect of analgesics.

We now present two examples of the use of fMRI to provide proof of biology and mechanistic information. The first is on fMRI investigation of pharmacological modulation of the co-agonist glycine site of the NMDA receptor. When investigating the effect of new compounds on a specific receptor target system, a study design that includes different pharmaceutical challenges to perturb the system in a predictable way can provide additional support for hypotheses on mechanism of action. This particular site has gained recognition as a promising therapeutic target to enhance glutametergic and dopaminergic system function with no or limited excitotoxic side effects. Compounds that inhibit glycine uptake via blockade of glial and neuronal transporter GlyT1 have been shown to increase extracellular glycine levels in rodents, and have shown clinical efficacy in ameliorating schizophrenic symptoms (Lechner 2006). The pharmacodynamic effects of novel therapeutics that work through indirect mechanisms, such as inhibition of GlyT1, are difficult to assess

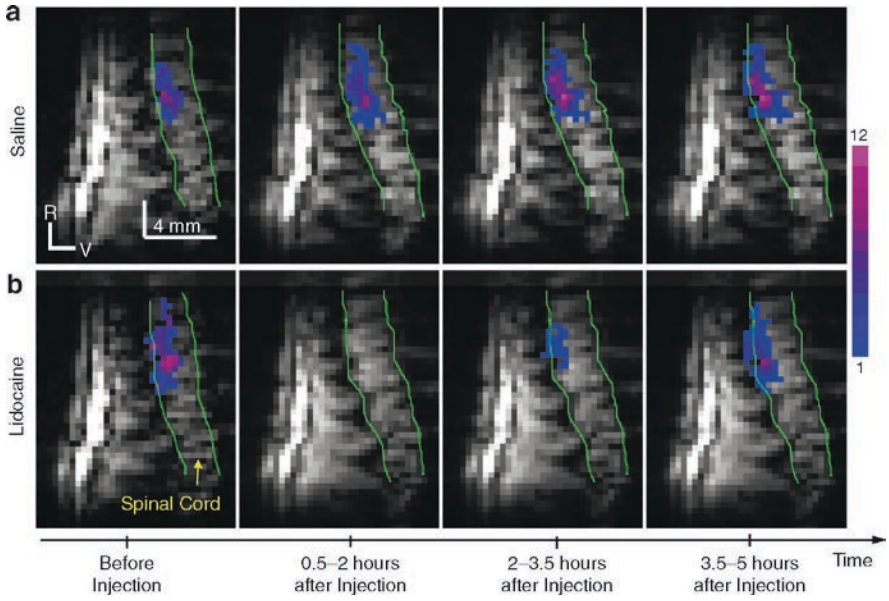

Fig. 3 BV-weighted fMRI demonstrating the effect of locally injected lidocaine in blocking electrical stimulation induced activity in the spinal cord in rat. Lidocaine was locally injected around the stimulating electrodes in one hindpaw, while the same amount of saline was similarly injected around the stimulating electrodes in the other hindpaw as a control (both paws were subjected to electrical stimulation simultaneously). (**a**) and (**b**) show activation maps obtained from two saggittal slices containing the respective dorsal horn before and after injection of saline and lidocaine. Negative ΔS/S changes (*blue/violet* as indicated by the color bar) detected in the BV-weighted fMRI indicate an increase in spinal blood volume. After local subcutaneous injection of lidocaine, fMRI signals in the slice ipsilateral to the paw injected with lidocaine were ablated within 2 h and slowly recovered with time (**b**). However, the fMRI signals after saline injection were relatively constant (**a**). The spinal cords were outlined by the *green lines*. *R* rostral, *V* ventral

in vivo, requiring invasive techniques and/or terminal studies with large numbers of animals. In this context, fMRI is of great value in that it represents a noninvasive method providing a physiologic biomarker for the pharmacodynamic activity of such compounds for proof of concept, dose selection, etc. Here we explore the use of ASL to assess dynamic cerebral perfusion following administration of compounds that modulate the NMDA receptor function (Welsh et al. 2008b).

Figure 4 shows dynamic cerebral blood flow (CBF) measurements during administration of various pharmacological agents known to perturb the NMDA system. Administration of the specific NMDA glycine site agonist D-serine, increased cerebral perfusion compared to baseline perfusion and vehicle controls (not shown). Blockade of the glycine site with a highly selective NMDA glycine site blocker reduced this perfusion response. In contrast, administration of two doses of the GlyT1 inhibitor produced a robust decrease in cerebral perfusion compared to baseline perfusion and vehicle controls. Prior blockade of the glycine site with a

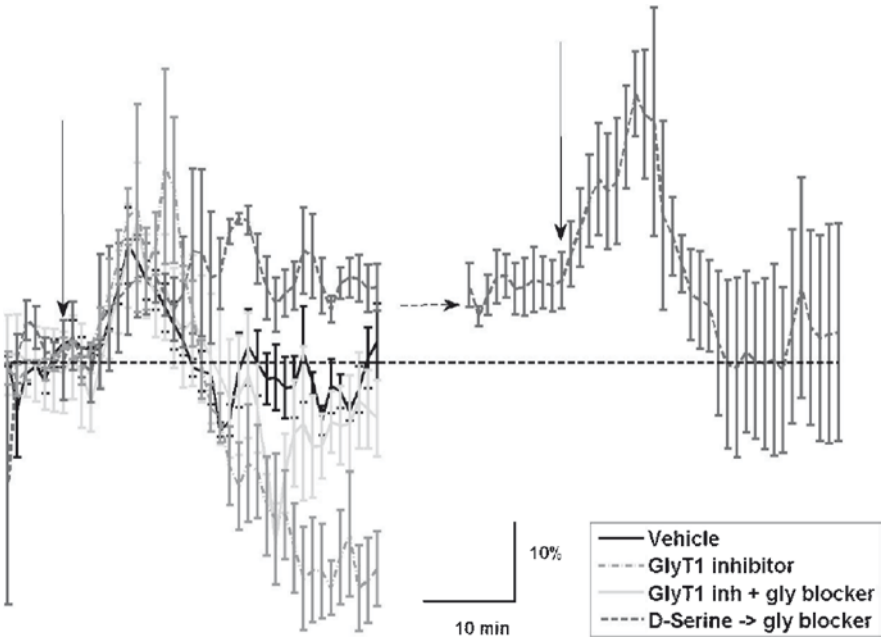

Fig. 4 Dynamic ASL measurements on CBF in rat during administration of pharmacological agents known to perturb the NMDA system. Plots show temporal evolution of blood flow following administration of D-serine and a GlyT1 inhibitor, with and without blockade of the NMDA glycine site via a highly selective glycine site blocker. Scale bars represent 10 min and 10% change in CBF with respect to baseline (*dotted line*). *Solid arrows* denote injections

highly selective glycine site blocker attenuated this decrease. A structurally different GlyT1 inhibitor also produced a robust decrease in perfusion (data not shown) supporting that the CBF increase is connected with NMDA activity and not due to a systemic effect. Arterial CO_2 remained constant for all animals during the course of scanning. Potential confounding covariates such as blood pressure were not measured during scanning, but separate bench experiments showed no increase in mean blood pressure after administration of the GlyT1 compound under the same anesthetic regimen (data not shown).

Given the positive response in perfusion elicited by D-serine, it is surprising that inhibition of GlyT1 produced a decrease in perfusion. Nevertheless, the NMDA receptor component of this response was demonstrated by the prior administration of the highly selective glycine site blocker. In addition, a similar perfusion decrease occurred after administration of a structurally different GlyT1 inhibitor, supporting that this response is a result of GlyT1 inhibition and not off-target activity. While further work is necessary to more fully characterize the effects of D-serine and the GlyT1 inhibitor on CBF, these data suggest that GlyT1 inhibitors indeed affect the NMDA receptor system. Furthermore, these data show that

changes in cerebral perfusion, measured in vivo with noninvasive ASL can provide a physiologic biomarker for assessment of the pharmacodynamic effects of novel psychoactive compounds.

The second example is an fMRI investigation of the mechanism of action of systemic lidocaine on noxious electrical stimulated CNS activity. Systemic lidocaine (e.g., intravenous infusion) has been increasingly used in the management of chronic pain syndromes such as neuropathic pain (Dirks et al. 2000; Bath et al. 1990; Mao & Chen 2000; Geha et al. 2007) since it was first introduced in 1961 for postoperative pain relief (Bartlett & Hutaserani 1961). The mechanism of action by which systemic lidocaine relieves neuropathic pain is unclear (Mao & Chen 2000). Neuropathic pain can result from peripheral nerve damage. The damaged nerve fibers spontaneously generate neural activity, ectopic discharges (Devor 1991), without activation of peripheral receptors. Previous studies have shown that systemic lidocaine suppresses ectopic discharges without blocking peripheral nerve conduction of the normal neural activity induced by electrical and mechanical stimulations (Puig & Sorkin 1995; Devor et al. 1992), suggesting that the action site of systemic lidocaine is the damaged nerves rather than the normal peripheral nerves which conduct the neural activity. We describe below, an fMRI approach to test if systemic lidocaine can block the peripheral nerve transmission of normal activity induced by electrical stimulation.

Lumbar spinal cord fMRI was performed in a 2 mm sagittal slice thickness covering the bilateral dorsal horns, before, during and after infusion of lidocaine at the rate of 1 mg/kg/min (volume rate: 0.1 ml/kg/min) over ~20 min. As a control study, saline was infused at the same volume rate in separate rats. Figure 5 shows fMRI results from one animal with saline infusion and one animal with lidocaine infusion. Fig. 5a features the activation maps of saline and Fig. 5b of lidocaine infusion before, during, and after infusion. Under identical statistical criteria, the activation volumes and activation strength during the lidocaine infusion are smaller compared to those before infusion and those after stopping infusion, indicating that the systemic lidocaine does suppress the stimulation-induced activation in spinal cord. As expected, no significant difference in the activation maps is observed for the rat receiving saline infusion (Fig. 5a).

The suppression effect by lidocaine in the spinal cord fMRI signal measured can be attributed to either a peripheral nerve conduction blockage, and/or to an effect of lidocaine directly on the spinal cord. However, previous studies have excluded the spinal effect of lidocaine due to the fact that the intrathecal lidocaine injection could not suppress the tactile allodynia in neuropathic rats (Chaplan et al. 1995) and the lower limb postamputation stump pain in patients (Jacobson et al. 1990). Therefore the data in this study appear to suggest that the suppression of fMRI activity is likely due to the peripheral nerve conduction blockage. In conclusion, systemic lidocaine, which is believed to only block the peripheral nerve transmission of *abnormal* neural activity (ectopic discharge) originating from the damaged peripheral nerves, also appears to block the peripheral nerve transmission of *normal* neural activity induced by transcutaneous noxious electrical stimulation.

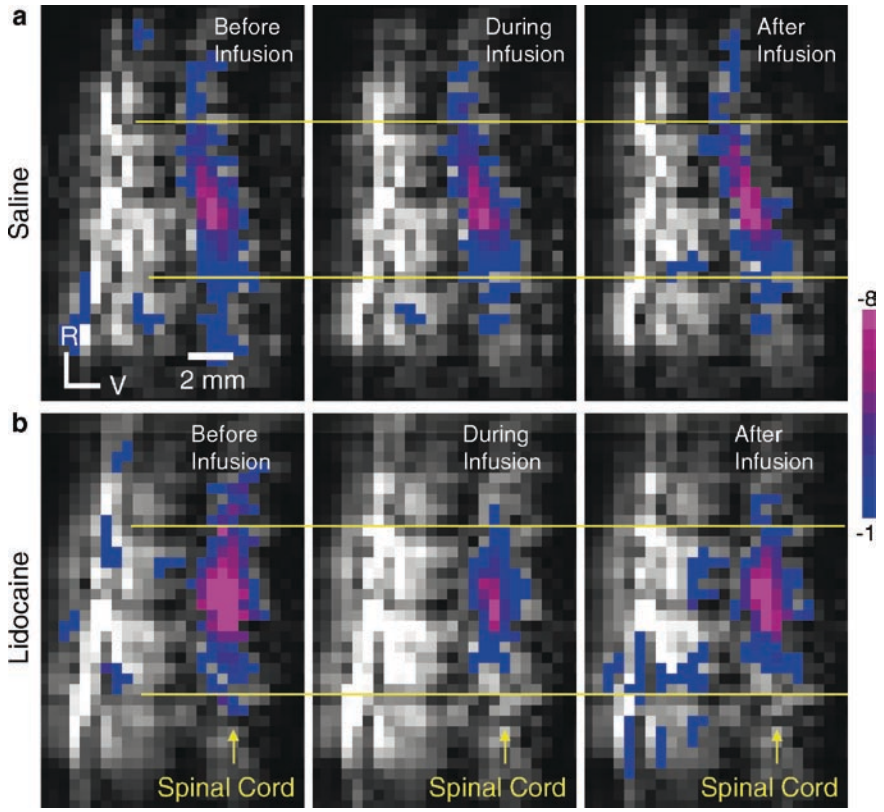

Fig. 5 BV-weighted fMRI demonstrating effect of intravenous infused lidocaine on the noxious bilateral hind paw stimulation-induced fMRI signals in spinal cord. Illustrative data from a saggittal slice covering dorsal horns in two representative animals, receiving intravenous infusion of saline (**a**) and lidocaine (**b**), respectively. Negative ΔS/S changes (*blue/violet* as indicated by the color bar) detected in the BV-weighted fMRI indicate an increase in spinal blood volume. For saline infusion (**a**), robust activations can be detected, and are highly reproducible before, during, and after the saline infusion with regard to both the activation pattern and activation strength. For lidocaine infusion (**b**), the activation becomes weaker during the infusion of lidocaine, and slowly recovers after stopping infusion. *Two horizontal lines* indicate the positions of disk between T_{13} and T_{12} (*top*) and disk between L_1 and L_2 (*bottom*). *R* rostral, *V* ventral

Concluding Remarks

In this chapter, we presented a practical approach to using fMRI for drug discovery using animal models. While fMRI can provide valuable information relating to CNS drug discovery, other MR methodologies such as volumetric MRI, perfusion and diffusion MRI, MR angiography, magnetic resonance spectroscopy (MRS), and the use of targeted contrast agents are being increasingly used by the pharmaceutical industry to help answer questions in nearly all disease areas. Rapid advances in new

techniques and instrumentation in magnetic resonance, and increasing investments made by the pharma on imaging methodologies should result in a rapid growth of imaging based research in the drug discovery process.

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Incorporating Functional MRI into Clinical Pharmacology Trials

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The Use of fMRI in Decision-Making for CNS Drug Development

Brain imaging approaches can help determine CNS drug penetration and distribution, target engagement, pharmacodynamics, proof of mechanism, proof of concept, and even proof of clinical efficacy (Hargreaves 2008; Wong et al. 2009). Positron emission tomography (PET) with radiolabeled ligands is already well incorporated into clinical pharmacology for determining CNS penetration and receptor occupancy of drugs. However, PET ligands cannot always be readily developed for every target and several novel drug classes have emerged for which a direct relationship between PET receptor occupancy and functional response is hard to predict. Thus, there is a growing need for reliable functional pharmacology methods to inform clinical dose setting for novel drug candidates. Functional MRI is suited for this purpose because it offers high spatial and temporal resolution, is safe and non-invasive, and is widely available. During early drug development, fMRI studies can examine acute effects of drugs on baseline neuronal activity and can determine drug impact on task-relevant neuronal activity. In this way, fMRI has the potential to validate targets in humans before expensive late phase clinical trials. However, there are many strategic and operational issues to be clarified before fMRI can start being used to make vital decisions during early phases of drug testing. The process for clarifying these issues will take significant time and resource investments before the benefits of routinely using fMRI in drug development are realized.

The major problem with using fMRI as a reliable reporter for CNS drug effects is that real-time neuronal activity cannot yet be directly detected using nuclear magnetic resonance. Instead, fMRI techniques indirectly report on neural activity by tracking secondary hemodynamic changes, which are several steps removed

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from the actual drug–target interaction (Iannetti and Wise 2007). This multi-step process, known as neurovascular coupling, links post-synaptic depolarization of neurons with subsequent metabolic and signaling events leading to a local change in perfusion that is delayed by several seconds from the initial neural response (Logothetis et al. 2001, 2008; Arthurs and Boniface 2002). Appreciation of this key point frames the major operational considerations for clinical fMRI studies. The use of delayed perfusion change as a marker for neuronal activity is subject to several pharmacological confounds including the potential of drugs to act on neurovascular coupling events, vascular tone, and blood flow. The way in which MRI techniques are deployed to infer CNS drug effects is also a concern since the brain normally has constantly ongoing and changing electrical activity and neurovascular coupling. These factors make the capturing of drug effect versus placebo under basal conditions or during experimental tasks highly dependent on the fMRI methodologies, task paradigms, study subjects, study design, experimental controls, study execution, and data interpretation.

Operational Issues for Using fMRI Methodology in Drug Studies

The appropriate fMRI methods, equipment, experimental design, and strategy to be used for a pharmacological study depends on whether the goal is to define dose ranging for novel drugs on CNS function, to validate the target through drug effect on specific neurobiology, or to develop surrogate measures of efficacy. There are also practical limitations that influence the selected approach since most early drug development studies face timeline pressures and regulatory constraints, have small study cohorts, incorporate novel incompletely understood drugs, and require careful tracking of pharmacokinetics and safety. An appreciation of the commonly used fMRI approaches and potential confounds posed by operational and pharmacological interventions is essential in planning and executing a successful study.

Blood oxygenation level dependent (BOLD) fMRI is the most commonly used fMRI technique and reports on neuronal activity-dependent perfusion through local changes in the amount of deoxyhemoglobin. Although change in neuronal activity is ultimately inferred from BOLD signals, the readout really represents a complex composite of changes in local metabolic rate of oxygen consumption (CMRO2), regional cerebral blood flow (CBF), and local cerebral blood volume (CBV). Since the measurement is a relative change in deoxyhemoglobin, the BOLD response associated with a given task depends heavily on the prevailing baseline level of neural activity, CMRO2, CBF, and CBV present prior to introduction of a task. So if any of these baseline values are modified by the drug under investigation, this may lead to over- or underestimation of the true drug effect during an fMRI task. For example, drugs such as anesthetics and sedatives, which lower baseline CMRO2, or opiates, which lower ventilation (thereby changing pCO_2 and dilating vessels) can alter the BOLD signal independently of their action on regional neuronal circuits. It is important, therefore, to think through the appropriate controls for the drug

mechanism to be studied and to plan on measuring potential drug-associated physiological changes in vital signs, level of arousal, motion artifacts, and cerebral hemodynamics that could confound interpretation of the fMRI data.

Reliance of BOLD signals on deoxyhemoglobin also impacts accurate neuronal signal localization. The greatest changes in BOLD signals are actually detected from venous blood downstream of active neurons. Therefore, in regions close to large veins, BOLD may not accurately localize functional activity to specific neuronal areas. The most popular fMRI sequence to measure BOLD signal (echo-planar imaging, EPI) is also very sensitive to macroscopic magnetic field inhomogeneities, which can lead to signal loss in parts of the frontal cortex and temporal lobes that are near anatomical air spaces. For these reasons, it is important to confirm ahead of time that task-associated changes in BOLD signals from the brain regions of interest are free of artifact. Simply put, by itself BOLD fMRI signal changes can only reliably report drug-induced effects on neural activity if the intermediate steps in the signal transduction from neurons to the fMRI scanner are not greatly altered.

Simultaneous quantitative measurement of CBF along with BOLD signals can help address some concerns regarding the specificity of drug action. Demonstrating a lack of direct drug effect on vascular function improves confidence in declaring that positive results reflect a real drug effect on neural tissue. Although not a standardized practice yet, CBF documentation is sure to gain consensus as fMRI begins to inform Go–No Go decisions for expensive drug development programs. Arterial spin labeling (ASL) is an emerging technique that can provide a convenient way to measure CBF in the same MR scanning session used for the BOLD fMRI measurements (Detre and Wang 2002; Williams et al. 1992). By noninvasively spin labeling water molecules in the blood entering the brain and visualizing this with MR scanning, ASL can allow quantitative calculation of CBF without relying on changes in deoxyhemoglobin levels and can also be used for functional imaging, although the approaches for doing so are not yet as widely standardized or adopted as for BOLD fMRI. ASL may eventually provide better spatial fidelity than BOLD in reflecting region of neuronal activity because the majority of the signal comes from the arterial side of the capillary bed rather than from veins. Of course, a drug could increase regional baseline CBF signal by enhancing baseline neural processes or via direct effect on vascular function. Direct examination of cerebrovascular function by trans-cranial Doppler ultrasonography may be useful in distinguishing the latter from the former. However, this approach requires additional equipment and expertise, and has limitations on the brain vascular regions it can access. A brief CO_2 breathing challenge provides another approach as this treatment is believed to produce direct vasodilation and increase cerebral perfusion without increasing neuronal activity or cerebral oxygen consumption signal. Any change in the perfusion response to this challenge seen with drug versus placebo treatment can potentially be useful for understanding specificity of drug effect and for making adjustments in the analysis of drug effect seen with BOLD signals. Standardized CO_2 challenges can also allow for calibration and quantification of BOLD signals (Chiarelli et al. 2007; Leontiev and Buxton 2007).

Operationally, each additional procedure added to an fMRI experiment presents a variable that can impact clinical pharmacology studies in unexpected ways. Thus

it is prudent to test all fMRI procedure for feasibility, reliability, and safety ahead of time through pilot studies. For example, the interleaving of BOLD and ASL sequences needs to be carefully constructed so as to maximally utilize the time that a subject spends in the scanner, without disrupting the critical task-related data acquisition, which typically forms the basis for the study's primary hypothesis. The length of time required inside the scanner can affect the subject's behavioral state and commitment to the imaging acquisitions. Even adding a minor procedure needs careful consideration regarding study impact. For example, CO_2 inhalation when added on top of an alerting drug in the claustrophobic environment of the scanner can potentially increase anxiety if the exposure is not well controlled. Use of breathing tubes to deliver air and CO_2 (5%) with end-tidal CO_2 measurement to document targeted exposures is useful in this regard, but the airflow resistance in the tube may need to be adjusted via the use of additional airbags so as not to labor breathing effort and generate artifact. Such operational details may best be recognized and corrected by having the clinical monitors, field monitors, or principal investigators personally undergo the imaging protocol firsthand during the preparation stages.

MR scanner technology is continuously evolving. For the clinical pharmacologist, it is important to appreciate that high magnetic field strengths can also increase susceptibility to artifacts within a given field of view. The potential advantages of acquiring high-resolution, high-quality images in individual subjects can even be lost in population studies where images from many individuals may be subjected to multiple registrations and normalizations. How important this is to the experiment and which techniques necessitate the normalization process is worth thinking through. Most pharmacologists are more interested in the statistically significant change in some signal for a given region of interest (ROI) than in the quality of image obtained. Thus, the choice of magnetic field strength and imaging sequences should be rationally pre-specified in the context of the experiment. Regular and well-characterized quality assurance of scanner hardware is required, with clear objective criteria from the manufacturer for determining acceptable and maintainable scanner stability. Given the current stage and evolution of pharmacological fMRI, significant post hoc analysis may be needed to identify drug effects in ways not previously considered. Sufficient brain coverage and flexibility should therefore be planned into the imaging parameters to allow for this. In an academic research setting, it is not uncommon to repeat fMRI scanning sessions to get the best quality data. However, given the extensive planning, coordination, costs, drug exposure risks and regulatory agency amendment requirements of industry-sponsored clinical pharmacology trials, repeating periods or entire studies to acquire additional data not previously planned for is a difficult option.

For these same reasons, it is opportunistically tempting to add on other biomarker measurements into the pharmacological fMRI study. Not only does this take advantage of the existing investments and commitment to the study, but this practice can also enrich the interpretation of the fMRI data by providing alternate complimentary measures of drug effect on CNS physiology. We have performed multi-modal experiments incorporating baseline and task-related assessment of cerebral hemodynamics (BOLD fMRI, ASL and trans-cranial Doppler, all with and without CO_2 challenge), electroencephalography (resting EEG, and evoked scalp potentials), and computerized

cognitive testing within a single session. While there are some efforts in the field to accomplish such multi-modal measurements all within the scanner environment, we used an assembly line or "car wash" approach in which subjects were moved from the scanner to adjoining rooms dedicated to the specific measurements. While this approach allowed each method to be worked out and performed in isolation from technical interference, it did require significant practice, choreography, and operational agility. Since more than one subject was put through this drill per day, our approach required impeccable attention to time allotted for the preparation and execution of each task and for each transfer between tasks. Moreover, this approach required the use of study drugs with predictable and sustained pharmacokinetic exposures. Performed as an operational experiment as much as a drug experiment, this experience taught us a great deal about how to plan, execute, and interpret CNS drug biomarker studies. If planning similar or even much less ambitious multi-modal studies, we highly recommend pilot practice sessions to get the operational details worked out. A clear rationale for adding additional measures is also an obvious pre-requisite.

Besides the MR scanner hardware and imaging sequences, other hardware and software are required for the performance of fMRI experiments. These include stimuli presentation software, button boxes to register behavioral data, two-way audio equipment to communicate with subjects in the scanner, a projector to display visual stimuli, apparel for CO_2 delivery and end-tidal CO_2 measurements, infusion pump for drugs, etc., not to mention any additional equipment for multi-modal studies. The combined equipment setup has to be very reliable and also very stable. This requires investment of time to optimize the setup, but also disciplined use and therefore serious education of all users. In most cases, fMRI systems are not only used for pharmacological research, but are predominantly used for other academic research projects and clinical applications. Many different people will therefore manipulate the system. A team of responsible operators to keep everything functional and in prime condition is absolutely necessary. Failure is always possible and adopting the mindset that "anything that can go wrong will go wrong" can maintain operational vigilance and ward off unexpected disaster. Back-up of the weakest components is useful and usually not expensive. "Emergency needs" for spares button boxes or a spare lamp for the projector are not rare. Also, having a spare stimulus personal computer with identical setup is a very good idea. Make sure you have an up-to-date service contract for the scanner and a good collaboration with the eventual service departments in your environment. You will need them. Do make sure your setup is 100% operational and the necessary spare parts and all service contracts are in place. Do not trust that everything will work. Make sure of it.

The Choice of fMRI Paradigms in Drug Studies

The choice of experimental fMRI paradigm is a key determinant for demonstrating CNS drug effects. Most often, crossover studies are performed in healthy volunteers who undergo scans in which either drug or placebo is administered in separate sessions.

The results of these sessions are compared to reveal regional differences in BOLD signal. Although the terminology still remains to be standardized, two general experimental paradigm approaches are currently popular for exploring brain drug effects through BOLD signals. One paradigm requires some form of task engagement by the subject to trigger a regional change in BOLD signals. Modulation of the specific task-induced BOLD signal change by drug versus placebo is looked for. The other more simple approach is performed with the subject maintained in an unstressed resting state while tracking regional changes in BOLD signal induced by drug administration.

The most commonly used paradigm examines the modulation by a drug of specific task-induced fMRI activity. The task may be chosen to be relevant to the expected clinical action or mechanism of the drug, or may represent systematic activation of sensory, motor, or cognitive brain regions. However, if the implicit hypothesis is that the drug modulates a specific brain function, then the inclusion of control task to evaluate a system which is not expected to be modulated by the drug is essential for demonstrating specificity. An elegant example is the demonstration of opiate inhibition of regional BOLD signal changes evoked by heat pain, with no drug effect seen on regional signal changes provoked with visual stimulation in the same experiment (Wise and Tracey 2006). Similarly, a finger-tapping motor task could be an appropriate control for a drug believed to solely impact cognitive processes. These control tasks can help qualify the fMRI experiment relative to some expected truth statement. Task development is not trivial. Issues such as task difficulty or practice effect can alter regionally appropriate BOLD signals both in volume and in intensity, and can also alter recruitment of additional brain areas. Operationally, the delayed hemodynamic response function for a given task must be known such that image capture can be timed appropriately. Also, subject performance on tasks should be verified. This can be done for example by observing finger tapping for a motor task or giving small memory test of images attended to for a visual task. There is little consensus on the best fMRI tasks for reporting drug effects. Efforts such as CNTRICS (Barch et al. 2009) are beginning to develop standardized approaches for testing cognition in certain disease conditions and a similar effort to develop a menu of fMRI tasks for early phase clinical pharmacology studies in normal volunteers is needed. Overall, fMRI tasks should be engaging, short, and appropriately matched to the subject's language and culture. Also, the effect of drugs on behavioral states such as anxiety, motivation, or sedation should be taken into account when evaluating task performance. Capturing of behavioral data through visual analog scales or other means is useful for allowing the psychological response to the drug to be used as a regressor for modeling drug effects in the brain.

Two variations of the resting state approach are popular. One simply requires an assessment of change in regional BOLD signal or regional blood perfusion prior to and just after drug infusion (Leslie and James 2000). This approach takes advantage of the fact that BOLD fMRI typically reports changes in signal between two juxtaposed conditions. The overall pattern of BOLD signal change is reported and ROIs showing prominent change can be evaluated for rapid pharmacokinetic/pharmaco-

dynamic relationships. Use of acute drug challenge phMRI is not meaningful for studying slowly absorbed, orally administered drugs. Instead, the drug needs to be infused intravenously and needs to enter the brain in a predictable and rapid manner over the time course of a single scanning session. Although rapid drug effects can be distinguished from vehicle infusion with this approach, the number of drugs that can be safely given to human subjects this way is limited, and special formulation is required for investigational drugs. Moreover, there may be significant differences in the way the brain reacts to rapidly rising doses of a drug versus the much slower rise seen after oral dosing. Measurements made in this manner may not be reflective of the desired pharmacology thereby making findings from this approach hard to relate to clinical conditions. Thus, acute dose-dependent fMRI effects observed for antidepressants or antipsychotic agents, which usually take several weeks to produce clinical benefit, may have little relevance to the required dose ranges and neurobiology impacted in chronic studies.

Another emerging resting state paradigm involves assessment of functional connectivity between brain regions, as indicated by the temporal coherence of changes in BOLD signals between brain regions (Bullmore and Sporns 2009; Rogers et al. 2007). If the spontaneous up and down fluctuations of BOLD signal over time in a given ROI are found to be highly synchronized with similar fluctuations in other brain regions, then these areas are believed to constitute a functionally connected network or circuit. With this approach, whole brain connectivity can be mapped using data-driven methods or the analysis can be applied to a restricted set of regions. One can ask whether novel functional connections have emerged between a given "seed" ROI and any other brain regions under drug versus placebo conditions, or whether connectivity in one of several known "default networks" is altered by drug exposure (Becerra et al. 2009). For either analytical approach, the degree of functional connectivity during drug exposure versus placebo can be used to infer drug effect on particular circuits. With either of the resting state approaches, the operational challenge is in ensuring that when the data is acquired, the subject's brain is indeed resting quietly and not distracted by subjective drug effects or the MR scanner environment.

Additional Logistical Considerations

There is always a shortage of scan time, and most departments with MR scanners have a waiting list and a policy for the reservation of scan time. On the other hand, pharmacological research companies often need speedy action and do not want to wait for months before a study can be piloted. This can create conflicts between researchers, clinicians and the institutional administrators. It is of utmost importance to negotiate scan time up front and to communicate the needs of all participants. Use of a calendar committee with clear rules to organize the scan time for all projects is useful. Time also needs to be reserved for a pilot study, which is the ultimate test to assure all parties that the complete study processes are 100% functional.

The pilot should serve to ensure that the staff are well trained, the data captured is of acceptable quality, the flow of procedures is optimal, enough buffers are included, all equipment and staff involved are ready for "high-throughput," and the effect size is appropriate for the powering of the study. Of course this latter part is difficult to discern for drug without giving drug, but the effect size of task-induced BOLD signal change with placebo can at least be estimated. Time also needs to be scheduled for familiarization visits, during which subject should be introduced to the fMRI setup and tasks to determine appropriate inclusion in the study following explanation and exposure to all procedures. Pharmacological studies are complex, and several procedures may have to be repeated due to system malfunction, logistical problems, etc. Reserving reasonable amount of repeat study time and stipulating this provision in the protocol is prudent.

Pharmacological fMRI studies generate a lot of data. Each subject contact for a recent study was almost 1 GB, with 16 subjects and 4 periods adding up to approximately 60 GB. If the hospital or radiology administrator allows internal storage for this then data back-up can be easy. Otherwise, a dedicated file server approach for storing information in a DICOM-compliant format or a PACS (Picture Archiving and Communication System) is recommended. These are typically best setup with back-up server, off-line back-up on site and appropriate audit trailing and regulatory provisions (e.g., US FDA's 21 CFR part 11, HIPAA compliance). Data security is also an issue. Even when made anonymous, data stored on some hospital systems can be viewed and even retrieved by anybody having access to the system. A careful selection of registration combined with an adequate access restriction policy makes inappropriate data access very unlikely. Data treatment is often performed at different locations for different segments of the study. Some paradigms might be developed in house and treated at the site of study, while data from other sequences and/or paradigms are treated at several different remote centers. Therefore, data should be made available via secure links to trusted sites. It is also a good idea to appropriately sort the data in such way that dedicated centers have access to the data they are going to process.

A good understanding of all the stakeholders' needs is crucial to success. Pharmaceutical companies and the research centers which accommodate their studies have both scientific and business interests at stake. In order to convince the research community and the hospital or university administrators of merit, industry-sponsored studies should always yield a good scientific output. Methodological papers are a good starting point if possible, but results of the study must be published and the participating center should be included in all these publications. This should be discussed upfront with the investigators involved, so as to avoid dissention at a later time.

In some ways, the use of fMRI for making neurosurgical patient management decisions can offer an important perspective for those considering its use in drug development decisions. In neurosurgery, specific resection of pathological tissue is required with minimal disruption of surrounding healthy brain tissue. Accurate identification of eloquent cerebral cortical regions near lesions using fMRI is essential for avoiding morbidity (Johnson and Stacey 2008). The vital nature of the neurosurgical

decision requires that meticulous pre-planning is done and that there are safeguards against fMRI equipment failure, magnetic field susceptibility artifacts and image distortions. Utilization of validated functional paradigms for specific cortical region activation, pre-specification of data analysis and interpretations, careful coordination of all efforts, and agreement by stakeholders on the decisions to be made from the fMRI study results are all key components of success. A similar level of operational rigor will be required before fMRI is routinely used to make Go–No Go decisions for multi-million dollar drug development programs. There are as yet no standard guidelines for pharmacological fMRI methodologies, functional paradigms, subject selection criteria, positive controls, or analytical approaches. Efforts such as the Imaging Consortium for Drug Development (Borsook et al. 2008) can fill some of these gaps but have only recently gotten underway. We believe that by leveraging operational best practices from their clinical pharmacology training, the pharmaceutical industry staff can play a key role in helping fMRI develop into a powerful and enabling tool for drug development.

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Imaging Placebo Responses in the Brain

Luana Colloca and Fabrizio Benedetti

Introduction

Any medical treatment that is performed in routine medical practice has two components, one related to the specific effects of the treatment itself and the other related to the perception that the therapy is being administered (Colloca and Benedetti 2005). The latter is labeled as placebo effect or placebo response. The study of the placebo effect is basically the analysis of the relationship between the complex psychosocial context surrounding the patient and its effects on the patient's brain (Benedetti 2002). The role of the psychosocial context in facilitating the cognitive and emotional modulation of a therapeutic outcome definitively emerges from the different outcomes arising from expected (open) or unexpected (hidden) administration of drugs (Colloca et al. 2004). A hidden treatment, of which the patient is completely unaware, is less effective than a treatment given overtly in accordance to routine medical practice. Thus, it appears clear that expecting a therapeutic outcome may affect the outcome itself. In this case, the placebo effect can be defined as the difference between the open and the hidden administration of the treatment, even when no placebo has been given.

Today, there is increasing evidence that beliefs and expectations, which are associated with the therapeutic procedure *per se*, can play a salient role in human health, and placebos can mimic, enhance, mask or prevent the beneficial responses to pharmacological agents (Colloca and Benedetti 2005; Benedetti et al. 2007).

The aim of this chapter is to give an updated account of the utilization of several brain imaging and mapping approaches in the study of the neurobiological mechanisms of placebo responses in pain, Parkinson's disease, depression and drug addiction. In fact, although the pharmacological approach with agonist and antagonist drugs has provided important information on the biochemical events triggered by placebo-induced expectations (e.g., Benedetti et al. 2005; Benedetti 2008; Colloca and

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Benedetti 2005, Hoffman et al. 2005), pharmacological studies based on behavioral and/or humoral responses cannot shed light on the brain regions involved in the placebo response across different medical conditions.

Pain and Analgesia

The involvement of endogenous opioids in placebo analgesia was first proposed by Levine et al. (1978) after studying dental postoperative pain. These investigators found that the opiate antagonist naloxone reduced the probability of a positive placebo response. Following this pioneering study, many accurate and carefully designed experiments confirmed the effect of naloxone in antagonizing placebo analgesia (Fields and Levine 1984; Levine and Gordon 1984; Benedetti 1996; Amanzio and Benedetti 1999), as well as the role of cholecystokinin (CCK)-antagonists in the potentiation of the placebo analgesic response (Benedetti et al.1995; Benedetti 1996; Benedetti et al. 1997). Following these pharmacological studies, a number of brain imaging studies started describing the brain areas involved in placebo analgesia.

PET studies. The first imaging study of placebo analgesia used PET and showed that a subset of brain regions are similarly affected by either a placebo or the μ-opioid agonist, remifentanil, supporting the hypothesis of a related mechanism in placebo-induced (psychological effect) and opioid-induced analgesia (pharmacodynamic effect) (Petrovic et al. 2002). In particular, the administration of a placebo induced the activation of portions of the anterior cingulate cortex (ACC), the orbitofrontal cortex (OrbF), and the anterior insula (INS). A significant co-variation in activity was found between ACC and the lower pons/medulla besides a sub-significant co-variation between the rostral ACC (rACC) and the periacqueductal gray (PAG), thus indicating that a descending rACC/PAG/pons/medulla pain-modulating circuit could be involved in placebo analgesia, as previously suggested by other authors (Fields and Price 1997; Fields 2004). In fact, an opioid neuronal network has been described as a descending pain-modulating pathway that connects, either directly or indirectly, the cerebral cortex to the brainstem (Fields and Price 1997; Fields and Basbaum 1994; Price 1999). In particular, ACC and OrbF project to PAG which, in turn, modulates the activity of the rostral ventromedial medulla (RVM). The "On" and "Off" cells of the PAG and RVM, which show opposite anticipatory pain-related activity, may be the possible target of this descending modulation (Fields 2004).

It is worth recalling that the μ-opioid receptors are heavily distributed in the cortical and subcortical regions relevant to pain and analgesia, including the thalamus (Th), the ACC, the nucleus accumbens (NAc), the amygdala (Amy), and the PAG, as well as some other nuclei in the brainstem (e.g. the parabrachial nuclei) (Willoch et al. 1999, 2004). Zubieta et al. (2005) have brilliantly confirmed the role of μ-opioid receptors in placebo analgesia. By using the selective μ-opioid receptor agonist [^{11}C]carfentanil and PET imaging, they investigated the effects of pain and placebo on the degree of μ-opioid receptor availability in different brain areas,

taken as an index of the activity of the endogenous opioid system. Painful stimulation was associated with significant changes of opioid receptor occupancy in the dorsal ACC, the medial prefrontal cortex (PFC), the rostral INS (controlaterally to pain), the ventral basal ganglia, bilaterally (NAc extending to ventral pallidum), the medial Th, the right Amy, the left subamygdalar temporal cortex, and PAG. Changes related to placebo were detected in the left dorsolateral prefrontal cortex (DLPFC), the rACC, the ipsilateral NAc, and the right anterior INS. This report is the first direct neurochemical evidence that a placebo procedure activates a pain and stress inhibitory neurotransmitter system, the endogenous opioid system. In another study, the same group demonstrated that the placebo-induced activation of the endogenous opioid system is modulated by the internal affective state. In fact, the emotional state of the subjects during pain, or the affective quality of experienced pain, were significantly associated with changes in placebo-induced endogenous opioid release measures, as gauged using PET and [^{11}C]carfentanil, in the DLPFC, the anterior INS, and the NAc (Zubieta et al. 2006). Overall, increases in endogenous opioid neurotransmission have been found in a number of key opioid-rich regions (Zubieta et al. 2005; Wager et al. 2007). During placebo treatments, the opioid activity increases in PAG, rACC, the pregenual ACC, multiple loci within OrbFC, the anterior INS, Th, DLPFC and Amy (see Fig. 1a). Conversely, the anticipatory phase just before the benefit is characterized by a decrease in opioid activity in the right PFC [DLPFC, superior frontal sulcus (SFS) and inferior frontal junction (IFJ)], the left amygdala, the left anterior INS, the pregenual ACC, the dorsal PAG and the caudate (see Fig. 1b). Interestingly, connectivity analyses on individual differences in opioid system activity revealed that placebo treatment increased functional connectivity between the PAG and rACC, as well as among a number of limbic and prefrontal regions (Wager et al. 2007).

Lieberman et al. (2004) used PET to assess the brain response of patients with irritable bowel syndrome (IBS), both before and after a 3-week placebo regimen. Following a pre-placebo scan, the patients were given inert pills to take on a daily basis for the next three weeks. However, they were told that the pills would decrease their recurrent abdominal pain and discomfort associated with IBS. Both in the pre- and post-placebo phase, the patients were scanned at rest and during rectal stimulation. A robust positive correlation was found between pre- and post-placebo regional cerebral blood flow (rCBF) increases in the right ventrolateral prefrontal cortex (VLPFC) and subjective reports of symptom improvement. A connectivity analysis revealed that increased activity in the right VLPFC was associated with decreased activity in the dorsal ACC.

As far as individual differences in the formation of placebo analgesic responses are concerned, the role of the mesolimbic dopaminergic pathway has been found. By combining PET and [^{11}C]raclopride and fMRI, Scott et al. (2007) demonstrated that high placebo analgesic responders were characterized by greater right NAc responses during expectation of monetary gain. Thus, variations in NAc reward processing may explain a substantial proportion of the variance in placebo effects. In a different study by the same group (Scott et al. 2008), placebo-induced activation of opioid neurotransmission was detected in the ACC, the OrbF and INS, the NAc,

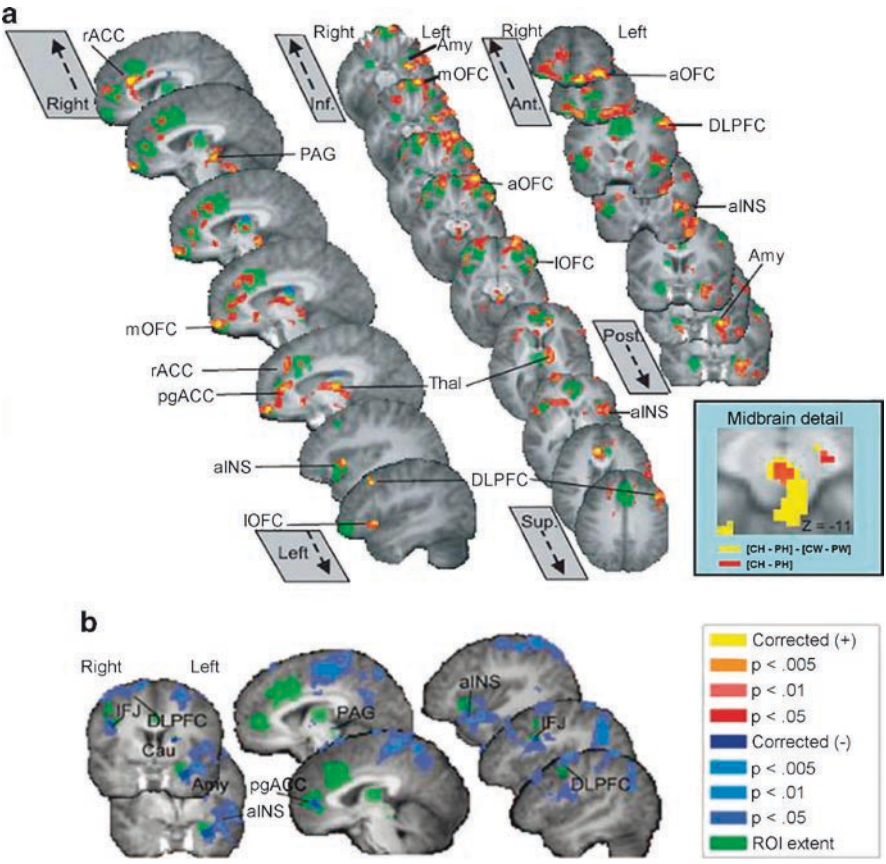

Fig. 1 (**a**) Placebo effects on human μ-opioid activity during pain. Note that the regions of interest (ROIs) show significant pain-specific opioid activation in the PAG, the rACC, the pregenual ACC, multiple loci within OFC, the aINS, the Th, the DLPFC and the amygdala. The opioid-rich ROI's extent is shown in green, and the significant voxels in or contiguous with the ROIs are presented in red/yellow (positive effects) or lavender/blue (negative effects). The insert shows the midbrain detail corresponding to the following placebo interaction contrasts: [(control painful heat, CH - placebo painful heat, PH) – (control nonpainful warmth, CW – placebo nonpainful warmth, PW)] and [CH-PH]. (**b**) Placebo effects on human μ-opioid activity during anticipation of benefit. Specific placebo-induced anticipation decreases of μ-opioid activity were found in the right PFC [DLPFC, superior frontal sulcus (SFS) and inferior frontal junction (IFJ)], the left amygdala, the left aINS, the pgACC, the dorsal PAG and the caudate. (Modified from Wager et al., 2007; Copyright © 2008 National Academy of Sciences, U.S.A.)

Amy, and PAG, and dopaminergic activation was observed in the ventral basal ganglia, including NAc. Regional dopamine and opioid activity were associated with the anticipated and subjectively perceived effectiveness of the placebo and the reductions in continuous pain ratings. Interestingly, whereas high placebo responses were associated with greater dopamine and opioid activity in NAc, nocebo

responses were associated with a deactivation of both dopamine and opioid release. NAc dopamine release accounted for 25% of the variance in placebo analgesic effects. Therefore, placebo and nocebo effects appear to involve opposite responses of dopamine and endogenous opioids in a circuit that is typically implicated in reward responses and motivated behavior.

fMRI studies. Wager et al. (2004) published the first fMRI work on the neural mechanisms underlying the effects of expectations on placebo analgesia. The authors showed that the activity in some regions of the pain matrix, particularly the thalamus, aINS and caudal ACC, was decreased by a placebo treatment after experimental noxious stimuli (either electrical or thermal) in healthy volunteers. In addition, during the anticipation phase of the placebo analgesic response, activation was found in a cognitive-evaluative network, including the DLPFC, the OrbF, the rostral medial and anterior PFC, and the superior parietal cortex (SPC), as well as the PAG (Wager et al. 2004).

By combining expectations and a validated sham acupuncture device, Kong et al. (2006) found that the fMRI signals decreased during placebo manipulation in the bilateral, lateral, and orbital PFC, the rACC, the cerebellum, the right fusiform gyrus, the parahippocampus, and the pons, and indicated that a complex neural network takes part in placebo analgesia, involving more regions than previously supposed. In this regard, the rACC has been found to play a key role in the maintenance of placebo analgesia, along with the subcortical structures involved in antinociception, such as the PAG and the bilateral Amy (Bingel et al. 2006). There is also some evidence that the activation of the right medial and ventral DLPFC may differentiate placebo responders from placebo non-responders (Colloca et al. 2008).

An important contribution of expectations to the magnitude of brain responses to noxious stimulation also emerges from the fMRI studies by Koyama et al. (2005) and by Keltner et al. (2006). The former study showed that expectations of decreased pain powerfully reduced both the subjective experience of pain and the activation of the primary somatosensory area (SI), the INS and Cing (Koyama et al. 2005). These findings were confirmed and extended by Keltner et al. (2006), who adopted a balanced experimental design with two levels of noxious thermal stimulation (high and low) and two corresponding levels of expectancy (high and low). Significant fMRI activations were found in nociceptive regions (the Th, INS, primary and secondary somatosensory cortices (SI-II), and ACC) and in the OrbF, the Amy, the ventral striatum, and the nucleus cuneiformis (nCF), when the high-intensity noxious stimulus was anticipated and accompanied by the high-intensity cue. By contrast, fMRI activation was significantly lower when the high-intensity noxious stimulus was anticipated and accompanied by the low-intensity visual cue.

In a more recent study, placebo analgesia was accompanied by large reductions in brain activation within the pain-related regions (the Th, SI-II, INS, and ACC) during rectal distension in IBS patients (Price et al. 2007). This study is important because it is the first to demonstrate the neural correlates of the placebo analgesic effect in a clinically relevant condition. Overall, these findings clearly demonstrate that expectations and beliefs influence pain perception and the activity of the pain

system. It is worth noting that these modulatory processes also seem to involve placebo responses other than placebo analgesia. In fact, a similar modulatory network, including the rACC and right OrbF, has been found to be activated during an anxiolytic placebo condition (Petrovic et al. 2005).

MEG/EEG studies. The neuroimaging approaches with both PET and fMRI have a relatively low temporal resolution and, consequently, they are limited in addressing the question as to whether placebo manipulation can affect early pre-evaluative nociceptive processing. In this regard, Lorenz et al. (2005) demonstrated that the amplitude of the laser-evoked MEG fields within the contralateral SII at about 165 ms after stimulus onset, were reduced if preceded by auditory cues indicating less intense stimuli (low expectation), and increased when they were preceded by auditory cues indicating more intense stimuli (high expectation). Conversely, the amplitude of the evoked responses peaking at about 300 ms after laser stimuli, with a single dipole source within a region encompassing the caudal ACC and posterior Cing, failed to show any cue validity effects.

Some other studies have described the placebo analgesic responses by using scalp laser evoked potentials (LEPs), which present the advantage of exploring the early central nociceptive processing non-invasively and investigating the peripheral Aδ fibers selectively. Wager et al. (2006) found that a placebo cream significantly reduced the P2 amplitude of LEPs which peaks at midline scalp electrodes and originates in the ACC (see Garcia-Larrea et al. 2003). Watson et al. (2007), suggested that both N2, generated by several cerebral areas, including the bilateral SII, INS and ACC (Garcia-Larrea et al. 2003), and the P2 component of LEPs are reduced by placebos.

These two studies tested the placebo responses after a pre-conditioning phase. However, the role of verbal suggestions (anticipation of benefit) has also been investigated in different neurophysiological contexts. Goffaux et al. (2007) demonstrated that opposite therapeutic expectations (analgesia or hyperalgesia) modulate pain at three levels: (1) the spinal cord (nociceptive RIII-reflex), (2) the brain somatosensory-evoked potentials (SEPs) reducing the N100, N150 and P260 components of SEP, and (3) the subjective perception of pain.

The loss of expectations following the impairment of cognitive functions has been found to disrupt the placebo component of treatments. In a clinical condition, Benedetti et al. (2006) applied a local anesthetic, either overtly or covertly, to the skin of patients with Alzheimer disease (AD) to reduce the burning pain after venipuncture. They correlated the placebo component with both cognitive status and functional connectivity among different brain regions. Therefore, in this case, the EEG was not used to study the responses to placebos, but rather to detect functional changes in demented patients. The researchers found that AD patients with reduced prefrontal executive control and decreased EEG connectivity of the prefrontal lobes with the rest of the brain, showed a reduced placebo component of the analgesic treatment. Remarkably, the loss of these placebo-related mechanisms reduced the treatment efficacy such that a dose increase was necessary to produce adequate analgesia.

Parkinson's Disease

Recently, Parkinson's disease has emerged as an exciting model to investigate the mechanisms of the placebo response. In fact, different approaches, ranging from PET to micromapping methods (microrecording and microstimulation) have significantly increased the body of knowledge of the placebo effect. Although micromapping methods in humans are highly invasive, as they are carried out in awake patients during neurosurgery, they have provided important insights at the single neuron level.

PET studies. By using PET imaging, de la Fuente-Fernandez and colleagues (2001, 2002) detected a significant drop in [^{11}C] the raclopride binding potential (BP) when Parkinson's patients were injected with a saline solution along with the suggestion of motor improvement. A reduction in [^{11}C] raclopride binding is indicative of an increase in extra-cellular dopamine concentration. In the study by de la Fuente-Fernandez et al. (2001, 2002), it occurred in the dorsal and ventral striatum. As the patients who experienced symptomatic benefit released more dopamine in the dorsal striatum than those who did not, the degree of placebo-induced dopamine release in the dorsal striatum seems to be related to the degree of perceived improvement by the patient (de la Fuente-Fernandez et al. 2001). Conversely, the level of placebo-dopamine release in the ventral striatum is independent of the perception of clinical benefit (de la Fuente-Fernandez et al. 2002). As the ventral striatum (NAc) is involved in the circuitry of reward mechanisms, de la Fuente-Fernandez et al. (2001, 2002) suggested that placebo-induced dopamine release might be related to the expectation of a reward. In the case of the placebo effect, the reward would be the clinical improvement.

Strafella et al. (2006) studied whether the expectation of therapeutic benefit from repetitive transmagnetic stimulation (rTMS) induced changes in striatal [^{11}C] raclopride BP. Placebo-rTMS induced a significant bilateral reduction in [^{11}C] raclopride BP in the dorsal and ventral striatum as compared to the baseline condition. With respect to previous studies (de la Fuente-Fernandez et al. 2001, 2002), they did not observe significant differences in [^{11}C] raclopride BP in the dorsal striatum between the group of patients who perceived the clinical benefit and the group who did not. In fact, placebo-rTMS induced a significant biochemical response in the striatum in all the patients, although only four of them perceived a certain degree of clinical benefit. Patient group characteristics, the type of given information, and / or previous medication exposure could explain this discrepancy.

Single-units recordings. The subthalamic nucleus (STN) is now the major target in the surgical therapy of Parkinson's disease, and its identification can require the recording of intranuclear electrical activity. The possibility of studying Parkinsonian patients implanted with electrodes for deep brain stimulation has been exploited to record from single neurons after the administration of a placebo. Benedetti et al. (2004a) first investigated the placebo effect at the level of single neurons. These authors recorded the activity from single neurons in the STN before and after placebo

administration to test whether neuronal changes were linked to the clinical placebo response. A placebo (saline solution) was administered in the operating room after several pre-operative administrations of apomorphine, according to a conditioning procedure. Those patients who showed a clear-cut clinical placebo response — as assessed by both the decrease of arm rigidity and the subjective report of well-being — also showed a significant decrease of neuronal discharge compared to the pre-placebo condition (Fig. 2a). None of the placebo non-responders showed these differences (Fig. 2b). Benedetti et al. (2004a) also found that the STN neurons of all the placebo responders shifted significantly from a pattern of bursting activity to a pattern of non-bursting discharge. None of the placebo non-responders showed any difference in the number of bursting neurons before and after placebo injection.

This is the first study of a placebo effect showing that a placebo procedure affects specific neuronal populations. These findings — decrease of frequency discharge and shift from bursting to no-bursting activity — were interpreted as a demonstration of drug-like effects following the pre-operative exposure to the treatment with apomorphine. Indeed, several studies have reported apomorphine-induced changes in the STN firing pattern of patients with PD (Lozano et al. 2000; Levy et al. 2001; Stefani et al. 2002). Although Levy et al. (2001) found a certain variability in the firing rates of single neurons under the effect of apomorphine, Stefani et al. (2002) reported that the administration of apomorphine is invariably followed by a reduction of firing activity from 40.4 to 27.2 Hz. Similarly, in the study by Benedetti et al. (2004a), a reduction in the firing rate was induced by a placebo (Fig. 2c).

Stimulation of STN. Benedetti et al. (2004b) also performed a micromapping stimulation study in PD patients during the implantation of electrodes. They found that the stimulation of the dorsalmost part of the subthalamic region, which includes the zona incerta and the dorsal pole of the STN, produced autonomic responses that did not differ in the hidden (unexpected) and open (expected) stimulation conditions. By contrast, the stimulation of the ventralmost region, which includes the ventral pole of the STN and the substantia nigra pars reticulata (SNr), produced autonomic and emotional responses that were inconstant over time and varied according to the covert or overt stimulation. It is interesting to point out that this ventralmost part of the subthalamic region is likely to be involved in limbic-associative functions (Alexander et al. 1986, 1990; Parent and Hazrati 1995). Thus, the stimulation of the ventral STN is likely to evoke effects that are related to associative-limbic functions rather than to a pure mechanism of cardiovascular control (Kaufmann et al. 2002; Thornton et al. 2002). In a similar study by the same group (Lanotte et al. 2005), it was found that the expected stimulation of the subthalamic region induced larger autonomic responses than unexpected ones if, and only if, the stimulation involved the ventral portion of the subthalamic region. These data suggest that expectation might increase the excitability of limbic structures, so that unexpected stimulations would require an increase in intensity in order to induce effects comparable to those induced by expected stimulation.

These micromapping studies, albeit highly invasive and difficult to perform, give us information about the effects of expectation and placebo mechanisms at the level of specific regions of the brain, such as the subthalamic region, and tell us that

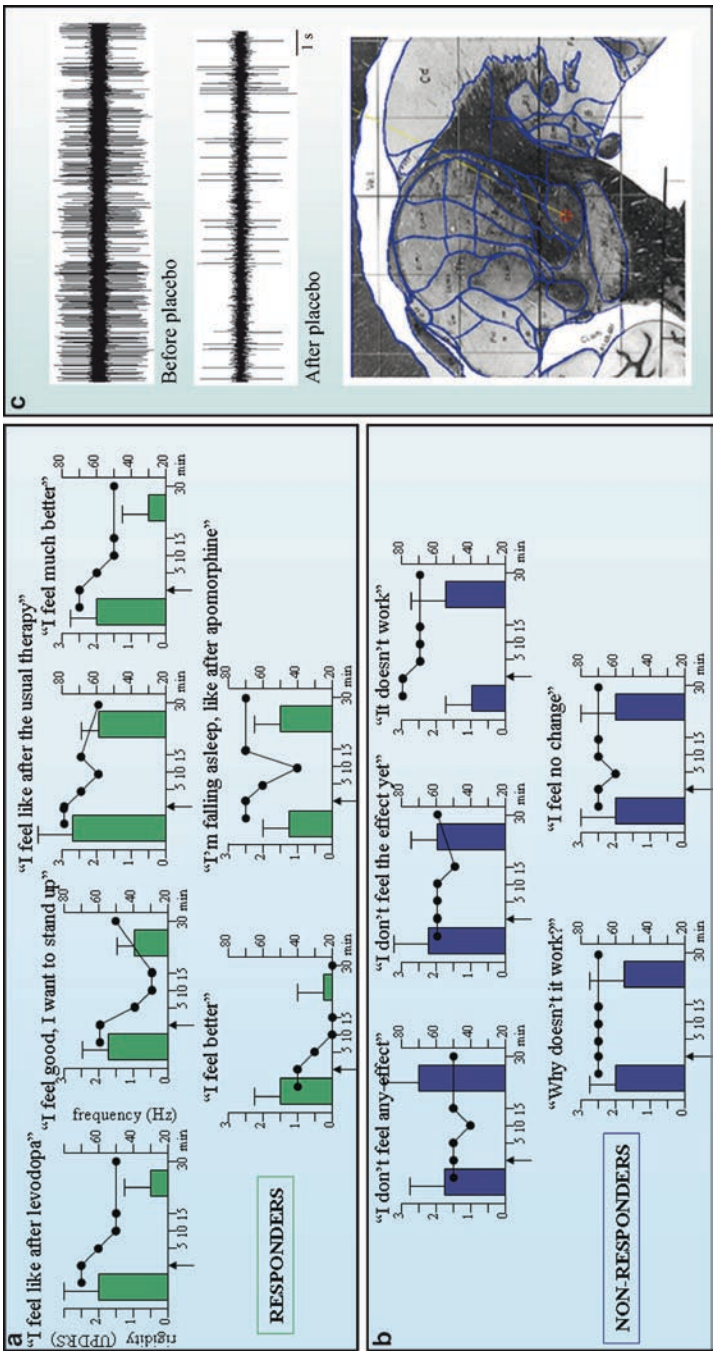

Fig. 2 Correlation among arm rigidity (*black circles*), STN neuronal frequency of discharge (*bars*), and subjective report in placebo responders (**a**) and non-responders (**b**). The *black arrow* on the abscissa indicates placebo administration. There was a decrease in neuronal activity after placebo administration in the placebo responders, but not in the non-responders. (**c**) A representative recording of a single neuron from the subthalamic nucleus before (above) and after (below) placebo administration. As shown in the table of the "Atlas for Stereotaxy of the Human Brain" by Schaltebrand and Wahren (1977), the electrode track was planned using a 58–63° anterior-posterior angle and a 14–20° lateral angle. The subthalamic nucleus was anatomically localized 2.5 mm posterior and 4 mm inferior with respect to the mid-commissural point, and 12 mm from the midline. Subthalamic activity showed an irregular pattern of discharge (bursting activity) at a frequency of 25–45 Hz. Note how firing rate and bursting activity decreased after the injection of placebo apomorphine (Modified from Benedetti et al. 2004a)

expectations can modulate the outcome of a treatment, be it a pharmacological agent or an electrical stimulation.

Depression and Drug Addiction

The neural mechanisms of placebo treatments have also been studied in psychiatric disorders, such as depression and drug addiction, although a few pieces of information are available in this case. There is a clear explanation for this. Unlike single-dose trials of an intervention, such as oral or intravenous analgesia or anti-Parkinson acute therapy studies, antidepressants do not work acutely, requiring on average a minimum of two-three weeks to manifest clinical effects. Therefore, investigating placebo effects in depression is more problematic from both an ethical and methodological point of view. In fact, if one wants to see what happens in the patient's brain by means of neuroimaging techniques, it is necessary to follow the patient for a long period of time, or else, to devise pre- and post-treatment assessment with adequate control groups. Of course, if one wants to compare a placebo group with a no-treatment group to rule out spontaneous remission, this requires that some patients need not to be treated for a long period of time, involving inherent ethical problems and limitations. This is one of the main reasons why depression, albeit an interesting and exciting model to study placebo effects, has not been investigated in detail so far.

EEG and PET studies in depression. Depressed patients who undergo a placebo treatment have been found to show both electrical and metabolic changes in the brain. Placebos induced EEG changes in the prefrontal cortex of patients with major depression, particularly in the right hemisphere. In fact, Leuchter et al. (2002, 2004) found distinct neurophysiological patterns in the placebo responders behind the prefrontal region by using an off-line elaboration of EEG recordings, labeled *cordance*, which is a method developed in their laboratory. Placebo responders also tended to have slightly enhanced cognitive processing speeds on a variety of neuropsychological tests, and they differed in the nature of their sleep complaints in comparison to non-responders.

By using PET, changes in brain glucose metabolism have also been documented in subjects with unipolar depression (Mayberg et al. 2002). Compared to baseline patterns, patients treated with drug (fluoxetine), regardless of response, showed changes in the subcortical areas, including the brainstem, and hippocampus, and cortical regions, including the posterior Cing, the DLPFC, the premotor cortex, the dorsal ACC, and the inferior parietal posterior INS. It was possible to note a suppression of activity in the subgenual cingulated (area 25). The placebo responders showed activity patterns in the cortex similar to those of the drug responders, but the magnitude of change was smaller in patients who received a placebo. Although these studies on depression need further research and confirmation as they did not

include appropriate control groups, they are a good example of the placebo effect in another pathological condition, and in particular, they show the similarity in the activation pattern of the brain by antidepressants and placebos.

PET studies in drug addiction. Another example of the powerful role of expectation in drug responses is the work by Volkow et al. (2003, 2006), who investigated the effect of placebos in both cocaine abusers and non-drug abusing subjects. In particular, they described the effects of methylphenidate on brain glucose metabolism, as measured by [^{18}F]deoxyglucose-PET, when subjects expected (1) to receive the drug and did receive the drug; (2) to receive the drug but received the placebo; (3) to receive the placebo but received the drug; (4) to receive the placebo and did indeed receive the placebo. The researchers found that when the subjects expected to receive the drug, the effects were about 50% greater than when the subjects did not expect the drug. In other words, unexpected methylphenidate induced smaller changes in the thalamic and cerebellar metabolism, thus indicating that expectation potentiates the pharmacological action of methylphenidate (Volkow et al. 2003). In non-drug abusing subjects, the changes of brain glucose metabolism occurred in regions involved in emotional reactivity and reward, such as the ventral gyrus (BA 25) and NAc (Volkow et al. 2006). The different findings in cocaine abusers and non-cocaine abusers suggest that in the first case, the enhanced thalamic and cerebellar responses reflect conditioned responses, whereas the changes in the striatum observed in the non drug-abusing subjects may indicate the prevalence of expectations in the absence of prior experience.

Conclusions

The recent brain imaging investigations have extensively elucidated the brain circuitry related to placebo responses in both health and pathological conditions. However, the placebo effect is an open field of research, which, it is hoped, will answer many questions about the effectiveness of various pharmacological, physical and psychological treatments. Multiple aspects deserve future scientific investigations to better understand the mechanisms through which the action of drugs is affected by top-down modulation. For example, today we know that dopamine and opioids are involved in the anticipation of analgesia (Scott et al. 2007, 2008), CCKs and opioids in the modulation of pain (Benedetti 2008) and emotion (Gospic et al. 2008), but little is known about the involvement of other neurotransmitters during placebo treatments. Therefore, the study of placebo mechanisms in pain, Parkinson's disease, depression, and drug addiction, as well as in other illnesses, will be important to understand both the action of drugs and the therapeutic response to their administration.

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Structural Imaging of Drug Actions in Neurodegenerative Diseases

Susanne G. Mueller

Background

Definition of Structural Imaging

At the first glance, structural imaging appears ill-suited to study drug actions. As implied by the name, structural or anatomical imaging provides information about brain structure. Drugs usually interact with their target by binding to an extra- or intracellular receptor. This induces a change of the functional state of the target cell within milliseconds – hours, i.e., an event which is not captured by structural imaging. However, over a longer time period some of these functional changes can result in changes of cell morphology or tissue composition, i.e., structural changes which are captured by structural imaging if they affect a large enough number of cells. Therefore, while structural imaging cannot usually detect the immediate effects of a drug, it can detect some aspects of their long term actions.

Structural information can be obtained either by computed tomography imaging (CT) or by magnetic resonance imaging (MRI). Both techniques have their strengths and weaknesses. Compared to MRI, CT exams are relatively inexpensive and can be acquired within a few minutes. Furthermore, CT exams are not hampered by metal implants or claustrophobia. MRI, however, has the advantage of higher image resolution, excellent gray/white matter discrimination and provides superior information about white matter disease. In addition to this, MRI does not suffer from bone hardening artifacts in the temporal lobe region and the fact that the imaging plane can be freely angulated allows for an optimal representation of the structure of interest. These properties give structural MRI an important advantage over CT;

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not only regarding sensitivity and specificity for the diagnosis of neurodegenerative diseases (e.g., sensitivity and specificity of MRI for the diagnosis of Alzheimer's disease (AD) is 80–94% and 60–100%, respectively compared to 63–88% and 81% of CT (Frisoni 2001)) but also for the assessment of potential treatment effects in those diseases. Based on the contrast mechanism, structural MRI can be divided into two main categories: (1) volumetric imaging, i.e., T1 weighted imaging, which provides good gray/white matter contrast and thus allows for optimal assessment of gray matter structures and T2, proton density weighted or FLAIR images, which depict white matter abnormalities as hyperintense regions and (2) diffusion tensor imaging, which provides information about white matter directionality and integrity. There are additional contrasts which can be derived from those basic contrasts, e.g., T1 or T2 relaxation measurements, magnetization transfer measurements, etc. However, their usefulness for diagnostic purposes or the assessment of drug effects still needs to be further explored.

Role of Structural Imaging in the Treatment of Neurodegenerative Diseases

The best method to establish the efficacy of a drug against a neurodegenerative disease would be to directly determine the number and function of neurons surviving due to the drug. However, this is impossible in human patients and instead surrogate outcome markers, which supposedly reliably reflect the number of surviving neurons, are employed. Currently, clinical measures of disease severity are most commonly used for this purpose, e.g., degree of cognitive impairment in AD or motor impairment in amyotrophic lateral sclerosis. While clinical measures unquestionably reflect very important aspects of the disease severity, they suffer also from limitations, e.g., they allow no distinction between symptomatic and truly disease modifying effects and have a low test–retest reliability due to non–disease-related factors which are difficult to control for, e.g., patient motivation, or subjective assessment of a function like muscle strength. As a consequence of these shortcomings, clinical measures are increasingly complemented with additional objective and quantifiable outcome markers.

The most important requirement for a good nonclinical surrogate biomarker is a well established relationship between the measure it provides and a clinical outcome meaningful to the patient, e.g., memory impairment is highly correlated with the degree of hippocampal volume loss in patients suffering from AD (Petersen et al. 2000). Furthermore, if the biomarker is to be used to assess drug effects, it should capture a feature of the disease process which is influenced by the drug, e.g., if the drug is supposed to reduce plaque accumulation in AD, the biomarker should ideally provide a measure of amyloid plaque load or at least a measure highly correlated to plaque load. It is also important, that there is a close temporal relationship between the disease process influenced by the drug and the biological event measured

by the biomarker, i.e., if a drug is supposed to be active during the earliest stages of a disease; the biomarker should reflect the disease process in the preclinical stage. Finally, the biomarker should provide an objective, quantifiable measure and have a high test–retest reliability.

Structural MRI fulfills many of these requirements and is thus increasingly used as a surrogate marker of disease progression in neurodegenerative diseases such as Alzheimer's disease, vascular cognitive impairment, amyotrophic lateral sclerosis, multiple sclerosis, etc.

Volumetric MRI

Volumetric MRI allows for the assessment of two important features: A. Volumetric measurements. In the context of neurodegenerative diseases it is predominantly volume loss in diseased subjects compared to healthy subjects, and the rate at which the loss occurs which is of interest. Volume loss can be diffuse and affect the whole brain or be restricted and affect only one tissue category, e.g., gray matter, or a well defined structure, e.g., the hippocampus. B. Imaging contrast of a brain structure. Disease processes often influence the relaxation properties of protons in a tissue and thus the image intensity. For example, ischemic damage to white matter leads to the appearance of white matter hyperintensities on T2 weighted images.

Measurement of volume loss or intensity changes on MRI provides meaningful information about the disease process. Several studies correlating these MRI features with autopsy findings found that gray matter loss in structural MRI is highly correlated with neuron loss or reduction of synaptic density and white matter hyperintensities with de-myelination, axonal rarefaction, and other disease specific characteristics, e.g., reduced vasculature in ischemic lesions (Bobinski et al. 2000; Brown et al. 2007; De Leon et al. 2004; Jack et al. 2002; Matsusue et al. 2007; Matthews and Arnold 2001; Moody et al. 2004; Petersen et al. 2000). Unfortunately, neuron loss or axonal rarefaction and consequently gray matter volume loss and white matter hyperintensities are not disease specific. Both can for example occur in vascular dementia and also in normal aging. However, large cross-sectional and longitudinal studies have shown that there are not only qualitative and quantitative differences in the regional pattern and rate of the development of volume loss/white matter intensities between normal aging and neurodegenerative diseases but also between different disease processes. For example, in normal aging rates of global brain atrophy typically increase from an annual rate of 0.2%/year at age 30–50 to 0.3–0.5%/year at age 70–80 and affect frontal and parietal gray matter more than occipital and temporal gray matter while changes in white matter are more diffuse (Resnick et al. 2003). In contrast, atrophy rates in neurodegenerative diseases are significantly higher, i.e., up to 2–3%/year (Fox and Schott 2004; Gunter et al. 2003) and affect different and more structures than in normal aging, e.g., AD is characterized by increase atrophy rates of limbic and temporal lobe structures but relatively maintained frontal lobe structures (Cardenas et al. 2003; Chatelat et al. 2002).

There are two fundamentally different approaches to assess volume changes in volumetric MRI. If the brain structure affected by the disease is known and anatomically well defined the so-called region of interest approach can be used in which this structure is marked either manually by a trained rater or automatically by a computer aided marking procedure. If the regions with volume changes are not very well defined or diffuse, computer aided whole brain volumetric techniques are used, e.g., tissue segmentation, global boundary shift integral method (Freebourogh and Fox 1997), voxel based morphometry (Karas et al. 2003), tensor based morphometry (Studholme et al. 2003) or cortical thickness measurement (Fischl and Dale 2000; MacDonald et al. 2000), etc. Manual and computer aided marking methods have both been shown to have high test–retest reliability (Hsu et al. 2002; Han et al. 2006).

Diffusion Weighted Imaging or Diffusion Tensor Imaging

Diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI) are sensitive to the random motion (diffusion) of water in brain tissue. If unrestricted, the motion of the water molecules is random or isotropic, i.e., is equally probable in any direction. In highly structured tissues the motion of the water molecules is hindered by the physical boundaries of the three-dimensional tissue microstructure and occurs preferentially parallel to those boundaries, i.e., becomes anisotropic. These properties make DWI ideally suited to detect the effects of acute ischemia, and DWI/DTI to detect neurological diseases affecting the integrity of highly structured tissues like white matter, e.g., multiple sclerosis. DTI allows for the calculation of three eigenvectors: λ_1 indicates the direction of the highest diffusivity and is thought to represent a measure of axonal integrity, λ_2 and λ_3 indicate the direction of intermediate, respectively lowest diffusivity which combined into the so-called radial diffusivity provide a measure of the myelin sheet integrity (Herrera et al. 2008; Song et al. 2002). Often, these three primary measures are also combined into a summary measure, e.g., apparent diffusion co-efficient (ADC), fractional anisotrophy (FA), relative anisotropy (RA), etc.

Although the exact mechanisms determining water diffusion in healthy and diseased brain tissue are not fully understood, several studies found that changes of MRI derived diffusion indices show an excellent correlation with abnormalities of white matter structure detected by histopathological examination (Budde et al. 2007; Simonyan et al. 2008; Song et al. 2005; Wu et al. 2007) and also with functional and clinical measures, e.g., disability indexes or cognitive functioning (Huisman et al. 2004; Makris et al. 2007; Kraus et al. 2007). Similarly as volumetric MRI, DTI changes can be measured using a region of interest approach or by whole brain voxel-based or tract-based analysis approaches (Smith et al. 2006; Snook et al. 2007). However, due to the special imaging requirements for DTI acquisition, DTI is more prone to imaging artifacts than volumetric MRI. These artifacts reduce its test–retest reliability and thus its usefulness for longitudinal studies. Nonetheless, preliminary studies have shown that DTI has reasonably good test–retest reliability

if acquisition, post-processing, and data analysis protocols optimized for longitudinal studies are employed (Bonekamp et al. 2007; Cheng et al. 2006; Ciccarelli et al. 2003; Kim et al. 2006; Muller et al. 2006; Pfefferbaum et al. 2003; Wakana et al. 2007).

Summary

Volumetric MRI and DWI/DTI provide biologically and functionally meaningful information in the context of neurodegeneration and thus fulfill the most important requirement of an effective biomarker. Volumetric MRI is currently the structural method of choice for the assessment of treatment effects in the CNS. Compared to DTI, volumetric MRI is less affected by imaging artifacts and several well established acquisition protocols and manual and automated parcellation procedures with high test–retest reliability already exist. One of the aims of the Alzheimer's disease neuroimaging initiative (ADNI), whose first cycle will be completed in 2009, is to identify those methods in the wealth of existing acquisition and analysis techniques which provide the most reliable and cost effective measures of disease progression in large multisite clinical trials with newly developed AD treatments. DTI provides structural information, which cannot be obtained by volumetric MRI, and preliminary studies have shown that it might provide an excellent measure for disease progression in amyotrophic lateral sclerosis and prediction of recovery after closed head traumatic brain injury. However, further research addressing such basic questions as for example which one of the different DTI measures reflects the progress of different disease mechanisms best and how they are influenced by a successful therapeutic intervention or which imaging and postprocessing methods are most effective and robust, etc. will be necessary to fully establish DTI as a surrogate biomarker for clinical trials.

Application of Structural MRI to Monitor Drug Action

Theoretically, there are two ways in which structural MRI can be used to monitor drug action in neurodegenerative diseases. A. Prediction of drug response. Not every drug is effective in all patients and one possible reason for treatment failure is that the target structure is too severely damaged by the disease and thus cannot interact appropriately with the drug. In such cases, structural MRI can provide important information regarding the condition of the target structure. B. Monitoring of drug efficiency. If a treatment is supposed to slow down or halt the neurodegenerative process, serial structural MRI before and after initiation of the treatment can be used to provide an objective measure of how the disease progression is influenced by the drug. For example, if a treatment is supposed to prevent the appearance of new white matter lesions in multiple sclerosis, serial structural MRIs can be used to monitor the progression of the lesion load under the treatment.

The following section gives an overview of how structural MRI has been used to either predict drug response or assess treatment efficacy in the two most common forms of dementia: Alzheimer's disease and cerebrovascular dementia.

Drug Effects in Alzheimer's Disease

Dementias are the most common manifestation of neurodegenerative diseases. In the population over 75 years, the prevalence of dementia is 15–21%. By far the most frequent form is Alzheimer's disease (AD) with an incidence of 5.9–10.8 cases per 1,000 above the age of 75. Consequently, the development of drugs against dementia has mostly focused on AD and several AD drugs have already been approved or are currently being tested in clinical trials. Treatments against AD can be divided into two main categories. A. Symptomatic treatments, which improve cognitive impairment, but do not modify the disease process itself. This group includes for example cholinesterase inhibitors like donepezil or NMDA receptor antagonists like memantine (Klafki et al. 2006). B. Disease modifying drugs, e.g., drugs preventing the deposition of amyloid plaques like beta-secretase inhibitors and neuroprotective drugs like caspase inhibitors and neurorestorative drugs, e.g., nerve growth factors, neurotrophins (Cummings et al. 2007).

Neuron loss and accumulation of neurofibrillary tangles and amyloid plaques in the perirhinal/entorhinal cortex and hippocampus are the earliest histopathological manifestations of AD (Braak and Braak 1996; Delacourt et al. 1999). In later stages, temporal, parietal, and frontal neocortical regions become involved as well. Volumetric MRI detects hippocampal, respectively perirhinal/entorhinal volume loss not only in subjects suffering from AD but also in subjects with mild cognitive impairment (MCI). Patients with MCI, while not yet fulfilling the criteria for dementia, show below average performance for age and education in one or more cognitive domain and have a significantly higher risk to develop AD than cognitively intact subjects (10–15% MCI convert to AD per year compared to 1–2% healthy controls (Petersen et al. 2001)). Longitudinal volumetric MR studies have shown that atrophy rates of hippocampus and entorhinal cortex and also of other brain structures, e.g., ventricle size, whole brain volume, cortical volume or cingulate gyrus (Cardenas et al. 2003; Chan et al. 2003; Killiany et al. 2000; Silbert et al. 2003; Thompson et al. 2004; Wang et al. 2003), are good predictors for conversion from healthy controls to MCI or from MCI to AD. The availability of highly automated and reliable computer-based methods for the measurement of atrophy rates of larger brain structures not only allows for a substantial reduction of the sample size required to detect differences between treatment groups but also for the efficient processing of the large number of data sets generated by such a trial.

The majority of clinical studies using volumetric MRI as outcome parameter were done in trials assessing the efficacy of drugs which modulate the cholinergic system. AD is associated with a reduction of the acetylcholine synthesizing enzyme choline transferase in neocortical regions and loss of cholinergic cells in the nucleus

basalis Meynert. The so-called cholinergic hypothesis assumes that some of the deficits observed in AD are a consequence of the resulting under function of the cholinergic system and thus improve if this imbalance is corrected by either inhibiting the degradation of acetyl-choline or by administering cholinergic agonists (Klafki et al. 2006). Jack et al. (2003) used serial MRI measurements of hippocampal and temporal horn volumes to monitor the effects of milameline, a cholinergic agonist, in a multicenter study. The MRI arm of the trial consisted of 192 patients (active drug: 100; placebo: 92) with probable AD who had a baseline MRI and a follow-up MRI 12 months later. Although milameline had no effect on hippocampal volume loss, this study was the first to demonstrate that volumetric measurements of the hippocampus and temporal horn volume can be obtained with high consistency across different study sites. Krishnan et al. (2003) studied the effect of the cholinesterase inhibitor donepezil over a treatment period of 24 weeks on the hippocampal volume in 67 patients with mild to moderate AD. About half of them were treated with donepezil, the other half with placebo. Both groups underwent cognitive and MR assessments every 6 weeks. Despite the short observation time and the fact that the study was not powered to detect small treatment effects, hippocampal volumes decreased significantly less (−0.4%) in the treatment group compared to the placebo group (−8.2%). That donepezil has a positive effect on hippocampal atrophy rate was confirmed by Hashimoto et al. (2005) who measured hippocampal atrophy rate using a semi-automated marking routine in 147 AD patients before and after 12 months of treatment (54 with donepezil 5 mg/day, 93 with placebo). They found that patients receiving the placebo had a higher annual hippocampal volume loss (−3.2%) than patients treated with the active drug (−2.8%). Venneri et al. (2005) studied 26 patients suffering from minimal to mild AD who were treated with either donepezil ($n=9$), rivastigmine ($n=9$) or galantamine ($n=8$) for 20 weeks. MRI was acquired before and after the treatment period; whole brain gray matter changes were assessed with voxel-based morphometry. Despite the small sample size and the short observation period, these authors found that patients treated with rivastigmine not only improved cognitively but also had less cortical gray matter loss than the patients in the two other treatment groups. They explained this difference by the fact that donepezil and galantamine selectively inhibit acetylcholinesterase but rivastigmine also inhibits butyrylcholinesterase which enhances the neurotoxic effects of amyloid plaques. Jack et al. (2008) determined annualized atrophy rate of the entorhinal cortex, hippocampus, whole brain volume, and ventricle enlargement in 131 subjects diagnosed with MCI – a subgroup of the 769 MCI patients enrolled in a large multisite trial assessing the treatment efficacy of donepezil and vitamin E (Petersen et al. 2005). While they found only a nonsignificant trend toward slowing of hippocampal atrophy rate in Apo E4 carriers treated with donepezil, the imaging variables otherwise behaved in the expected way, i.e., subjects who converted to AD had higher atrophy rates than subjects who remained stable and Apo E4 carriers had higher atrophy rates than noncarriers. Based on this, the authors concluded that although they were not able to demonstrate a treatment effect, the results of this study supported the usefulness of MRI as an outcome parameter for disease progression.

There are only two studies which used MRI outcome measures to assess the treatment efficacy of a disease modifying treatment. Fox et al. (2004)studied 280 subjects with probable AD, who were treated in a double blinded fashion with either amyloid beta immunotherapy or placebo, with serial MRI. The result of this study was surprising because patients with the expected immune response and a corresponding cognitive improvement had a greater ventricular enlargement than patients without treatment response. The significance of this finding is unclear. Larger patient groups need to be followed for longer time periods to determine if this is a consistent finding. Statins not only lower plasma lipid levels but also have antioxidative, anti-inflammatory, and antiamyloid properties, i.e., properties which are potentially disease modifying. Doraiswamy and Steffens (2004) studied hippocampal shape changes in 33 cognitively impaired but not demented subjects of whom 11 received treatment with different types of statins. After adjusting for differences in age, gender, and education, they found that hippocampal volume changes over a 2-year period in subjects treated with statins were not different from those in untreated subjects. However, the groups were rather small and thus it cannot be excluded that the study was underpowered.

Altogether three studies used volumetric MRI to predict treatment response. Hanyu et al. (2007) measured the thickness of the substantia innominata, which contains the nucleus basalis Meynert in AD patients treated with donepezil, and found that cognitive improvement after 12 weeks was inversely correlated with the thickness of the substantia innominata, indicating that patients with severe atrophy of the nucleus basalis Meynert were less likely to benefit from treatment with donepezil. Csernansky et al. (2003) correlated hippocampal volume and shape characteristics of patients diagnosed with very mild and mild Alzheimer's disease with cognitive improvement during donepezil therapy and found that smaller hippocampal volume and a greater degree of shape deformation in the subicular and CA1 region was associated with less cognitive improvement during the treatment period. Visser et al. (2005) finally also found that cognitive decline during treatment with rivastigmin was more pronounced in AD patients with more progressed medial temporal atrophy than subjects with milder degrees of atrophy.

Drug Effects in Cerebrovascular Disease and Vascular Dementia

Vascular dementia (VD) is probably the second most frequent form of dementia with a prevalence of 4–10% in European and North American autopsy series and 22–35% in Asian autopsy series (Jellinger 2002). However, different definitions of the clinical syndrome, of the imaging criteria and different subtypes of VD (small vessel disease vs. large vessel disease), make it difficult to determine its true incidence and prevalence. The fact that cerebrovascular disease and other forms of dementia, e.g., AD, can occur together results in additional diagnostic and therapeutic problems. The histopathological hallmarks of cerebrovascular disease are small and/or large vessel disease resulting in complete and incomplete ischemic lesions manifesting themselves as periventricular and deep white matter lesions,

small lacunar strokes, and large cortical–subcortical strokes or microscopic cortical microinfacts. Depending on the predominant lesion type, subtypes of vascular dementia have been proposed, e.g., subcortical vascular dementia (predominantly white matter lesions, lacunes in basal ganglia, internal capsule, corona radiata) or multi-infarct dementia (multiple large cortico-subcortical strokes) (O'Brien 2006). Current treatment strategies consist in the attempt to modify the progression of cerebrovascular disease by rigorous treatment of the known risk factors such as, hypertension, hypercholesterolemia, diabetes, and/or in a symptomatic approach by treating the manifestations of cognitive impairment/dementia.

The efficacy of risk factor treatment can be directly assessed, e.g., in the case of antihypertensive therapy by measuring blood pressure. Its effect on cerebrovascular disease, however, has to be indirectly assessed by quantifying the end organ damage caused by it. Several studies have shown that MRI measures of this end organ damage, e.g., white matter hyperintensities or lesions (WML), and whole brain or regional brain atrophy rates are well correlated with the presence of vascular risk factors and provide excellent surrogate measures for the severity of cerebrovascular disease (Korf et al. 2007; Schmidt et al. 2004; Den Heijer et al. 2003; Wiseman et al. 2004). Successful treatment of vascular risk factors can result in a quantifiable reduction of the amount and/or progression rate of these surrogate measures. For example, several MRI studies demonstrate that a successful antihypertensive therapy results in significantly lower amounts of WML and brain atrophy. Dufouil et al. (2001) using a visual quantification strategy studied 845 elderly subjects enrolled in the Epidemiology of Vascular Aging study and found that the risk of having severe WML load in the 4-year MRI follow up was significantly reduced in subjects in whom blood pressure was controlled by antihypertensive medication compared to subjects whose blood pressure was not controlled. This finding was confirmed by De Leeuw et al. (2002), who also used a visual rating scheme to study 1,077 subjects enrolled in two large Dutch observational studies. Subjects with poorly controlled hypertension had an about two times higher risk of severe deep or periventricular WML than successfully treated hypertensive subjects. Firbank et al. (2007) acquired serial structural MRIs in 41 normotensive and in 92 hypertensive elderly subjects. The latter were treated either with placebo and open label hydrochlorothiazid if necessary to achieve a target blood pressure of <160/90 or with the angiotensin receptor blocker candesartan in combination with open label hydrochlorothiazid if necessary to achieve the target blood pressure. Using an automated quantification method, they found that although total WML and brain atrophy progressed during the 2-year observation period in all groups, the progression was more pronounced in hypertensive subjects than in normotensive subjects and that deep WML load on the first scan was a strong independent predictor of further WML increase. The effects of lipid lowering and antidiabetic treatments on brain structure have been less well studied and are less consistent. Soljanlahti et al. (2005), for example were able to show that 30 patients at risk for fatal or nonfatal stroke because of familial hypercholesterolemia treated with lipid lowering drugs (statins) did not have more white matter lesions than age matched controls despite incomplete control of their hypercholesterolemia. However, Ten Dam et al. (2005), who studied 535 elderly subjects with increased risk for cerebrovascular events,

found that, although low density lipoproteins were reduced by 34%, subjects treated with pravastatin had still a similar increase of WML after 33 months as subjects treated with placebo. There exists currently no studies that systematically assessed the effect of blood glucose control of the progression of WML or brain atrophy but a positive correlation between the severity of WML and atrophic changes and glycosylated hemoglobin has been demonstrated (Murray et al. 2005; Musen et al. 2006).

Similarly as in AD, cortical gray matter loss was found to be the most consistent predictor of cognitive decline in cerebrovascular impairment/dementia (Boyle et al. 2003; Cohen et al. 2002; Mungas et al. 2001, 2002; O'Brien et al. 2001) and thus is used in drug trials aiming to improve the cognitive consequences of cerebrovascular disease. Although cerebrovascular impairment and dementia have been the object of several treatment trials (Pantoni 2004), the number of studies using neuroimaging methods as outcome measures is still small. Broderick et al. (2001) studied the effects of a treatment with aspirin and pentoxyfylline vs. a treatment with aspirin alone. Of the 105 patients suffering from multiinfarct dementia included into this study, only 25 had comparable MRI of good quality at baseline and at the completion of the trial. Ventricular volume and the ischemic volume significantly increased in all patients independently from the treatment regimen and the neuropsychological test scores did not change. Sweet et al. (2003) used changes in WML volume and whole brain volume to assess treatment effects of citicoline in 23 patients with cerebrovascular dementia. During the 12-month treatment trial (12 placebo, 11 citicoline), the volume of WML increased and the whole brain volume decreased significantly while the cognitive outcome parameters changed only insignificantly in both groups.

Conclusion

Structural MRI provides biologically and clinically meaningful surrogate measures of disease progression in neurodegenerative diseases. However, the measures provided by structural MRI are not disease specific and provide at best a delayed and indirect measure of the drug action. Therefore, structural MRI is less suited than other imaging modalities to further the understanding of how a drug interacts with the brain. However, the measures provided by structural MRI have been successfully used in a number of clinical trials in which they allow for a significant reduction of the number of subjects necessary to detect treatment effects.

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Molecular Imaging of the CNS: Drug Actions

Thomas Mueggler and Markus Rudin

Introduction: Neuroreceptor and Functional Imaging

Imaging has always played a major role in the diagnosis and staging of CNS disorders and in the evaluation of therapeutic interventions. While in the past imaging applications were focused on the structural, physiological, and metabolic characterization of CNS pathologies, in the last two decades methods have emerged that enable to noninvasively study the neurochemistry of the living brain. These methods, predominantly based on nuclear imaging modalities such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), use radioligands targeting specific neuroreceptors. The ever-increasing availability of radiotracers for neuroactive compounds (neurotransmitter synthesis/metabolism, enzymes, transporters, receptors, neuromodulators, and second messengers) provides an attractive toolset both for basic neuroscience, diagnosis and patient care. Complementary to such specific molecular (PET) probes in diagnosis of diseases and patient management, generic tracers can be used to derive physiological and/or metabolic information. [^{18}F]-2-fluoro-2-deoxyglucsoe (FDG) PET provides information on the regional cerebral metabolic rate of glucose ($rCMR_{glu}$) (Phelps et al. 1979), while [^{15}O]-H_2O can be used to assess regional cerebral blood flood flow (rCBF) have been widely applied to assess brain activity. Alternatively, brain function can be assessed using functional MRI (fMRI), which measures hemodynamic changes elicited through the neurovascular coupling (Ogawa et al. 1992). Both PET and fMRI methods have emerged to become standard techniques for the clinical evaluation of drug candidates providing an indirect readout (via hemodynamic coupling) of the drug effects on CNS activity. This topic has been the subject of many reviews.

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In this chapter we will focus on the applications of molecular (PET) imaging probes to characterize specific CNS disorders, to evaluate the outcome of therapeutic interventions in neuropsychiatric disorders such as mood, anxiety, and psychotic disorders (categories according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition). In the second part we will discuss current attempts to use molecular imaging for diagnosing and staging (diagnose) of neurodegenerative disorders such as Parkinson's (PD) and Alzheimer's disease (AD) and for evaluating the outcome of treatment.

Application of Neuroreceptor and Functional Imaging to Psychiatric Disorders

Major Depression and Bipolar Affective Disorder

Contemporary models of mood disorders, i.e., major depression disorder (MDD) and bipolar affective disorder (BD), emphasize that mood symptoms arise from disruptions in the interactions of limbic/paralimbic neural networks involved in emotional and cognitive processing. The understanding of how the neurotransmitter systems within those affected neuroanatomical structures are involved together with the modulatory role of glucocorticoids, inflammatory cytokines, and brain-derived growth factors led to an integrated model of depression (Maletic et al. 2007). Symptoms of mood disorders are viewed as a result of disturbances in the ability to regulate emotions, attention, and memory supported by these networks under normal conditions. Mayberg's limbic-cortical model of depression involves dorsal, ventral, and rostral compartments, each of which accounts for some portion of the constellation or symptoms associated with depression (Mayberg 1997). Consistent with this model resting state PET and SPECT studies in patients with MDD and BD have consistently found decreased frontal lobe function as reflected by decreased regional cerebral glucose metabolism (rCMRglu) and decreased rCBF in the dorsal compartments of the prefrontal cortex and the cingulate. Increases in rCMRglu and rCBF have been observed in the ventral compartment including orbitofrontal and sugenunal prefrontal cortex, amygdala, and insular cortex. Increases in rCMRglc and rCBF in the limbic/paralimbic system also include the anterior temporal cortex. In addition, alterations in basal ganglia and thalamic function have been reported. These imaging read-outs indicative of aberrant energy turnover have been complemented by information on the integrity of specific neurotransmitter systems. In fact, the dominant hypothesis guiding research and treatment in MDD and of depressed phases in BD is that of decreased monoaminergic function: norepinephrine, dopamine and, in particular, serotonin (Fuller and Wong 1990; Vermetten and Bremner 2002a, b; Nutt 2008) as reflected by the modern classes of antidepressants being, the selective serotonin reuptake inhibitors (SSRIs), the serotonin and norepinephrine reuptake inhibitors (SNRIs), norepinephrine reuptake

inhibitors (NET), and the norepinephrine-dopamine reuptake inhibitor (NDRI) bupropion. Several specific molecular (PET/SPECT) ligands to target monoaminergic function have been developed and are used for the management of patients suffering from MDD and BD.

The evidence supporting the role of serotonin (5-hydroxytryptamine, 5-HT) dysfunction in MDD includes studies of neurochemical markers in post mortem brain of the neuroendocrine response to acute pharmacologic interventions of the serotonin system and of pharmacologic manipulations of serotonin systems and their effects on mood (as reviewed by (Kalia et al. 2005). Progress in medical chemistry of drugs interfering with the serotonergic system has promoted also the development of imaging agents for PET and SPECT. Besides the alpha-[^{11}C]-methyl-l-tryptophan as surrogate marker of the cerebral 5-HT synthetic rate, most of the available selective or nonselective radiotracers to date target 5-HT$_{1A}$ or 5-HT$_2$ receptors or the serotonin re-uptake transporter (SERT) (Fig. 1). SERT is

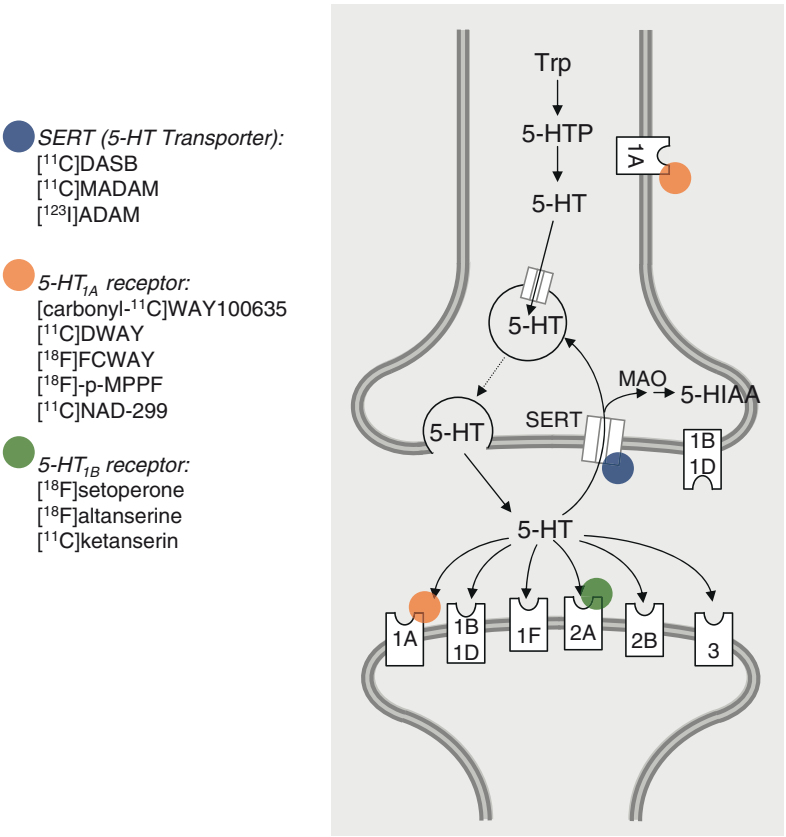

Fig. 1 Available radiotracers targeting 5-HT transporter, 5-HT$_{1A}$ and 5-HT$_{1A}$ receptors

the primary target for selective serotonin reuptake inhibitors (SSRIs) used in treatment of mood and anxiety disorders. SSRIs bind to the transporter thereby blocking the reuptake of 5-HT which raises extracellular serotonin levels and stimulating a cascade of intracellular events including the desensitization of 5-HT_{1A} autoreceptors. This leads to an increase in neuronal firing rate and to downstream trophic effects that are correlated with the clinical response (Blier and de Montigny 1999; Duman et al. 1997). Multiple attempts have been made to synthesize suitable radiolabeled tracers targeting SERT; mostly, clinically approved SSRIs have been labeled with the positron-emitting isotopes, ^{11}C or ^{18}F, and their value as PET tracer evaluated in primates. Currently, two structurally related PET radiotracers suitable for imaging SERT levels in the human brain are at hand: [^{11}C]*N*,*N*-dimethyl-2-2-amino-4-cyanophenylthiobenzylamine ([^{11}C]DASB) (Frankle et al. 2006; Kim et al. 2006; Wilson et al. 2002; Houle et al. 2000) and [^{11}C]-*N*,*N*-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine ([^{11}C]MADAM) (Lundberg et al. 2006; Halldin et al. 2005; Larsen et al. 2004). In parallel, [^{123}I]-2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine ([^{123}I]-ADAM) has been reported to be a suitable tracer for SPECT imaging of SERT in humans (Catafau et al. 2005; Kauppinen et al. 2003). Most of the 5-HT_{1A} receptor antagonist radioligands which have been evaluated for PET imaging (Pike et al. 2000; Cliffe 2000) bear structural similarity to the 5-HT_{1A} antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)-cyclohexane carboxamide (WAY100635). Among them the [carbonyl-^{11}C]WAY100635 (Hall et al. 1997), [^{11}C]DWAY (Pike et al. 1998) and their fluorinated analogs [^{18}F]FCWAY (Lang et al. 2006) and [^{18}F]-p-MPPF (Shiue et al. 1997) appear to be the most useful PET ligands for the quantification of 5-HT_{1A} receptors in humans (Kumar et al. 2007). Robalzotan (NAD-299), a structurally different compound, has been labeled with carbon-11, and preliminary results show that [^{11}C]NAD-299 is a promising imaging probe for the 5-HT_{1A} receptors (Sandell et al. 2002). 5-HT2_{A} receptor antagonism is a property of certain antipsychotic and antidepressant drugs (Moresco et al. 2006; Bockaert et al. 2006). Specific radioligands for PET such as [^{18}F]setoperone (Blin et al. 1988), [^{18}F]altanserine (Lemaire et al. 1991), ^{11}C-ketanserin (Baron et al. 1985), and for SPECT ([^{123}I]5-I-R91150) (Peremans et al. 2003) are available allowing to investigate in vivo the exact role of the 5-HT_{2A} receptor availability in the time course of symptoms of depressive, mood, or eating disorders (Moresco et al. 2006; Moeller et al. 2006), and borderline personality disorder (Soloff et al. 2007). A selective 5-HT_{2A} receptor antagonist MDL-100907 [(2,3-dimethoxy-phenyl)-{1-[2-(4-fluorophenyl)-ethyl}-piperidin-4-yl}-methanol] has been introduced (Lundkvist et al. 1996) and validated in a number of studies in human and nonhuman primates (for review see Moresco et al. 2006).

In vivo imaging studies on neuroreceptor expression levels have confirmed the hypothesis of decreased 5-HT neurotransmission in depressed patients. In an early study, Agren et al. (1991) reported reduced uptake of [^{11}C]-5-hydroxy-tryptophan in depressed patients. Further investigations on the 5-HT transporter or 5-HT receptor have only recently been possible as a result of major advances

in ligand development. The majority of these studies have been conducted in midlife depressed patients. The current study is to assess the binding potential (BP) of SERT an index of the transporter density and affinity. PET/SPECT studies comprising a number of different SERT ligands, among them the state-of-the-art [^{11}C] DASB, have been reviewed by Meyer et al. It can be concluded from these imaging readouts that the contributing mechanism to extracellular serotonin loss is excessive 5-HTT rather than loss of serotonin neurons through degenerative processes. Imaging studies reporting on regional decreases in 5-HTT BP include other comorbid axis I illnesses (presence of a substance use, anxiety, or eating disorder) and disorders in their sampling. This reflects effects of common comorbid illnesses rather than MDD alone, whereas a significantly greater 5-HTT BP can been found in MDD subjects with more severe pessimism (Meyer et al. 2007). A further important aspect is receptor (i.e., transporter) occupancy. Endogenous displacement of [^{11}C]DASB may occur on the 5-HTT with large magnitude changes in extracellular 5-HT but would not be expected to occur with extracellular 5-HT changes that are physiologically relevant for humans. Studies using [^{11}C]DASB carried out in the rhesus monkey, the cat, and the rat brain showed decreased 5-HTT BP in several brain regions after a pharmacologically induced increase in the interstitial serotonin (5-HT) concentration. In vivo binding of [^{11}C]DASP was studied before and after having increased interstitial 5-HT concentrations using tranylcypromine (TCP), which inhibits the enzyme (monoamine oxidase, MAO). The critical point here is that the rise in extracellular serotonin with high doses of tranylcypromine is enormous, with a several hundred to thousand percent rise being typical. This magnitude of serotonin change may exceed what is physiologically relevant in humans. In fact the effect of tryptophan depletion upon 5-HTT has shown no effect, demonstrating that endogenous serotonin occupancy is unlikely to appreciably influence [^{11}C]DASB43 under physiologically tolerable conditions (Meyer et al. 2007).

The investigation of serotonin transporter occupancy by SSRIs using SERT PET ligands has revealed a high degree of SERT occupancy at relatively low plasma concentrations of SSRIs paroxetine, citalopram (Meyer et al. 2001, 2004; Voineskos et al. 2007), clomipramine, and fluvoxamine (Suhara et al. 2003) venlafaxine, and sertraline (Voineskos et al. 2007). It could be inferred that 80% occupancy of the 5-HTT is a necessary condition for demonstrating successful SSRI treatment for depressive episodes in clinical trials and, therefore, has a relationship to the clinical effect. However, the exact relationship between SERT occupancy and clinical efficacy of SSRIs has not yet been evaluated.

The development of 5-HT$_{1A}$ R-selective positron emission tomography (PET) radioligands has enabled in vivo measures of presynaptic and postsynaptic 5-HT$_{1A}$ R binding in depression. Drevets et al. demonstrated that the mean 5-HT$_{1A}$ R-binding potential (BP) was reduced in the mesiotemporal cortex (MTC) and raphe in unmedicated depressives relative to controls using PET and [carbonyl-^{11}C]WAY-100635 (Drevets et al. 1999). Similar reductions were evident in the parietal and medial occipital/posterior cingulate cortices. These data were consistent with those of Sargent et al. (2000), who found decreased 5-HT1AR BP,

measured using PET and [^{11}C]WAY-100635, in unmedicated depressives relative to controls in the raphe, MTC, insula, anterior cingulate cortex, temporal polar cortex, and orbital cortex. Similarly, Meltzer et al. (2004) found significantly decreased [^{11}C]WAY-100635 binding in the raphe in elderly depressed patients relative to age-matched controls. Several studies reported that these reductions in 5-HT$_{1A}$ R binding also were evident in currently remitted patients with a history of MDD (Sargent et al. 2000; Moses-Kolko et al. 2007; Bhagwagar et al. 2004), suggesting that this abnormality persists across illness episodes in recurrent depression.

An important property of 5-HT$_2$ receptors is that their receptor density has an inverse relation to extracellular serotonin levels, such that the density of 5-HT2 receptors in the cortex increases after chronic serotonin depletion and decreases after chronically raising extracellular serotonin (Meyer et al. 2007). To investigate to what degree a reduction in 5-HT$_{2A}$ receptor availability can be used to predict the clinical efficacy of antidepressant agents several PET studies in patients suffering from MDD have been reported. However, the measured changes in the 5-HT$_{2A}$ receptor availability during the course of antidepressant therapy (i.e., SSRI) are contradictory (Attar-Levy et al. 1999; Meyer et al. 2001; Mischoulon et al. 2002). Based on a binding study using the nonselective 5-HT$_2$ receptor PET ligand [^{18}F] fluoro-ethyl-spiperone (FESP) (Coenen et al. 1988) in patients classified according to their clinical response and treated with paroxetine it has been stated that the 5-HT$_2$ receptor behaves more as a state marker (presence or absence of symptoms) than a trait marker (presence of MDD) (Moresco et al. 2006).

The physiological actions of DA in the brain mediate functions that are commonly disturbed in MDD, such as reward, motivation, initiation of movement, and cognitive functions (Nestler and Carlezon 2006) implicating the involvement of dopamine in the pathophysiology of MDD, and many antidepressant drugs have shown to increase dopamine D$_2$ and D$_3$ receptor (D$_2$R/D$_3$R) density and affinity specifically in the mesolimbic dopamine system. Radionuclear Imaging has been used to study the involvement of striatal dopamine in the etiology of MDD (for review see Hirvonen et al. 2008) using [^{11}C]- or [^{18}F]-DOPA to assess presynaptic DA turnover, the SPECT DA transporter ligands [^{99m}Tc]TRODAT-1 and [^{123}I]β-CIT or the D$_2$R ligands [^{11}C]raclopride and [^{123}I]IBZM for PET and SPECT, respectively (Fig. 2). The assessment of striatal D$_2$R density is of interest, considering that this index can be used to indirectly estimate changes in the synaptic concentration of dopamine (Laruelle 2000) and the postsynaptic responsivity of the nigrostriatal dopamine system. These studies have shown either higher (Shah et al. 1997; Meyer et al. 2006), lower (Montgomery et al. 2007), or unchanged (Parsey et al. 2001; Kuroda et al. 2006) striatal D$_2$ receptor density in MDD as compared with controls. However, most of the studies have been performed in patients who have a history of antidepressant pharmacotherapy. Higher D$_2$ receptor density in the striatum has also been found in postmortem brain tissue of MDD diagnosed and medicated suicide victims but not in medication-free MDD patients (Bowden et al. 1997). Many imaging studies have recruited and analyzed patients with a specific subtype of

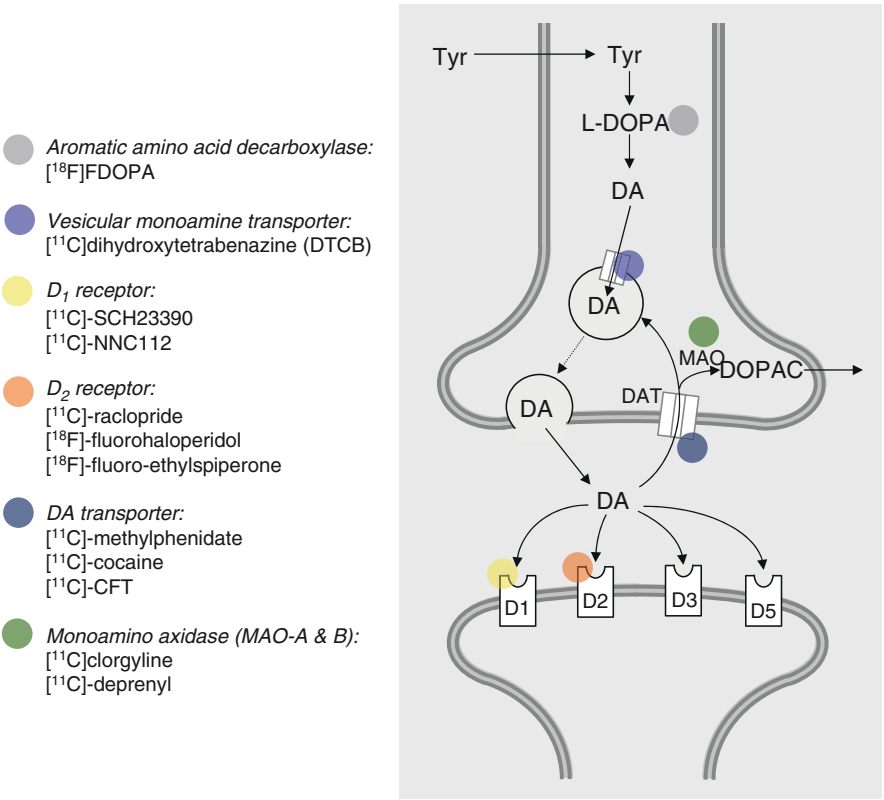

Fig. 2 Selective and nonselective PET radiotracers targeting the dopaminergic system

MDD (such as those with motor retardation or the melancholic subtype). Thus the results may not be generalizable to the population of depressed patients as a whole. In a recent PET study using [^{11}C]raclopride Hirvonen et al. (2008) reported striatal *and* thalamic D_2 receptor-binding values in treatment-seeking drug-free patients MDD patients and healthy controls. In all regions analyzed no statistically significant differences in [^{11}C] raclopride BP were observed between the two groups. Correlation analysis of depressive symptoms severity vs. [^{11}C]raclopride BP showed no association in any region. Unaltered thalamic D_2 receptor binding in MDD is consistent with the results of Montgomery et al. (2007) which show no differences between MDD patients and controls using the high affinity $D_{2/3}$ receptor ligand [^{11}C]FLB-457. However, the lack of altered dopamine D_2 receptor density in the sample patients is *not* in disagreement with the dopamine hypothesis in antidepressant effect. According to this hypothesis, the sensitivity of the postsynaptic dopamine receptor system is increased, specifically in the mesolimbic dopamine system targeting the nucleus accumbens (Gershon et al. 2007). It should be noted

that if the affinity of D_2/D_3 receptors is altered in MDD even before antidepressant treatment, it cannot be detected using antagonist radioligands such as [^{11}C]raclopride (which bind with equal affinity to receptor configured in low- and high-affinity states). A difference could only be observed with receptor agonist tracers that prefer the high-affinity state (Finnema et al. 2005; Hwang et al. 2005; Willeit et al. 2006).

Selective NE reuptake inhibitors (NRIs) (e.g., reboxetin) have found use for the treatment of depression, and many antidepressants have substantial potential occupancy of the NET at recommended dosages or are combined as SNRIs [i.e., duloxetine; Gupta et al. 2007). Given that the NE transporter (NET) is also a binding site for cocaine and drugs of abuse, there is a great need for a probe to assess the densities of NET in vivo by brain imaging with either positron emission tomography (PET) or single photon emission tomography (SPECT). However, despite the importance of understanding this transporter's role in psychiatric disease and treatment, a suitable radioligand for studying NET has been slow to emerge. Several potent NET-selective antidepressants, among them nisoxetine, oxaprotiline, lortalamine, and analogs of reboxetine, have been labeled for in vitro or in vivo mapping of brain NET (Ding et al. 2005; Kung et al. 2004; Schou et al. 2003; Seneca et al. 2006; Kanegawa et al. 2006; Tamagnan et al. 2007). Unfortunately, most of these candidate ligands do show a high in vivo nonspecific binding excluding their utility as NET imaging agents. So far only the (S,S)-reboxetine-based agents such as *O*-methyl ((S,S)-[^{11}C]-MeNER) (Schou et al. 2003), *O*-fluoromethyl ((S,S)-[18F] FMeNERD2) (Seneca et al. 2006), and 2-iodo ((S,S)-[123I]IPBM) (Kanegawa et al. 2006; Tamagnan et al. 2007) analogs showed many desired in vivo properties for imaging. In an attempt to further optimize (S,S) isomers of the reboxetine motif (i.e., shorter time-to-peak equilibrium) new structures and new analogs have been synthesized and their in-vitro-binding affinities measured (Zeng et al. 2008). Results of comparative microPET studies of these tracers in nonhuman primate brain are needed to estimate the potential of theses radiotracers for their application in clinical studies.

Anxiety Disorders

Pathological anxiety is classified into five types: obsessive-compulsive disorder (OCD), phobias, panic disorder, post-traumatic stress disorder (PTSD), and generalized anxiety disorder. Notably, a large proportion of psychiatric disorders comprise pathological anxiety as a comorbidity which can for example in the case of mood disorder condition demonstrate a considerable overlap of clinical symptoms and pathophysiological processes. The neurobiological basis of anxiety has been reviewed extensively (Vermetten and Bremner 2002a, b; Kent et al. 2002; Ressler and Nemeroff 2000). For example, the role of the benzodiazepine (BZD) receptor has been studied in patients suffering from panic disorders and post-traumatic stress

disorder (PTSD). Neuroimaging studies in panic disorder patients using either the SPECT ligand [^{123}I]-iomazenil or the PET ligand [^{11}C]-flumazenil have revealed decreased BZD receptor binding relative to normal controls. In particular, [^{123}I]-iomazenil binding was found to be decreased in the left hippocampus and precuneus of the patients (medication free for six weeks and BZD free for four weeks prior to scanning) binding was higher than control values in the right caudate, cuneus, right middle frontal gyrus, and left middle temporal gyrus. In patients who experienced a panic attack during scanning binding decreased throughout all brain structures when compared to patients with no panic attack. Besides the BZD receptor system attention has been drawn on the 5-HT$_{1A}$. Using [^{11}C]WAY-100635 PET a significant relationship has been reported between 5-HT$_{1A}$ BP and anxiety (Tauscher et al. 2001)

Psychotic Disorders

Schizophrenia is a mental disorder associated with abnormal perception or expression of reality (hallucinations, paranoia, disorganized speech, and thinking). From a neurochemical perspective, a critical role is attributed to dysfunctional DA neurotransmission involving the mesolimbic system. The importance of DA becomes obvious as drug-induced inhibition of DA function was found to reduce psychotic symptoms. Hence it was hypothesized that excessive DA neurotransmission was associated with the positive symptoms of schizophrenia. Today, the efficacy of atypical neuroleptics, which interfere less with the DA system but also affect 5-HT, has demonstrated that DA dysfunction alone does not account for the disorder (Jones and Pilowsky 2002). In addition, abnormal low levels of glutamate receptors in the brains of deceased schizophrenia patients implied an important role of glutamate and impaired function of NMDA receptors (Konradi and Heckers 2003). In addition it was shown that inhibition of NMDA receptors may cause psychosis-like behavior (Lahti et al. 2001), as a severe side effect that has prevented the development of NMDA inhibitor drug for other CNS indications, e.g., acute cerebral ischemia.

One of the best examples documenting the value of receptor imaging refers to treatment of schizophrenia patients with D_2R inhibitors. In line with the increased activity of the DA system, it has been found that D_2R densities are upregulated in the brains of approximately 70% of schizophrenia patients (Verhoeff 1999). First-line antipsychotic therapy is therefore aimed at inhibiting D_2R-induced signaling. In order to achieve therapeutic efficacy, a significant percentage of D_2R has to be occupied by the inhibitor. Yet initial studies revealed that there is a narrow range between therapeutic doses and doses causing severe adverse motor side effects (extrapyramidal side effects) (Halldin et al. 1998). PET studies using the D_2R ligand [^{11}C]raclopride in schizophrenic patients treated with the D_2R antagonist haloperidol revealed that a minimal striatal receptor occupancy (RO) of 60% is

required to produce an optimal antipsychotic response, while receptor occupancies exceeding 75–80% prompted side effects (Verhoeff 1999). Clearly the availability of a D_2R PET ligand has made a significant contribution to the clinical management of schizophrenia patients. [^{11}C]raclopride is reproducible and reliable (Hirvonen et al. 2003), and this paradigm has been used to show altered thalamic D_2 receptor binding in patients with schizophrenia (Talvik et al. 2006).

In addition to its important clinical role, it may represent an attractive tool for basic biological research and in particular for drug discovery and development. For example, [^{11}C]-raclopride in combination with microPET imaging has been used to study D_2R-binding characteristics in rats. It has been found that D_2R receptor binding can be studied reproducibly in a quantitative manner. Injections of 9.25 MBq of [^{11}C]-raclopride (approximately 250 μCi) provided sufficient sensitivity for quantitative determination of the distribution volume ratio (Alexoff et al. 2003). Animal PET using this radiotracer constitutes an attractive approach to study D_2R-ligand-induced modulation of dopaminergic transmission, enabling the determination of the temporo-spatial distribution of candidate drugs, the assessment of their receptor occupancy, and the relationship between receptor interaction and downstream (therapeutic) efficacy. The relative high activity of radiotracer that is commonly used in these studies due to sensitivity reasons constitutes a potential confound. Hence in the data analysis it should be considered that the concentration of the tracer is not necessarily much smaller than that of the drug candidate (Alexoff et al. 2003). The sensitivity of the method was evaluated in comparing D_2R binding of [^{11}C]-raclopride in normal wild-type and D_2R knock-out (KO) mice. KO mice showed significantly lower binding in the strital reaclopride binding than wild-type animals. The striatal-to-cerebellar activity ratio was 1.33 ± 0.13 for wild-type mice and 1.05 ± 0.03 for KO mice (Thanos et al. 2002).

A very different application of PET imaging of D_2R binding is to use it as reporter system for studying gene expression in vivo as demonstrated, for example, in adenoviral delivery systems and in tumor xenografts. Overexpression of dopamine D_2R in the rat striatum through gene transfer mediated through an adenoviral vector led to significantly higher local activity of D_2R PET ligands [^{11}C]-raclopride, [^{11}C]-nemonapride, and [^{11}C]*N*-methylspiperone, when compared to the contralateral striatum, which was injected with a control vector. Coinjection with an excess of "cold" raclopride inhibited the binding of [^{11}C]-raclopride. On the other hand, the uptake of neither a D_1R-specific nor a DAT-specific PET ligand was not different between the two striata demonstrating the specificity of the readout (Ogawa et al. 2000). An important characteristic of using D_2R as a reporter gene is to uncouple the ligand receptor from potential downstream effects, e.g., the modulation of cyclic AMP levels. This can be achieved through mutations: it has been demonstrated using various assays including PET with the ligand 3-(2'-[^{18}F]-fluoroethyl)-spiperone (FESP) that the D2R80A mutant has still the full capability as a PET reporter gene, i.e., efficiently binding the radioligand FESP, while it does not modulate cAMP levels following ligand binding (Liang et al. 2001).

Application of Neuroreceptor and Molecular Imaging to Neurodegenerative Disorders

Alzheimer's Disease

Diagnosis and Imaging Targets

Early detection of AD is imperative for studying the pathophysiological mechanisms leading to disease and for efficient clinical treatment of this disorder. The characteristic signature of AD is the deposition of amyloid-β plaques and neurofibrillary tangles (NFTs) in the patient's brain, which parallels the disease progression, but can only be diagnosed with certainty by autopsy. Key pathological features of AD – senile plaques (SPs) and NFTs (Hardy and Selkoe et al. 2002) – are identified using either histopathological dyes such as Congo Red (CR) or Thioflavin T (ThT) or by immunohistochemistry. SPs and NFTs are mainly composed of aggregated (polymeric) forms of amyloid-β (Aβ) peptide (composed of either 40 or 42 amino acids) and hyperphosphorylated Tau (phospho-Tau) proteins, respectively (Glenner and Wong 1984). Beta amyloid (Aβ 1–42) in its fibrillar form (Hardy and Selkoe et al. 2002) or as soluble oligomers (Selkoe 2002) has been suggested as the primary cause of AD. It is still an open question whether soluble or insoluble oligomers or mature amyloids are more toxic. The amount of soluble Aβ in brain seems to correlate better with impairment of cognition than do plaque counts (Näslund et al. 2000). Other potentially concomitant processes especially tau phosphorylation and downstream events such as oxidative stress, inflammatory reactions, microglia activations, play a crucial role in the AD pathology (Mattson 2004). It was also recently suggested that tangles may precede amyloid plaques (Schoenheit et al. 2004). The ultimate effects of complex inflammatory, ionic, and oxidative changes that occur in affected brain regions are neuritic dystrophy, as well as selective synaptic and neuronal loss. Presumably, these processes occur gradually over many years in the preclinical asymptomatic phase of AD, which may involve early synaptic dysfunction, and then continue during its clinical progression.

Imaging of Energy Turnover

First and second generation of imaging biomarkers included mainly altered glucose metabolism (assessed by [^{18}F]FDG-PET) as surrogate of synaptic dysfunction and [^{15}O]PET for rCBF deficits along with general and/or region-specific structural abnormalities as predictor of gross neuronal atrophy. PET studies have thereby been performed under baseline conditions to assess resting state metabolic activity, as well as during the performance of various cognitive tasks. Using FDG-PET the largest reduction in resting state $CGMR_{glu}$ has been found in the posterior cingulate cortex (Minoshima et al. 1997). Despite some promising results, it was soon realized from these clinical imaging studies that patterns of brain atrophy and

hypometabolism offered only limited diagnostic specificity for differentiating AD form other types of dementia; these nonspecific imaging readouts are therefore of limited value as surrogate markers of early disease, i.e., for mild-to-moderate stages of AD.

PET Imaging of Cholinergic Neurotransmission: Radioligands for Muscarinic and Nicotinic Acetylcholine Receptors and Acetylcholine Esterase

An obvious result of the synaptotoxicity is loss of cholinergic enzymes (choline acetyltransferase and acetylcholinesterase AChE) leading to massive deficits in the cholinergic system (Davies and Maloney 1976; Whitehouse et al. 1981) followed by an impairment of other neurotransmitter and neuromodulator systems. AchE blockers have been shown to be beneficial as symptomatic treatment in AD. Thus in vivo imaging of AChE activity might yield immediate insight into the drug-induced modulation of cholinergic function. Not surprisingly, a significant number of imaging studies conducted thus far in AD patients have focused on evaluating the hypothesis of cholinergic hypofunctionality in AD.

A considerable number of imaging agents targeting the cholinergic system in vivo have been developed. This includes radiotracers developed for measurements of the vesicular acetylcholine transporter ([^{123}I]-iodovescamicol; Kuhl et al. 1994), AChE activity such as *N*-[^{11}C]methylpiperidin-4-yl propionate ([^{11}C]-PMP (Koeppe et al. 1999), [^{11}C]-physostigmine (Pappata et al. 1996), as well as ligands for the acetylcholine receptors (AChR = The AchR of the muscarinic type (mAChR) can be assessed using PET and SPECT labels such the C11 labeled ligands [^{11}C]-benztropine (Dewey et al. 1990), [^{11}C]-scopolamine (Frey et al. 1992), [^{11}C]-*N*-methyl-4-piperidylbenzilate [^{11}C]-NMPB) (Suhara et al. 1993), the F18 labeled muscarinic agonist, 3-(3-(3-[^{18}F]Flouropropyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine ([^{18}]F-TZTP/[^{18}]FP-FTZP) (Kiesewetter et al. 1995; Podruchny et al. 2003), or the iodinated compounds 3-quinuclidinyl 4-[^{123}I]-iodobenzylate [^{123}I]-QNB (Eckelman et al. 1984), [^{123}I]-4-iododexetimide, and [^{123}I]4-iodolevetimide (Muller-Gartner et al. 1992). With the exception of the radiotracer [^{18}F]FP-FTZP that is selective for the M2 muscarinic receptor subtypes, the other muscarinic radiotracers are not subtype specific (Eckelman 2001). For the AchR of the nicotinergic type (nAChR) ligands have been developed such as [^{11}C]-nicotine (Nordberg et al. 1995) or [^{18}F]fluoro-A-85380 (Horti et al. 2000). It has to be mentioned that the cholinergic receptors and therefore radiotracers have a widespread distribution in the cortex, subcortical, and limbic structures consistent with the distribution observed in post mortem autoradiographic studies in human brain. As a result, it is difficult to identify a region that is devoid of AChE or of the receptors to use as a nonspecific binding reference, rendering the quantitative analysis more difficult.

These PET ligands have been extensively used for diagnostic purposes and in particular for demonstrating pharmacological proof of principle of cholinergic drugs, which is of clinical relevance as AChE inhibitors are currently the most commonly used class of medications to treat cognitive and behavioral symptoms in AD. Several studies have also evaluated a potential implication of the monoamine systems in

AD or the use of radiotracers for the DA system to eventually distinguish Lewy body dementia (DLB) from AD.

The involvement of nAChRs in vivo has so far been studied by analyzing nicotine binding. In an earlier PET study, it was demonstrated that [^{11}C]-nicotine binding was lower in the brains of AD patients compared with that in control subjects, reflecting a loss in high- and low-affinity nAChRsites (Nordberg et al. 1990). In addition, significantly lower levels of [^{11}C]-nicotine binding have been observed in the frontal and the temporal cortex, and hippocampus of patients with AD compared with controls (Nordberg et al. 1995). After treatment with AChE inhibitors such as tacrine, an increase in [^{11}C]-nicotine binding has been found in cortical regions in patients with AD after short-term (3 months) treatment (Nordberg et al. 1997). A few AChE inhibitors such as galantamine and physostigmine are known to interact with nAChRs as allosterically potentiating ligands. Their efficacy is due to slowing down of the receptor desensitization as well as by sensitizing the nAChRs, which increases the probability of channel opening induced by acetylcholine or by nicotinic agonists (Maelicke et al. 2000).

Imaging of Abeta

Despite the relevance of the cholinergic system in AD and the importance of imaging cholinergic transmission in AD patient care, such studies are only indirectly related to the pathophysiological processes leading to AD. Therefore significant efforts have been undertaken to develop noninvasive imaging tools that are directly probing molecular species in AD pathophysiology, e.g., to assess the Aβ plaque load in vivo. It is without saying that such methods would be of tremendous benefit for tracking disease progression and for monitoring therapy's efficacy in clinical trials. So far four such radioligands, out of a significantly larger number of tracer candidates, have been advanced toward clinical evaluation using cohorts of AD patients: [^{11}C] PIB (Klunk et al. 2004), [^{18}F]FDDNP (Shoghi-Jadid et al. 2002), and [^{11}C]SB13 (Verhoeff et al. 2004), and [^{11}C]BF-227 (Kudo et al. 2006). All four ligands display uptake and retention in areas of the brain that are known to contain high densities of plaques (for review see Furumoto et al. 2007; Nordberg et al. 2004). At present, the tracer with most supporting in vivo data is [^{11}C]PIB.

Parkinson's Disease

Diagnosis and Imaging Targets

Parkinson's disease (PD) is a progressive multifocal central nervous system (CNS) degenerative disease. Clinical features of PD patients are associated with degeneration of dopaminergic nigrostriatal neurons; they include the typical motor symptoms of bradykinesia, rigidity, and tremor. In advanced disease stages, additional symptoms

emerge, which result from degeneration of nondopaminergic as well as dopaminergic pathways. The clinical onset of motor dysfunction related to PD is linked to a depletion of DA levels by 60–80%, which corresponds to the death of approximately 30–40% of the neurons in the substantia nigra pars compacta (SNc). A further histopathological hallmark of the disease is the presence of intracytoplasmic inclusions called Lewy bodies in the remaining dopaminergic neurons of the substantia nigra. These eosinophilic aggregates are predominantly composed of aggregated forms of the protein α-synuclein (Goetz et al. 2006), which is normally located presynaptically.

Common pharmacological therapies are mainly based on increasing cerebral DA levels by administration of the precursor L-DOPA (levodopa, L-3,4-dihydroxyphenylalanin), which is taken up through the blood-brain barrier and converted to DA through decarboxylation by the aromatic amino acid decarboxylase (AAAD). L-DOPA treatment as such is limited to relieving the symptoms of PD without modulating the course of the disease.

Imaging in PD

PD is associated with reduced neurotransmission through the dopaminergic system, which might arise either from reduced dopamine availability or due to impaired dopamine sensitivity at the postsynaptic neuron or both. It is obvious that the PET probes targeting the molecular players of the DA system together with functional readouts of brain activity such as FDG-PET and fMRI play an important role both for diagnostics and for guiding therapy regimens in these patients. With the availability of radioligands to monitor both presynaptic and postsynaptic DA function (Mazière et al. 1992) noninvasive imaging of PD has largely been in the domain of positron-emission tomography (PET) and single-positron-emission tomography (SPECT) imaging in rat models. These PET studies have been complemented by pharmacological fMRI experiments that assessed the neural activity induced by administration of L-DOPA (Jenkins et al. 2002), the D_1R and D_2R agonists apomorphine (Zhang et al. 2001), amphetamine or a dopamine transporter agonists (Chen et al. 1997).

Many of the PET probes to test various aspects of the dopaminergic system have been already discussed (see the section on schizophrenia). In view of the clear correlation of low DA levels with the severity of PD symptoms, a noninvasive readout of the local DA synthesis rate would be clinically relevant. DA synthesis rate can be estimated from PET studies using a labeled DA precursor, [^{18}F]-fluoroDOPA ([^{18}F]-FDOPA) (Firnau et al. 1987). Cerebral tracer activity depends on the uptake kinetics and on the activity of the AAAD. As discussed earlier, receptor density at the postsynaptic neuron can be assessed using radiolabeled receptor ligands such as the D_2R ligand [^{11}C]-raclopride. Other aspects of the system can be studied using radiolabels; the design template has been borrowed from compounds with known pharmacological activity. For example, cocaine is known to inhibit reuptake of DA via DAT. Hence, [^{11}C]-labeled cocaine derivatives could be used as PET probes to study the density and binding capacity of DAT. The benzoquinolizine compound

3,4-dihydrotetrabenazine (DTBZ), which has been shown to deplete cerebral monoamines in rat brain by reversibly inhibiting vesicular monoamine transporter 2 (VMAT2) (Pettibone et al. 1984), was originally used as an antipsychotic drug and more recently to treat hyperkinesia disorders. Correspondingly, the labeled [^{11}C]-DTBZ might serve as a tool to probe VMAT2 distribution (Goswami et al. 2006). Similarly, [^{11}C]-labeled clorgyline enables measuring of the MAO-A activity, an enzyme involved in monoamine degradation. Finally, in vivo imaging of rodents can be used to characterize the chronic effects of drug treatments using a single animal, thus mimicking long-term drug therapy in humans. For example, PET imaging using [^{11}C]raclopride of the rat brain has been used to help elucidate a neurochemical basis for fluctuations in the efficacy of chronic L-dopa treatment of Parkinson's disease (Opacka-Juffry et al. 1998; Hume et al. 1995).

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Part IV
Imaging CNS Disease States

Translational MRI in CNS Drug Discovery

Markus von Kienlin and Céline Risterucci

Introduction

The expression "translational" was coined a few years back in medical research, to express the potential benefit that modern technologies may bring to the discovery and to the development of new and improved therapeutic options for the most burning medical needs. As is true for many fashionable terms, the exact meaning of "translational" may vary, depending already on who says it and in which context. One current definition of "Translational Medicine" reads (http://en.wikipedia.org/wiki/Translational_medicine):

> Translational medicine attempts to more directly connect basic research to patient care. (…) In the case of drug discovery and development, translational medicine typically refers to the "translation" of basic research into real therapies for real patients. The emphasis is on the linkage between the laboratory and the patient's bedside. (…) Many pharmaceutical companies are building translational medicine groups to facilitate the interaction between basic research and clinical medicine, particularly in clinical trials. Traditionally, basic research has been separated from the clinical practice of medicine by a series of hurdles or fences. New drugs were developed independent of the clinic, and often "thrown over the fence" for safety testing and clinical trials. The move toward translational medicine is focused on removing these fences, and stimulating "bench to bedside" research.

This is the typical definition of "forward translation." Equally important, however, is the complementary route "from bedside to bench": how can the experience and the technologies available in clinical centers be best capitalized on, in order to direct and to enrich preclinical drug research? How can all the information obtained from patient studies be utilized to explore more efficiently the mechanisms of neuropathology and to identify potential new drug targets? A close linkage from clinical research back into early drug discovery will enhance the chances to successfully create new therapies for the ultimate benefit of the patient.

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The various imaging modalities such as CT, US, PET and MRI by their very nature are well suited to enhance the translational bridges to and fro preclinical drug discovery and clinical development: they can be applied equally well both in human patients and in animal models of disease. The same imaging read-outs that eventually will demonstrate efficacy in a clinical trial can be utilized to assess the action of new compounds in animal models. Vice versa, the experience gained by imaging in animals will help to increase the confidence in image measures and will facilitate and accelerate the discovery and the validation of innovative imaging biomarkers.

In the past decade, many pharmaceutical companies have invested in preclinical imaging laboratories, in order to enhance their drug discovery programs. In this chapter, the value as well as some possible pitfalls of "translational imaging" will be elucidated with tangible examples from two domains, Alzheimer's disease (AD) and psychiatric disorders.

Translational Imaging in Alzheimer's Disease

The ever increasing number of elder by patients with AD, the associated burden on families and the health care system, the lack of a validated understanding of the underlying pathology, and finally the complete lack of any cure make AD a (dismal) textbook example for the potential value of translational imaging markers in the discovery and development of new therapeutic concepts. The slow progression of the disease and its high variability render conventional clinical trials very tedious and expensive, to the point of being impractical: when using some clinical cognitive rating scale as the endpoint to demonstrate the benefit of a new medical entity, hundreds of patients need to be enrolled in a year-long trial before the possible efficacy in patients can be assessed. Any marker providing a more quantitative and reliable insight into the disease process and predicting future progression in the patient would significantly enhance the process of elaborating new treatment concepts against this devastating disease. The availability of similar tools for preclinical pharmaceutical research would accelerate the assessment of potential new targets and innovative approaches to modulate the pathologic pathway, prior to testing new medical entities in humans. Before analyzing the applications of MRI techniques in transgenic mouse models of the disease, the following section very briefly reviews the main MRI approaches pursued in AD patients.

Clinical Imaging in AD patients

The objectives of current imaging approaches in AD patients fall in two, conceptually fundamentally different categories. The imaging protocols in the first category aim at finding (early!) indicators of the progression of the disease, correlating with the clinical status. Such a marker would serve not only as a predictor for the future

evolution of the disease in a patient by enhancing the diagnostic accuracy but would also have the potential to serve as an objective marker to assess treatment efficacy. If some imaging read-out in Alzheimer patients would reflect, for instance, neuronal integrity, and if one could demonstrate that neuronal loss is stopped by some new medical entity, the confidence to test this treatment in a larger clinical trial would be significantly enhanced, to assess the ultimate clinical benefit for the patients.

The second category of the imaging approaches strives to obtain mechanistic rather than diagnostic insight: these modalities assess parameters in the specific pathologic pathways involved in the disease, without directly reflecting the status of the patient. Such a mechanistic marker can increase the confidence in the concept of a new therapeutic approach by demonstrating that indeed the targeted pathway is modulated by some intervention. This kind of marker, however, provides little or no evidence that the status of the patient will indeed improve after the intervention.

The most prominent example in the first category is three-dimensional anatomical brain imaging to measure the volume of the total brain, of some specific substructures in the brain (typically the entorhinal cortex) or the ventricles. Among others, these approaches have been pioneered by the group of Nick Fox who introduced the boundary-shift-integral to follow the evolution of total brain volume in individual persons (Fox and Freeborough 1997; Scahill et al. 2003; Chan et al. 2003; Fox and Schott 2004), and by Clifford Jack and his team who focused more on measuring the volume of substructures in the brain (Jack et al. 1997; Jack et al. 1998; Grundman et al. 2004; DeCarli et al. 2007). The potential value of these volumetric imaging measures over clinical cognitive tests or rating scales is emphasized by statistical power calculations which show that the required sample size to detect significant effects is much reduced for imaging readouts (Fox et al. 2000; Jack et al. 2003; Jack et al. 2004a; Ezekiel et al. 2004). Finally, a study testing the effects of Donepezil treatment detected a slowing of the rate of hippocampal atrophy (Krishnan et al. 2003). The findings from all these pioneering studies, and the conceptual face value of measuring neuronal loss, were so convincing that volumetric imaging became one of the main pillars in a large public-private partnership initiated by the National Institutes of Health, the "Alzheimer's Disease Neuroimaging Initiative (ADNI)" (http://www.adni-info.org; Jack et al. 2008a). One of the main goals of this initially five-year-long endeavor is to improve the design of clinical trials in AD by generating a large database linking the imaging readouts to the clinical status and progression of patients. Ultimately, the findings and methods validated in this venture should help to lead to effective treatment against the disease.

Another example in the first category is hydrogen magnetic resonance spectroscopy (^{1}H-MRS). One of the main pioneers of ^{1}H-MRS in AD patients is Brian D. Ross who found typical changes in the spectra from AD patients compared to normal, age-matched controls (Miller et al. 1993; Shonk et al. 1995): a reduction of the amplitude of the signal of N-acetyl-aspartate (NAA), most likely reflecting the neuronal loss in the advanced stages of the disease, and an increase of the signal of myoinositol. The relevance of these findings again is increased by the observation of a (temporarily) higher NAA level after treatment with Donepezil in a double-blind, placebo controlled clinical study (Krishnan et al. 2003). Because of the

higher complexity of conducting MRS examinations in a clinical environment, however, the database of spectroscopic studies in human patients as of today is still much slimmer than for volumetric imaging.

The second category of clinical imaging markers in AD includes all the approaches which provide some mechanistic insight into the pathologic process or some treatment effect, but without any measure, which would somehow reflect the patient status. Many of the treatment approaches which are currently pursued in pharmaceutical R&D focus on the amyloid hypothesis, that is, the neurotoxicity induced by the aggregation of amyloid plaques in the patient's brain. Following this hypothesis, any new treatment approach has to demonstrate a decrease of the plaque load in patients in a proof-of-concept study – if the new therapy has no influence on amyloid, no benefit for the patient is conceptually to be expected, and the investment in a phase III trial is not warranted. Plaque load imaging is mainly within the realm of nuclear imaging, using plaque specific ligands carrying isotopes which can be detected either with PET or SPECT-cameras. As of today the best characterized ligand for plaque imaging both in vitro, in animals, and in humans is the "Pittsburgh-compound B (PIB)" [e.g., (Klunk et al. 2003; Jack et al. 2008b)], but there are a number of other ligands in development at various sites which may have different properties, in particular in terms of amyloid binding specificity. In addition, various concepts to use MRI for the direct or indirect visualization of the plaque burden are being explored [e.g., (Benveniste et al. 1999; Poduslo et al. 2002; Higuchi et al. 2005; Bartlett 2005)].

Preclinical MRI in Transgenic Mouse Models of AD

How can these clinical imaging concepts be translated back into preclinical pharmaceutical discovery, exploiting the "bedside-to-bench" route? The first requirement is the availability of some model system which mimics essential aspects of the pathology, and which allows one to examine the action of innovative therapeutic concepts. Once past the early experimental stages in test tubes or Petri dishes, pharmaceutical drug discovery in AD focuses mainly on transgenic mouse models with mutations that generate similar alterations in the mouse brain as the human disease. The creation of these transgenic mouse models follows the experience and the hypotheses gained from clinical research: the rationale behind most transgenic models for AD are based either on the amyloid-hypothesis, the tau-hypothesis, or both [e.g., (Hsiao et al. 1996; Lewis et al. 2000; Ishizawa et al. 2003)]. The purpose of these animal models is to obtain a better understanding of the pathophysiologic pathways of the disease process, to test the effects of potential treatments, and also to develop and to validate new biomarkers ultimately for human use. In particular for the latter two goals, preclinical in vivo imaging plays an essential role. The list of successes and failures in this area also provides more conceptual insight in the huge potential – but also the pitfalls – of translational imaging in pharmaceutical R&D.

The detection of neuronal loss by volumetric brain imaging would be the first obvious approach to phenotype and to characterize these transgenic animal models.

Indeed, some studies have described smaller brain areas in transgenic mice, some even before onset of plaque formation (Weiss et al. 2002; Redwine et al. 2003). Reports on total brain volume are conflicting: a smaller total brain volume – albeit not accompanied by a loss of neurons – has been recently detected in the APP T714I mouse model (Van Broeck et al. 2008), but our own studies have not revealed any significant reduction of total brain volume or ventricle size in PS2APP mice (von Kienlin et al. 2005). Furthermore, these studies showed one clear distinction between mice and man: whereas the healthy human brain reaches its largest size in late adolescence and then starts shrinking, the brain of mice grows continuously throughout their life span (see Fig. 1). In mice, any mechanism of neuronal loss thus may be compensated or masked by the natural growth of the organ. Unless tissue atrophy can reliably be detected in some specific transgenic mouse model and can – with high confidence – be associated to some specific pathologic mechanism, preclinical volumetric brain imaging has low translational value.

The second-best characterized parameter in human AD patients is magnetic resonance spectroscopy (MRS) to characterize the neuron-chemical profile. MRS has also been applied in transgenic mouse models and has detected differences between transgenic animals to controls [e.g., (von Kienlin et al. 2005; Dedeoglu et al. 2004)]. These studies reported lower levels of NAA and Glutamate in the frontal cortical areas of aged transgenic mice; these findings are consistent with reduced neuronal viability and in line with the findings in human AD patients. Although this change of the metabolic profile is significant and may provide further insight into the disease pathology, it has to be recognized that its value for preclinical

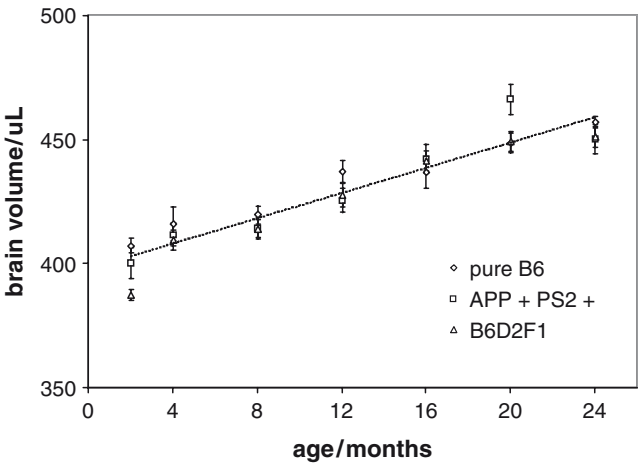

Fig. 1 Growth of murine whole brain volume throughout the lifespan, monitored by volumetric MRI in a transgenic mouse model for Alzheimer's disease (PS2APP), and in two wild-type control strains (pure B6 and B6D2F1). In contrast to the human brain which reaches its maximal size in early adulthood, the brain of mice grows continuously. This growth may mask potential brain atrophy induced by neurodegeneration–no difference in the rate of total brain growth could be detected between these three strains

drug discovery is limited for practicality reasons: the amyloid pathology in the PS2APP mice starts early and leads to behavioral deficits already detectable at age 8 months (Richards et al. 2003), but the reduction of NAA in the MR spectrum reaches significance only after an age of 16 months. The duration of studies testing the effect of new compounds using MRS as read-out thus is in the range of at least one year, which is prohibitive in the usual time-frame of preclinical discovery departments.

Functional imaging appears to be very promising to characterize the phenotype of transgenic animals. PET or SPECT imaging consistently detects hypo-metabolism or reduced blood flow, respectively, in particular in the temporoparietal cortex of AD patients [review in (Johnson and Albert 2000)]. It is still under debate whether this is due to some vascular impairment or a reduced neuronal energy demand – the "hen-or-egg" question. Translating these findings into the preclinical research environment, the Novartis group – among others – has run some very interesting studies in the transgenic mouse strain APP23: first, they have shown age dependent, progressive cerebrovascular abnormalities which may contribute to the pathogenesis of the disease; from the absence of transgene overexpression or amyloid plaque in the vasculature, they concluded that the vascular deficit might be because of the deleterious effects of soluble A-beta (Beckmann et al. 2003). In addition, in a functional study stimulating the somatosensory cortex, they found the hemodynamic response decreasing with age, which they attributed to a compromised cerebrovascular reactivity in this disease model (Mueggler et al. 2003). In the B6.PS2APP model, we could also detect consistent hypoperfusion in the dorsal parts of the cortex as early as from age 10 months (Weidensteiner et al. 2008). If these findings are confirmed and further validated, these functional parameters may become early markers of the pathologic status of the animals, and thus serve as potential readouts for functional improvement when testing the efficacy of new compounds.

The second category of imaging approaches defined above provides mechanistic insight into the disease pathology; in this category, for instance, all methods to visualize amyloid plaques in vivo, in the brain of transgenic mice (Wadghiri et al. 2003; Jack et al. 2004b; Vanhoutte et al. 2005; Braakman et al. 2006) belong. High-field MRI instruments and modern coil technology have improved the sensitivity to the point that individual larger plaques can be detected through MR microscopy. It nevertheless needs to be recognized that the data acquisition time required to achieve sufficient spatial resolution is still quite long. Furthermore, other imaging modalities such as optical imaging may be better suited to measure plaque load in small rodents (Hintersteiner et al. 2005).

Translational MR Imaging in Psychiatric Disorders

A second domain in CNS drug R&D in which translational imaging modalities are playing a critical role is the huge field of psychiatric disorders. Imaging is expected to provide a better understanding of the etiology of these diseases, the

brain areas involved, and the underlying disease mechanisms. This should lead to the development of quantitative imaging markers providing differentiated information about the patient status, markers which are less variable and less subjective than current clinical rating scales. Translational imaging research in psychiatric disorders has been adopted by several pharmaceutical companies as a pragmatic, efficient approach to extend predictivity of animal models and treatment outcomes. These imaging biomarkers are used to assess the efficacy and differentiation of new drug candidates in animal models allowing a more focused, faster early drug development. Neuroimaging findings in schizophrenia, as an example of translational approaches used for psychiatric disorders, are developed in the following sections.

Clinical Imaging in Schizophrenia

Several imaging techniques have been extensively used in schizophrenic patients to better understand pathophysiology and identify new potential biomarkers. Impaired functional connectivity is one of the main brain alterations measured in schizophrenic patients. A large set of data implicates the frontal lobes as key areas involved in this dysfunctional neurocircuitry. fMRI studies reveal dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) dysfunctions in individuals with schizophrenia (Yoon et al. 2008; Tamminga et al. 1992). This alteration in DLPFC and ACC responses has been associated with impaired attention and cognition. A third cortical subregion, the medial prefrontal cortex (mPFC), was abnormally hyperactive in patients performing socioemotional paradigm, reflecting reality distortion processes (Taylor et al. 2007). Functional abnormalities have not been measured in cortical areas only. Indeed, patients performing a contextual memory task showed neuropathology in frontohippocampal circuitry. Despite similar memory performance, these patients activated neuronal pathways different from healthy individuals (Weiss et al. 2006). Efficacy of antipsychotic treatments on abnormal brain activity was reported in schizophrenic patients. Interestingly, distinct effects were measured with typical and atypical antipsychotics (Braus and Brassen 2005).

In addition to impairment in executive functions (i.e., attention, cognition), schizophrenic patients could present negative symptoms such as anhedonia, a loss of interest or pleasure in daily activities. Anhedonia is associated with dysfunction of the brain reward system. Patients performing a reward-learning association task showed inappropriately stronger activations in the ventral striatum, one of the key brain areas involved in reward processing, consistent with abnormal assignment of motivational salience to neutral stimuli (Jensen et al. 2008). Abnormal ventral striatum activity was also measured during an incentive monetary task in medication-free schizophrenics. In this study, the reduced striatal activity during presentation of reward- and loss-indicating stimuli correlated with the severity of symptoms (Juckel et al. 2006).

To better understand neuropathology, schizophrenia-like symptoms could be induced in healthy volunteers. Indeed, pharmacological challenges with glutamatergic

N-methyl-D-aspartate (NMDA) receptors antagonists such as ketamine or phencyclidine (PCP) induce in healthy humans symptoms that resemble schizophrenia and exacerbate them in patients (Umbricht et al. 2000; Lahti et al. 2001; Krystal et al. 2003). A recent pharmacological magnetic resonance imaging study measuring blood oxygenation level dependent (BOLD) signal changes during ketamine administration in healthy humans reveals selective regional brain activity changes (Deakin et al. 2008). The author describes a ketamine-induced activity decrease in ventromedial frontal cortex (i.e., orbitofrontal cortex, subgenual cingulate) and increase in areas such as mid-posterior cingulate cortex, thalamus, or temporal cortical regions. These effects were reversed by pharmacological treatment. Indeed, attenuation of neuropsychiatric effects and dysfunctional neuronal circuitry of ketamine have been shown in healthy volunteers treated with lamotrigine, a sodium channel blocker that decreases glutamate release (Deakin et al. 2008; Anand et al. 2000). Ketamine could be an attractive translational pharmacological challenge to assess neuronal circuitry involved in NMDA antagonist-induced schizophrenia symptoms and to evaluate potential antipsychotic efficacy.

As schizophrenia is a neurodevelopmental disease, anatomical connectivity could be an important marker for disease progression and efficacy of therapeutics. Diffusion tensor magnetic resonance imaging (DTI) noninvasively assesses neuronal tractography, that is, abnormal circuitry, neuronal degeneration or demyelination. Indeed, DTI studies consistently suggest altered intra- and interhemispheric connectivity in schizophrenic patients such as corticocortical, frontotemporal and transcallosal altered connectivity (Seal et al. 2008; Brambilla and Tansella 2007). Further investigations are needed to better characterize neuropathology and evaluate effects of antipsychotic treatments.

Another translational neuroimaging technique is magnetic resonance spectroscopy (MRS), allowing measurement of brain metabolites. In schizophrenic patients, the main reported alteration is a reduced NAA level in the temporal and frontal lobes and in the thalamus. Such a reduction in NAA level is considered as surrogate of neuronal loss or dysfunction and correlates with increased symptoms (Callicott et al. 2000; Ende et al. 2000). In addition to this effect, higher cortical level of glutamate has been measured in schizophrenia (Olbrich et al. 2008). Interestingly, antipsychotic treatments normalized such alterations in metabolites levels. For example, atypical antipsychotic treatments such as clozapine or risperidone showed efficacy by increasing NAA levels in both cortical areas and in the thalamus (Ertugrul and Uluğ 2007; Bertolino et al. 2001; Szulc et al. 2005; Braus et al. 2001). Lower NAA levels measured in patients under typical medication may be caused by either the progression of the disease or by a direct action of these drugs (Bustillo et al. 2001). However, more recent evidence showed no direct "toxic" effect of typical antipsychotics such as haloperidol on NAA concentrations (Bustillo et al. 2007). MRS technique could also be used to assess effect of pharmacological challenges. An increase in anterior cingulate glutamine, a putative marker of glutamate release, was indeed measured in healthy humans treated with ketamine (Rowland et al. 2005).

Preclinical MRI in Animal Models of Schizophrenia

Within the last few years, numbers of preclinical neuroimaging studies have emerged in the field of psychiatry. Indeed, brain alterations in animal models of schizophrenia have been evaluated and compared to human findings. A variety of animal models of schizophrenia have been developed that are aimed at gaining a better understanding of the etiology and pathophysiology of this disorder. These models are classically divided into two broad categories – the pathophysiological models, relying on deficits induced by abnormal neurodevelopment, dysfunction of cortical glutamatergic systems, or genetic susceptibility and the neuropharmacological models related to specific neurotransmitter systems (typically dopamine and glutamate) and postulating that a dysfunction of these systems is underlying the disease. The neuropharmacological models rely on the use of psychotomimetic substances (dopamine agonists, NMDA antagonists) to produce schizophrenic-like symptoms in animals. In both types of models, similar schizophrenia-like symptoms are generated such as hyperlocomotion, sensorimotor deficits, or deficits in latent inhibition, impaired performance in cognition and memory tasks, and altered social and reward processes (Le Pen et al. 2002; Lipska and Weinberger 2000).

Similar functional brain alterations have been measured in a neurodevelopmental and a pharmacological animal model of schizophrenia (Risterucci et al. 2005). Indeed, rodents with neonatal ventral hippocampal lesion or acutely treated with the NMDA antagonist PCP showed abnormal activity in corticosubcortical circuitry involving brain areas such as temporal and prefrontal cortex, ventral striatum, or thalamus. This dysfunctional activity in regions that may play a role in schizophrenia-related behavior of rats, are reminiscent of neuroimaging findings in schizophrenic patients. Specificity of dysfunctional neurocircuitry was demonstrated as antipsychotic drugs like olanzapine, haloperidol, and risperidone normalized the PCP-induced activity changes whereas no reversal was measured with the anxiolytic diazepam (unpublished observation). Similar findings were reported by other groups. Indeed, dysfunctions in cortico-limbo-thalamic regions were measured in PCP- and ketamine-treated animals (Gozzi et al. 2008; Littlewood et al. 2006). The authors showed efficacy of the antipsychotic clozapine in the PCP model (Gozzi et al. 2008). Interestingly, as described by Deakin's group in healthy volunteers (Deakin et al. 2008), the anticonvulsant lamotrigine suppressed NMDA antagonist-induced activity changes in rodents as well (Gozzi et al. 2008). NMDA receptor antagonist models offer a good translational approach to evaluate efficacy of new potential antipsychotic drugs. They offer reasonable face validity with respect to the clinical disorder, and predict to some degree the efficacy of drugs in patients (Large 2007). As these pharmacological models are related to specific neurotransmitter systems, it is essential to combine findings from different animal models to increase construct validity for schizophrenia. MRI appears to be a valid noninvasive tool to improve translation between preclinical and clinical findings, to better understand brain alterations in schizophrenia, and to establish early markers for successful treatment.

Pharmacological MRI studies could also be done in naïve animals to identify brain signature of specific drug treatments (Nordquist et al. 2008). Activity profile of a new drug (i.e., activity changes measured in a network of brain areas) could be compared to antipsychotics profiles. This information could help to better understand the mechanism of action and further extend predictivity of treatment outcomes.

Anatomical connectivity can also be assessed in animals. DTI recently allowed the evaluation of early postnatal development of rat brain (Bockhorst et al. 2008). This technique has a great potential to evaluate white matter integrity in neurodevelopmental models of schizophrenia. Translation to human was not yet demonstrated as no DTI study has been reported in animal models of psychiatric disorders.

Alterations in brain metabolites have been shown in animal models of schizophrenia. For example, MRS revealed reduced NAA levels in prefrontal cortex of adult rats with neonatal hippocampal damage (Bertolino et al. 2002). This finding correlates with decreased NAA levels measured in frontal areas in schizophrenic patients (Callicott et al. 2000). Translational aspect should be further evaluated using pharmacological treatment.

Conclusion

Translational aspects are a critical element in the design of preclinical imaging studies for drug discovery. Taking clinical expertise and the imaging protocols that are applied in human patients into account will enrich preclinical experimentation and will facilitate the consistent interpretation of data across species. The main issue that is consistently raised when exploring new potential imaging markers, however, is about appropriate validation: how much confidence is required to trust that data obtained in animals are predictive of the human situation? In most CNS disorders – with multiple sclerosis being the possible exception confirming the rule – the relationship between imaging findings and patient status has not been established. For years to come, it is very unlikely that the health authorities will accept any imaging read-out as "surrogate," that is, as primary endpoint in phase III trials for CNS disorders. In disorders such as AD, for which no efficacious treatment is available, no "positive control" data can be generated for assessing the statistical power of the imaging modality, that is, its signal amplitude and its variability. The level of confidence in the imaging protocol thus depends on the face value of the measurement concept–insight in a well-known disease mechanism will generate much more convincing results than exploratory studies which merely provide some correlation to the (patho-) physiologic status of the patient. The main value of clinical imaging for pharmaceutical R&D lies in mechanistic proof-of-concept studies during phases I and II, and in adjunct studies such as for dose finding.

In our aging population, slowly developing chronic disorders without efficacious treatment options, such as neurodegenerative diseases or psychiatric disorders and also diabetes, arthritis etc., are a major concern for the people and a threat for the

health care system. In particular the slow progression of these disorders makes clinical trials very long and costly before demonstrating the benefit of some new potential treatment. Proof-of-concept imaging studies in human patients will have an essential role in deciding which projects will be carried forward to full clinical development. Similarly, translational preclinical studies providing mechanistic insight into drug effects in animal model systems will generate the confidence as to which compounds will be moved into man. They also serve to identify and to validate innovative imaging approaches as early, quantitative, and statistically more powerful biomarkers. The better characterization of the in vivo properties of new medical entities will reduce the attrition rate in pharmaceutical portfolios and will enhance the discovery of better treatment options for the most urgent medical needs. High expertise in the concepts and the execution of translational imaging studies constitutes a significant competitive advantage for pharmaceutical companies.

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In Vivo Mouse Imaging and Spectroscopy in Drug Discovery

Nicolau Beckmann, Catherine Cannet, Martin Rausch, Rainer Kneuer, and Hans-Ulrich Gremlich

Introduction

The knowledge about the pathophysiology of a disease as well as its early diagnosis and characterization stands at the center of drug research. The better the mechanisms of a disease known, the higher the probability of finding an appropriate therapy. Also, the better and earlier a disease can be diagnosed and characterized, the greater will be the chance to interfere in the pathological process with a chemical entity. This reasoning sets the framework for the use of imaging in pharmaceutical research.

In a simplified view, the drug discovery and development process can be divided into four phases (Fig. 1). In vivo imaging may play an important role in three of these phases. In target validation and pre-clinical research, which encompasses all endeavors previous to testing compounds in humans, a significant portion of the in vivo activities is performed in mice. Since mouse and man are similar at the genetic level, it is possible to measure similar disease parameters in mice and in humans. The ability to manipulate the mouse genome has allowed and will allow us to define molecular pathways describing the processes of disease initiation and progression. These models may serve as an excellent platform for the identification of novel molecular targets for therapy as well as for the evaluation of the efficacy of targeted therapies. Indeed, genetic alterations in the mouse often result in functional changes through which relevant pharmacological effects in man can be predicted (Tornell and Snaith 2002; Zambrowicz and Sands 2003; Zambrowicz et al. 2003). A retrospective evaluation of the knockout phenotypes for the targets of the 100 best-selling drugs indicates that effects in murine models of human disease correlate well with known clinical drug efficacy (Zambrowicz and Sands 2003), suggesting a productive path forward for discovering future drug targets.

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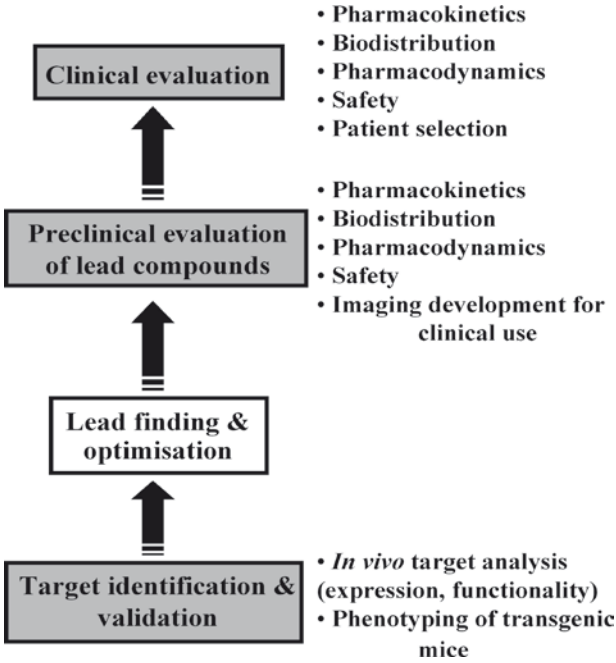


Fig. 1 Simplified view of the drug discovery process. Except for the lead finding and optimization phase, possible uses of in vivo imaging at the several phases are indicated on the right

In this chapter, we discuss the use of mouse imaging in the context of drug discovery. First, a brief description of imaging techniques currently adopted for imaging mice is provided. The design of imaging probes, which is becoming more and more important in this context, is addressed next. Then, we turn our attention to the specific roles played by transgenic mice in drug discovery. Finally, we present examples of mouse imaging in Alzheimer's research, selected to illustrate some key points reflecting the advantages, challenges and limitations of in vivo imaging in preclinical drug research.

Techniques for in Vivo Brain Imaging and Spectroscopy of Mice

An overview of current imaging modalities of interest within drug research is provided in Table 1. In this section we briefly outline the main characteristics of these techniques, for the scope of imaging mice in vivo. The reader is referred to other reviews to obtain more detailed information on mouse imaging methods including practical aspects and challenges (Acton and Kung 2003; Chen and Henkelman 2006; Contag and Bachmann 2002; Gambhir 2002; Hammoud et al. 2007; Nieman et al. 2007; Ntziachristos et al. 2003; Ritman 2004; Rudin 2006).

Table 1 Current imaging modalities of interest in drug research and discovery

Technique	Spatial resolution; time scale	Clinical imaging	Application	Main characteristics
Ultrasound	50 μm; s to min	Yes	Anatomical, functional; drug delivery	Microbubbles as contrast agent; difficult to image through bone or lungs
CT	50–100 μm; s to min	Yes	Anatomical, functional	Poor soft tissue contrast
MRI	80–100 μm; s to min	Yes	Anatomical, functional, metabolic, cellular	High spatial resolution and soft tissue contrast; low sensitivity (~1 mmol)
PET (high energy γ-rays)	1–2 mm; min	Yes	Metabolic, functional, molecular	Most common isotopes have short half-lives; high sensitivity (pmolar concentrations); cyclotron needed
SPECT (low energy γ-rays)	1–2 mm; min	Yes	Functional, molecular	Radioisotopes have longer half-lives than those used in PET; sensitivity 10–100 times lower than PET
Bioluminescence	1–3 mm; s to min	No	Molecular	High sensitivity (~1 nmol); transgene based approach; light emission prone to attenuation with increasing tissue depth
NIRF	1–3 mm; s to min	Yes[1]	Molecular, functional	High sensitivity (~1 nmol); excitation and emission light prone to attenuation with increased tissue depth

[1]Applications in clinical evaluation of breast cancer and arthritis under evaluation

Ultrasound and Drug Delivery to the Brain

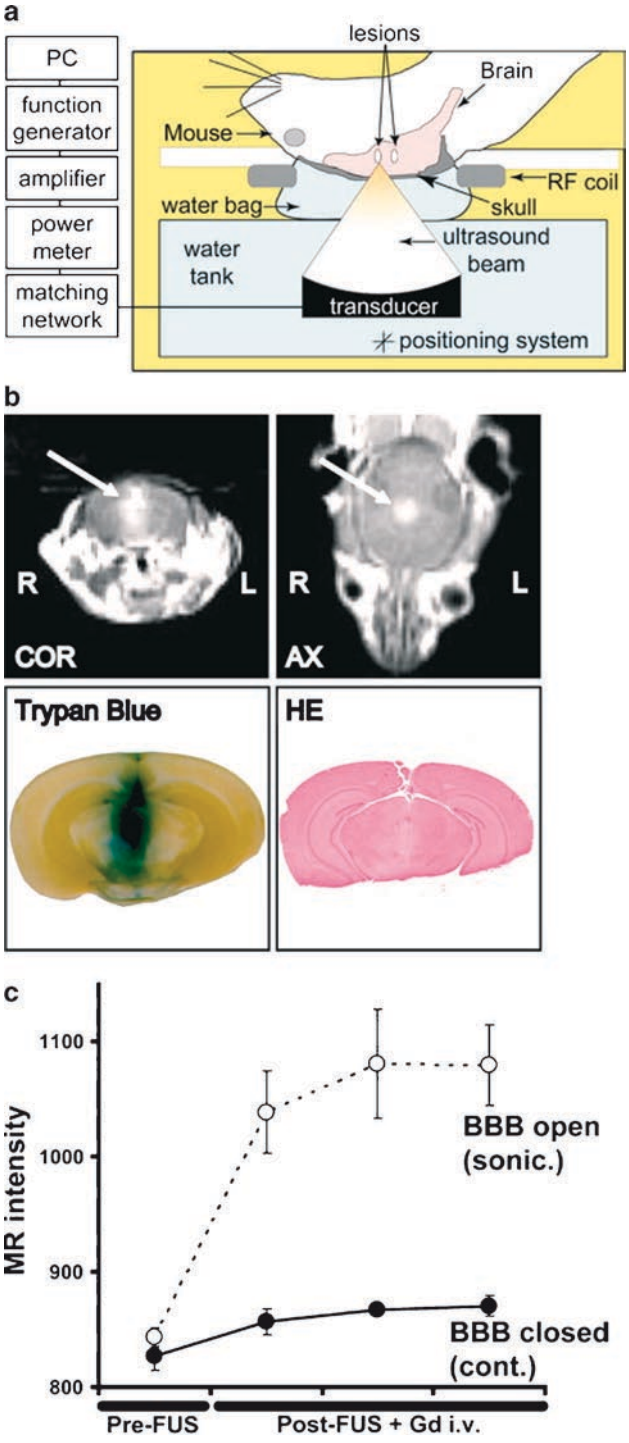
The central nervous system (CNS) is protected from the entry of foreign substances by the almost impenetrable blood–brain barrier (BBB), which hampers the delivery of potentially effective diagnostic or therapeutic agents. Because antibody-based agents with a molecular size of approximately 150 kDa are easily blocked by the BBB, their delivery to the CNS requires the temporary suspension of the physiological role of the BBB to bar larger molecules from the CNS.

Advances in acoustic technology have made ultrasound a modality with therapeutic as well as diagnostic applicability. The combined use of ultrasound and gas bubble-based ultrasound contrast agents induces bioeffects, such as transient changes in cell-membrane permeability (Kinoshita and Hynynen 2005). This approach has been shown to facilitate the reliable disruption of the BBB in murine models (Choi et al. 2007; Kinoshita et al. 2006a). The technique has been applied to examine in mice the feasibility of delivering Herceptin through the BBB (Kinoshita et al. 2006b) (Fig. 2). Contrast-enhanced high-resolution magnetic resonance imaging (MRI) revealed BBB opening.

Microcomputerized Tomography

Microcomputerized Tomography (micro-CT) systems providing high-resolution images (~50 μm) and rapid data acquisition (typically 5–30 min) are emerging as a cost-effective means for detecting soft-tissue structures, skeletal abnormalities and tumors in small animals (Badea et al. 2004; Cavanaugh et al. 2004; Ford et al. 2005; Ritman 2004). The use of iodinated contrast agents enhances the weak endogenous contrast between different soft tissues. However, the difficulty in designing CT contrast agents limits the utility of the technique for molecular imaging applications, and at least in the near future, micro-CT will be used essentially to supplement data from other molecular imaging techniques.

Fig. 2 Experimental settings and BBB disruption in mice by MRI-guided focused ultrasound. (**a**) Diagram and protocol for BBB opening used in this experiment. Mice in the supine position were placed on the sonication table in the MR scanner. The ultrasound beam was focused through the intact skull on the target in the brain. (**b**) Representative example with BBB disruption achieved by 0.6-MPa (peak negative-pressure amplitude) focused ultrasound exposure. (*Upper*) The BBB opening was easily monitored by leakage of the MR contrast agent into the brain parenchyma on axial (AX) and coronal (COR) MR images (*arrows*). (*Lower Left*) The location of the BBB opening was confirmed by trypan blue staining of the affected area. (*Lower Right*) No apparent macroscopic damage related to BBB disruption can be seen. (**c**) Magnitude of BBB disruption in the animal presented in B monitored by the MR-intensity change. Absolute values of the MR intensity of the sonicated target (*open circle*) and the contralateral side (control; *filled circle*) are plotted for repeated image acquisitions after sonication. Data are presented as the mean ± SD of four voxels. Courtesy of Kullervo Hynynen, Harvard University, Boston. Reproduced with permission from Kinoshita et al. (2006b).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R
L
COR
R
L
AX
Trypan Blue
HE
c
MR intensity
1100
1000
900
800
BBB open (sonic.)
BBB closed (cont.)
Pre-FUS
Post-FUS + Gd i.v.

Accuracy in the images is determined by the X-ray dose given to the animal. One concern of micro-CT is therefore radiation dose, which despite not being lethal, may be high enough to induce changes in the immune response and other biological pathways, so that experimental outcomes could be affected (Boone et al. 2004; Ford et al. 2003).

Magnetic Resonance Imaging and Spectroscopy

The principal strengths of magnetic resonance (MR) techniques are noninvasiveness, high spatial resolution – of the order of 100 μm for small rodent studies – and excellent soft tissue contrasting capabilities. The signal is governed by a number of parameters, and this wealth of information renders MR a valuable tool for diagnosis, tissue characterization and in vivo morphometry, for obtaining physiological and functional readouts, and for deriving metabolic and, to some extent, target-specific tissue characteristics.

A major limitation of MR is its low sensitivity, and in general terms, the role of in vivo MR imaging (MRI) and spectroscopy (MRS) in pharmacological research is to study the effects of a drug on tissue morphology, physiology and biochemistry rather than to study the fate of the drug itself in the organism. In other words, MR methods yield primarily pharmacodynamic readouts (Beckmann 2006; Beckmann et al. 2001, 2004; Rudin et al. 1999).

Development of gradient systems with improved design (Dodd and Ho 2002; Leggett et al. 2003), of specialized radiofrequency coils for microimaging (Bilgen 2006; Webb 1997), including cryogenic coils (Darrasse and Ginefri 2003; Ratering et al. 2008; Voehler et al. 2006), and of devices for appropriate anesthesia and physiological control (Brau et al. 2004; Hedlund et al. 2000) are significantly improving the quality of mouse MRI/S. Most of the studies are performed at high magnetic fields (≥4.7 T), however, an interesting alternative for translational purposes could be the use of cryogenic coils in combination with magnets operating at lower fields (≤1.5 T), compatible with clinical settings (Poirier-Quinot et al. 2008) (Fig. 3). Despite the practical challenges in performing MRS studies in living mice [see (Choi et al., 2003; Heerschap et al. 2004) for reviews], several groups have shown the feasibility of applying the technique on a routine manner in the neuroscience area (Jenkins et al. 2005; Marjanska et al. 2005; von Kienlin et al. 2005). High resolution proton spectra from the mouse brain can be obtained at high (9.4 T) (Miyasaka et al. 2006; Tkac et al. 2004) and low fields (2.35 T) as well (Schwarcz et al. 2003).

Positron Emission Tomography

Positron Emission Tomography (PET) produces images of the body by detecting the radiation emitted from substances injected into the body and labeled with positron emitting radioactive isotopes such as carbon-11, fluorine-18, oxygen-15,

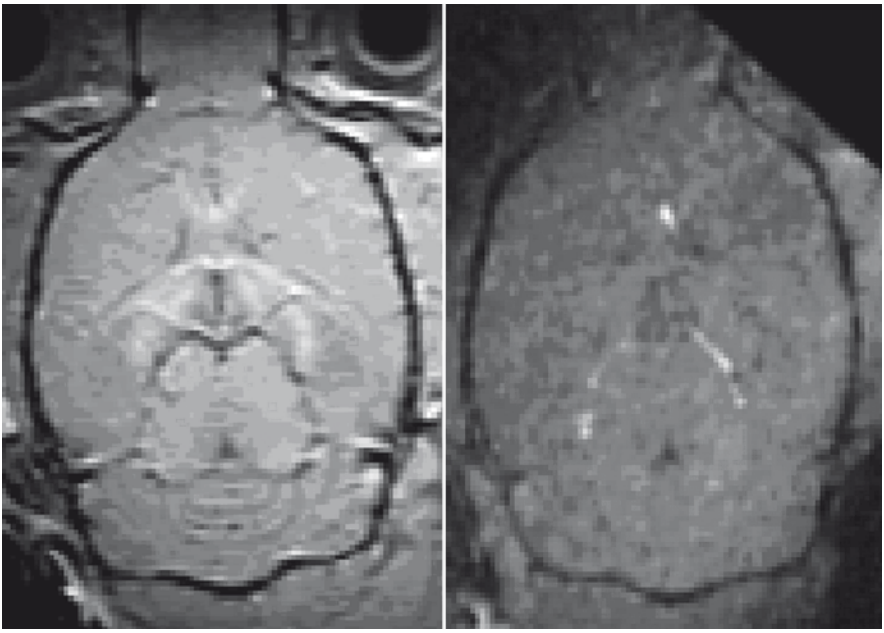

Fig. 3 A MRI of the mouse brain in vivo using a high temperature superconducting radiofrequency coil (*left*) and a conventional copper coil of the same geometry (*right*), both operating at 1.5 T in a clinical scanner. Acquisitions were performed using a gradient-echo sequence. See Poirier-Quinot et al. (2008) for details. Courtesy of Marie Poirier-Quinot, Jean-Christophe Ginefri and Luc Darrasse, Université de Paris Sud – CNRS, Orsay, France

or nitrogen-13. As isotopic substitution does not affect the physicochemical and binding properties of a compound, PET is the method of choice for pharmacokinetic studies of biologically active compounds, for instance drugs or drug candidates, by labeling them with e.g. carbon-11. The short half-life of PET radionuclides implies that scanners have to be located near particle accelerators (cyclotrons) that produce the radioisotopes. PET imaging is one of the most sensitive imaging approaches and picomolar amounts of radiolabel can be readily detected and quantified in vivo, irrespective of tissue depth. For comparison, SPECT and MRI require respectively 10^1–10^2 and 10^7–10^8 higher amounts of probe. Recent advances in radionuclide labeling allow the design and development of a large variety of radiopharmaceuticals including macromolecular structures (Duatti 2004). The availability of such tools enables target-specific studies of label biodistribution, pharmacokinetics, and excretion to be carried out in vivo, as well as to visualize and quantify target expression levels and target function.

Preclinical studies using small laboratory animals demand high spatial resolution provided by dedicated micro-PET systems. Typical voxel volumes achieved with such systems are between $(1.1\ \text{mm})^3$ and $(1.5\ \text{mm})^3$, rendering them adequate for studies in mice, rats, and nonhuman primates (Tay et al. 2005; Weber and Bauer 2004; Yang et al. 2004).

Single Photon Emission Computed Tomography

Single Photon Emission Computed Tomography (SPECT) radionuclides (xenon-133, technetium-99, iodine-123) are characterized by relatively long half-lives. They stabilize by emission of single gamma rays. SPECT is used to collect physiological information (e.g., blood flow) and to measure the biodistribution of radioactive substances. In addition to being less expensive than PET, a SPECT instrument does not need to be located close to a particle accelerator. Commercial small animal SPECT systems capable of measuring mice, typically use pixelated crystal arrays coupled to position sensitive photomultiplier tubes and a pinhole collimator (Beekman et al. 2002; Cao et al. 2005).

In Vivo Optical Imaging

Bioluminescence

Bioluminescence refers to the generation of (visible) light by living organisms, commonly due to an enzymatic reaction (Contag et al. 1997; Zhang et al. 2001). Reporter genes are used to study the expression of a gene of interest. This is achieved by inserting into the host cell genome a gene cassette containing the reporter gene construct under the control of the target gene. Bioluminescent reporters yield exquisite sensitivity as there is no endogenous background signal in mammalian cells resulting in high signal-to-background ratios: using sensitive detection devices such as photomultiplier tubes or cooled charge-coupled devices (CCD) sensitivity is sufficient to count only a few emitted photons.

A prerequisite for bioluminescence imaging is genetic engineering of the tissue cells of interest, i.e. the incorporation of an exogenous reporter gene. The most commonly used bioluminescent reporter is luciferase from the North American firefly that catalyze the transformation of D-luciferin (injected e.g. intraperitoneally) into oxyluciferin in the presence of both O_2 and Mg^{2+}-ATP leading to the emission of radiation at wavelengths larger than 600 nm (Rice et al. 2001), which falls into the window of reduced tissue absorption. Reporter gene assays have been demonstrated to yield fundamental biological information on e.g. transcriptional regulation, signal transduction, protein-protein interactions, cell trafficking or targeted drug action (Choy et al. 2003; Piwnica-Worms et al. 2004).

Fluorescence Imaging

Related to bioluminescence imaging is fluorescence imaging, an attractive tool due to its operational simplicity, safety, and cost-effectiveness. Exogenous fluorochromes (dyes or genetically engineered fluorescent proteins) are excited by e.g. laser diodes operating at a frequency close to that of the detected light; the emitted fluorescent light is then detected in a spatially resolved manner by a CCD camera.

The near-infrared window is particularly suitable for in vivo investigations. Near-infrared fluorescence (NIRF) imaging takes advantage of the low absorbance of tissue chromophores such as oxy- and deoxy-hemoglobin, water, melanin and fat, for light of wavelengths between 650 and 900 nm, to study in vivo biological processes at the cellular and molecular levels. At these wavelengths scattering of photons is a more significant attenuation factor than absorption.

A difficulty of in vivo optical imaging in general, is spatial resolution: the light intensity distribution measured at the surface critically depends on the depth of the light source within the tissue. A population of luciferase-expressing cells near the surface of the skin will appear both brighter and more focused than the same number of cells growing at deeper tissue sites. Tomographic approaches are being devised to improve data quantification (Ntziachristos et al. 2005; Wang et al. 2004; Zacharakis et al. 2005).

Multimodality Imaging

In many respects the imaging techniques discussed above are complementary; there is no "all-in-one" imaging modality providing optimal sensitivity, specificity and temporo-spatial resolution. Due to its relatively low sensitivity, MRI is of limited value for detecting molecular processes in vivo; nevertheless, its high spatial resolution provides a good anatomical reference for molecular data obtained with high sensitivity, low resolution imaging modalities. This might be achieved by postprocessing of data obtained in different imaging sessions or by simultaneous multimodality small animal imaging such as PET-MRI (Benveniste et al. 2005; Lucas et al. 2006; Pichler et al. 2006; Raylman et al. 2007; Slates et al. 1999), PET-CT (Del Guerra and Belcari 2002; Deroose et al. 2007; Hsu et al. 2008; Nahrendorf et al. 2008) and SPECT-CT (Merron et al. 2007; Müller et al. 2008; Seo et al. 2007). Combining imaging data requires compatibility of data formats for the various modalities as well as sophisticated software tools for image coregistration (fusion), data visualization and integration across modalities. The integration of multimodal imaging information into bioinformatics platforms comprising nonimaging data (gene/protein expression data, pharmacodynamic, pharmacokinetic, and pharmacogenetic databases, histological data, atlases) will be mandatory in the future for handling the ever increasing complexity of biomedical information.

Contrast Agents, Molecular Probes and Tracers

For ultrasound, CT and MRI, the administration of exogenous agents serves to enhance the quality of anatomical data or to provide additional information, usually related to the measurement of physiological parameters. On the other hand, for optical imaging and PET/SPECT an imaging agent is a necessity for signal generation. There are two main interests to develop a target-specific contrast agent or tracer in the context of drug discovery:

- Imaging and measuring the drug biodistribution: Early information on drug biodistribution and pharmacokinetic properties is essential during lead optimization and profiling. Conventionally, such data are obtained in rodents by blood and tissue sampling, or by autoradiography. More recently, nuclear imaging methods, in particular PET/micro-PET (Fischman et al. 2002; Phelps 2000), have been regularly used to derive such information in humans and animals as isotopic substitution with 11C or 18F does not affect the physicochemical properties of the compound (Fischman et al. 2002; Phelps 2000). Alternatively, one could label molecules with fluorochromes and use the far more accessible optical imaging techniques as preliminary, fast readouts of drug biodistribution. Compounds selected at this preliminary step would then be submitted to the significantly more involved PET examinations. This approach might be limited to visualize the distribution of large molecular weight compounds such as biopolymers [e.g. monoclonal antibodies (mAb), proteins, siRNA] as the reporter groups for optical imaging are bulky dyes that may affect the properties of the labeled molecule. This influence will be less pronounced on these macromolecules compared to conventional small molecule drugs;
- Imaging the target distribution/density and pharmacodynamic effects of drugs: Demand is for specific reporter probes and amplification strategies in order to differentiate target information from nonspecific background signal and to cope with the low (subnanomolar) target concentrations. Minimization of background signals requires elimination of the unbound and possibly of the nonspecifically bound fraction of the label, which implies a waiting period following injection of the reporter probe. Modulations of the signal from the reporter probe after administration of a drug candidate can be used to assess the compound binding to the target (receptor occupancy) or the effect of the drug on a certain molecular pathway. Reporter probes include targeted agents [e.g. small molecules, peptides, metabolites, antibodies or other molecules labeled with (a) 11C and 18F for PET, (b) ^{111}In or ^{99m}Tc for nuclear imaging, (c) fluorochromes for optical imaging or (d) magnetic reporter probes and activatable probes (Hogemann et al. 2002; Sipkins et al. 1998; Weissleder et al. 2000)]. The latter undergo chemical or physicochemical changes upon interacting with their target. Examples include caged near-infrared fluorochromes (Bornhop et al. 2001; Weissleder and Ntziachristos 2003), protease-activatable dequenching probes (Tung et al. 2000), paramagnetic agents that change spin–lattice relaxivity on activation (Louie et al. 2000), and superparamagnetic sensors (Perez et al. 2002).

Despite the poor sensitivity of MRI there is plenty of evidence that molecular imaging approaches using targeted contrast can become a routine tool for in vivo pharmacological studies in the near future. This requires that a “hot spot” of paramagnetic or superparamagnetic centers be delivered efficiently to the target, which can be achieved by using labeled nanoparticle platforms conjugated to targeting vectors (Lanza et al. 2004; Morawski et al. 2005). These platforms are optimized by different surface modification techniques to have reasonable circulation times to

reach their targets in the tissue by escaping the organism's particle filter (reticuloendothelial system). Among others, lipid-based nanoparticles, such as liposomes or micelles, extensively used as drug carrier vehicles, constitute a promising strategy for molecular imaging applications using MRI [see (Mulder et al. 2006) for a recent review]. Targeting ligands can be conjugated to lipidic particles by incorporating lipids with a functional moiety to allow a specific interaction with molecular markers and to achieve accumulation of the particles at diseased sites.

Reporter molecules for optical imaging consist of a near-infrared fluorescent dye, which can be coupled to target specific ligands/carriers such as antibodies, nanoparticles or polymers, proteins, peptides and small molecules, analogous to radiolabeling methods but with certain limitations due to the bulky dye molecules (Frangioni 2003; Licha 2002). In addition, fluorescence detection allows researchers to design smart sensor reporters based on fluorescence quenching mechanisms, which are not detectable in their native state but are activated by interaction with their target (e.g. protease sensors) to increase signal-to-background ratios (Funovics et al. 2003). Another way to increase the sensitivity of detection is the introduction of biocompatible superbright quantum dots into small animal research (Ballou et al. 2004; Gao et al. 2004) [see (Portney and Ozkan 2006) for a review]. Quantum dots of different colors tuned to target different biological process by coupling to corresponding carrier molecules will potentially enable multiplexed imaging in vivo.

Imaging of Mouse Models of Alzheimer's Disease

In this section, we select imaging of mouse models of Alzheimer's disease (AD) to illustrate issues of particular relevance within the pharmaceutical research context. Pathological features of AD are amyloid-beta (Aβ)-peptide-containing plaques, neurofibrillary tangles consisting of aggregated, hyperphosphorylated tau, extensive neuritic degeneration, and distinct neuron loss. Vascular abnormalities coexist commonly with the histological features of AD (de la Torre 2002; Farkas and Luiten 2001; Iadecola 2004). Deposition of Aβ-peptide in cerebral vessel walls, known as cerebral amyloid angiopathy (CAA), is very frequent, but its contribution to the onset of dementia is unknown (Castellani et al. 2004; Nicoll et al. 2004).

Diagnosing AD remains an imperfect science. No definitive biomarker is currently available (Thal et al. 2006) and this hampers clinical diagnosis and drug discovery involving transgenic mice modeling AD. Various quantitative MR techniques that measure the anatomical, biochemical, microstructural, functional and blood-flow changes are being evaluated as possible surrogate measures of disease progression (Kantarci 2005; Mueggler 2006).

Quantitative volumetry based on MRI is an important approach to assess the disease progression in patients. Correlation between antemortem MRI assessments of the hippocampal volume and postmortem analyses suggest that the hippocampal atrophy, although not specific for AD, is a fairly sensitive marker of both the pathologic stage and the hippocampal neurofibrillary tangle burden (Silbert et al. 2003).

Reduced hippocampal volume and corpus callosum length were detected by MRI in PDAPP mice before Aβ deposition, suggesting that overexpression of APP and amyloid may initiate pathologic changes before the appearance of plaques (Redwine et al. 2003).

Amyloid deposits may affect diffusion properties of the brain interstitium with implications for the transport of endogenous signaling molecules during synaptic and/or extrasynaptic transmission. Using diffusion-weighted MRI, Mueggler et al. (2003b) showed that fibrillar amyloid deposits and associated gliosis in brains of 25-month-old APP23 transgenic mice were accompanied by a reduction in the apparent diffusion coefficient (ADC). This decrease was most pronounced in neocortical areas with a high percentage of congophilic amyloid and was not significant in the caudate putamen, an area with only modest and diffuse amyloid deposition (Fig. 4). These findings suggest that extracellular deposition of fibrillar amyloid and/or associated glial proliferation and hypertrophy cause restrictions to interstitial fluid diffusion. Reduced diffusivity within the interstitial space may alter volume transmission and therefore contribute to the cognitive impairment in AD.

MRI approaches to analyze functional or hemodynamic changes related to the development of AD have also been successful in the process of characterizing transgenic mice in vivo. Functional MRI (fMRI) has been applied to assess brain functionality (Mueggler et al. 2002, 2003b) in APP23 mice. The cerebral hemodynamic response to infusion of the GABAA antagonist, bicuculline, was significantly reduced in aged APP23 mice compared with age-matched wild-type littermates. The decreased response was attributed to a compromised cerebrovascular reactivity associated with perivascular amyloid deposition. For fMRI, not a trivial procedure in small rodents, mice need special preparation (intubation, artificial ventilation, paralysis) which can be a substantial burden especially to aged animals. In addition, the throughput of the experiment is low. Despite providing only semiquantitative information, MR angiography of the mouse brain (Beckmann et al. 1999; Beckmann 2000), performed in spontaneously respiring animals and without administration of contrast material, has proven to be an interesting alternative for analyzing the vascular changes in transgenic mice. High resolution MR angiograms acquired in 16 min demonstrated flow perturbations in major arteries at the Circle of Willis in old but not in young APP23 transgenic mice (Beckmann et al. 2003; Thal et al. 2008). Corrosion casts (Krucker et al. 2004, 2006; Meyer et al. 2008) revealed that, at sites where flow voids were detected in vivo, vessel elimination, substitution and/or deformation had taken place. The loss of vascular integrity revealed by angiography might provide the basis for the age-related impairment of the cerebral blood volume response to pharmacological stimulation in the fMRI studies. Also, changes in relative cerebral blood volume (rCBV) and flow (CBF) determined with ^{2}H MRS and gradient-echo contrast enhanced MRI were reported recently in the brains of APP/PS1 mice subjected to different lipid diets (Hooijmans et al. 2007). Overall, these results support the idea that cerebral microcirculatory abnormalities evolving progressively could contribute to AD pathogenesis and cognitive impairment.

The most consistently reported neurochemical abnormality detected by ^{1}H-MRS in humans is a decrease in *N*-acetylaspartate (NAA) [for reviews see (Kantarci et al.

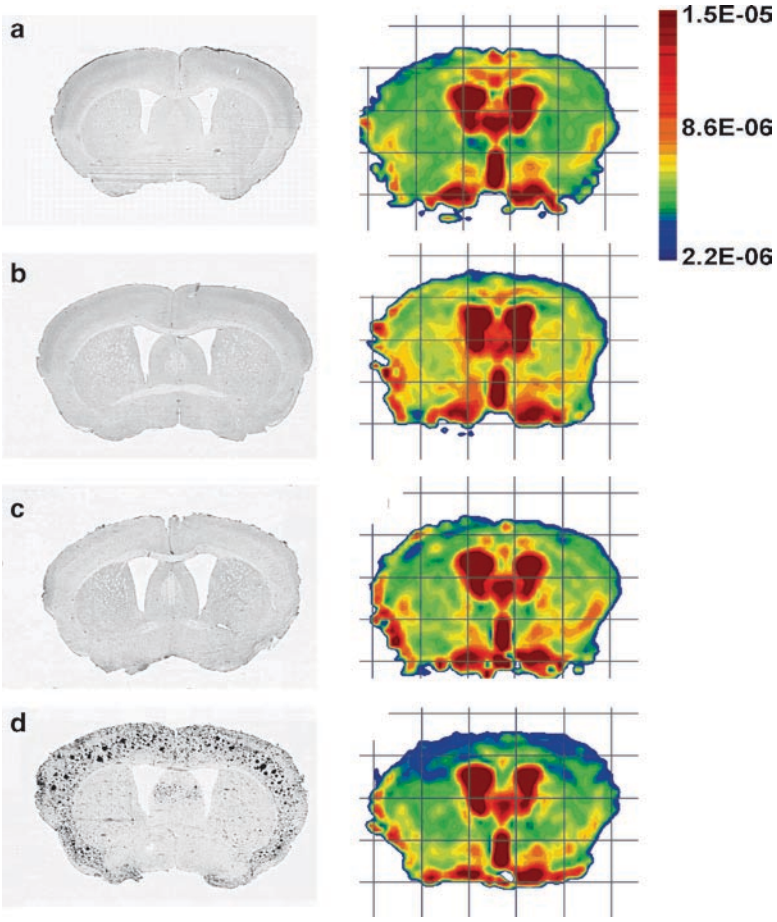

Fig. 4 Color-coded representative ADC maps of a coronal brain section at the level of Bregma for a 6- (C) and 25-month-old APP23 mouse (D) and age-matched controls (6 months, A; 25 months, B). The ADC scale on the right is in 10^{-6} cm^2/s. A significant ADC decline, color-coded in blue, is detectable in the medial and dorso-lateral cortex of aged APP23 mice. In the dorso-lateral cortex of the 6-month-old APP23 mouse, few areas with reduced ADC are visible. Courtesy of Thomas Mueggler, ETH Zurich, Switzerland. Modified with permission from Mueggler et al. (2003a). © Federation of European Neuroscience Societies

2004; Kantarci 2005)], considered as an indicator of neuronal number and health. An increase of the signal from myo-inositol, which may either be a marker for osmotic stress or astrogliosis, has also been reported in AD patients. Thus, myo-inositol may be an earlier marker of pathological change in AD than NAA. Proton MRS has also been applied to transgenic mice in AD research (Marjanska et al. 2005; von Kienlin et al. 2005), with spectral acquisition times between 10 and 35 min. A reduction in NAA and glutamate levels, compared with total creatine levels, and an increase in the concentration of myo-inositol was found in transgenic

mice with advancing age. The spectroscopic measures in vivo correlated well with the plaque load in the frontal cortex.

The ultimate readout of AD would be the visualization of cerebral plaques in vivo. Several groups have pursued the detection of amyloid plaques with MR microimaging in mice without administering contrast media (Braakman et al. 2006; Dhenain et al. 2002, 2007; Helpern et al. 2004; Jack et al. 2005; Lee et al. 2004). Initial trials involved very long acquisition times, up to several hours, however, recently, plaques have been resolved in vivo and at high magnetic fields (≥7 T) in shorter measurement times of the order of 2 h (Jack et al. 2005; Lee et al. 2004) and even of 25 min (Braakman et al. 2006). Another approach involved enhancing contrast-to-noise by using molecular probes that specifically target Aβ plaques (Higuchi et al. 2005; Kandimalla et al 2007; Poduslo et al. 2002; Wadghiri et al. 2003). From the perspective of drug research, these approaches are hampered by either relatively long acquisition times – several hours (Higuchi et al. 2005; Poduslo et al. 2002) – or by the necessity to open the BBB by mannitol (Wadghiri et al. 2003). However, Gd[N-4ab/Q-4ab]Abeta 30, a novel MRI agent based on a derivative of human Aβ peptide shown to cross the blood–brain barrier (BBB) and bind to amyloid plaques in APP/PS1 mice, holds promise for shorter acquisition times (Kandimalla et al. 2007).

Reduced T2 and T2* values have been reported in plaque-like structures in the cortex and hippocampus in several mouse models of AD (Braakman et al. 2006; El Tannir El Tayara et al. 2006; El Tayara Nel et al. 2007; Falangola et al. 2007; Helpern et al. 2004; Vanhoutte et al. 2005). This reduction could be explained by the presence of iron in plaques (Falangola et al. 2005). Despite not detecting plaques directly, this could potentially provide an interesting readout for following with a reasonable throughput age-related plaque load in AD models. A decreasing trend for T2 with age, while plaque area, number and size increased markedly, has been described (Braakman et al. 2006; El Tayara Nel et al. 2007).

Much more sensitive PET and NIRF approaches were successfully applied to detect plaques in vivo. For instance, the Pittsburgh Compound-B (PIB; [11C]6-OH-BTA-1), a hydroxylated derivative of an amyloid-binding dye thioflavin-T, has been developed as a PET tracer for plaque detection in the clinics (Klunk et al. 2004; Mathis et al. 2003). Recently, Maeda et al. (2007) showed the feasibility of quantitatively mapping by micro-PET the amyloid accumulation in the brain of APP23 mice modeling AD using [^{11}C]PIB (Fig. 5). Micro-PET investigations of transgenic mice over an extended range of ages, including longitudinal assessments, demonstrated an age-dependent increase in radioligand binding consistent with progressive amyloid accumulation. The approach has then been used to test therapeutic approaches for AD in APP23 mice. A reduction in amyloid levels has been observed in the hippocampus of transgenic mice during the course of antiamyloid treatment using an antibody against Aβ peptide. Moreover, micro-PET scans with [^{18}F]fluoroethyl-DAA1106, a radiotracer for activated glia, were conducted parallel to amyloid imaging, revealing treatment-induced neuroinflammatory responses, the magnitude of which intimately correlated with the levels of preexisting amyloid estimated by [^{11}C]PIB (Maeda et al 2007). Surprisingly, no signifi-

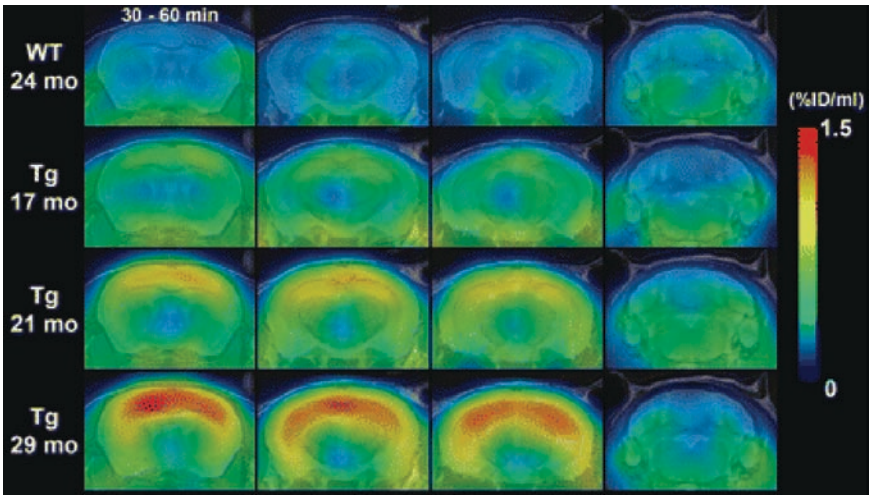

Fig. 5 In vivo detection of amyloid plaques in APP23 mice at different ages. PET images were generated by averaging dynamic scan data at 30–60 min after administration of [^{11}C]PIB and were overlaid on the MRI template. From *left* to *right*, panels represent coronal images at 0, 2, 3, and 7 mm posterior to the bregma. mo, Month. Courtesy of Makoto Higuchi, National Institute of Radiological Sciences, Chiba, Japan. Reproduced with permission from Maeda et al. (2007). © 2007 Society for Neuroscience

cant retention of [^{11}C]PIB was seen in other mouse models, e.g. in presenilin-1/amyloid precursor protein (PS1/APP) (Klunk et al. 2005) and in Tg2576 animals (Toyama et al. 2005) despite an excellent brain uptake of the probe. Possible reasons for this discrepancy could be differences in the secondary structure of Aβ for the transgenic lines.

A NIRF oxazine dye, AOI987, has been demonstrated to readily penetrate the intact BBB and to bind to amyloid plaques (Hintersteiner et al. 2005). Using NIRF imaging, a specific interaction of AOI987 with amyloid plaques was shown in APP23 mice in vivo, and confirmed by postmortem analysis of brain slices. Quantitative analysis revealed increasing fluorescence signal intensity with increasing plaque load of the animals, and significant binding of AOI987 was observed for APP23 transgenic mice aged 9 months and older. Thus, AOI987 is an attractive probe to monitor noninvasively disease progression in animal models of AD and to evaluate the effects of potential drugs on the plaque load.

Final Remarks

Three main roles can be defined for noninvasive mouse imaging in preclinical research (a) phenotyping transgenic animals developed as disease models or for target selection/validation; (b) performing in vivo validation of pharmacological

targets; and (c) testing compounds in murine models of diseases. In all cases, the noninvasive nature of imaging can be exploited to either cope with the high variability in transgenic animal models, or to reduce the biological variability in the pharmacological experiments since the same animal may be used as its own control.

Ideally, an animal imaging laboratory in a pharmaceutical environment should provide access to several techniques. The opportunity to apply a diverse range of imaging techniques to a given problem is a strength. The complementarity of the techniques should be explored to address questions of pharmacological relevance. The choice of technique will be dictated by several factors, including e.g. its spatial and temporal resolution, sensitivity, or availability in clinical diagnosis as well. As a rule, imaging should provide robust and reproducible readouts, and measurements should interfere minimally with the animal's physiology since in most cases repetitive acquisitions are required. Because of the unique challenges encountered in imaging small rodents, not only a thorough understanding of the imaging modality but also of the animal's physiology and the biological/pharmacological question to be addressed is required. Colby and Morenko (2004) have recently addressed several important aspects in small rodent bioimaging, including the choice of appropriate anesthetic regimens, monitoring and supporting the animal's physiologic balance, biosecurity and radiation safety.

A fundamental driving force behind activities involving the use of imaging to characterize animal models of human disease in drug discovery is that the methods may facilitate the translation between preclinical and clinical drug research and development. Once potential biomarkers are identified and validated (qualified), similar study designs can be applied to preclinical and clinical investigations involving a given compound. Moreover, studies in animals can serve as the basis to rationalize experimental findings in humans through the use of analogous biomedical readouts. Helping to bridge the two intimately connected, however in practice often too distant, areas of preclinical and clinical research is a major contribution of imaging in pharmaceutical research.

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Neuroimaging in Understanding Chronic Pain Mechanisms and the Development of New Therapies

Karolina Wartolowska and Irene Tracey

Introduction

Chronic pain is a significant medical problem, seriously affecting the quality of life of patients and creating a financial burden on the health services and the economy. It is estimated that about 20% of adults in Europe suffer from chronic pain, 40% of whom do not receive satisfactory pain relief (Breivik et al. 2006). The pathophysiology of chronic pain conditions is not fully understood, which hinders the development of new mechanism-based analgesic therapies. Unlike acute pain, there is no specific and effective medication to treat chronic pain. New drugs that appear on the market are mostly just refinements of existing drug classes.

Pain is not just a warning symptom informing our body of actual or potential damage to the tissue. It is a complex, unpleasant sensation with sensory, emotional, and cognitive dimensions. In this chapter, we are concerned mostly with chronic pain. Pain is defined as chronic when it accompanies chronic disease or has lasted longer than 3 months, despite resolution of the disease or healing of the injury that caused it. The important characteristic of chronic pain is that it loses its warning function and becomes a disorder in its own right (Loeser and Rolf-Detlef 2008).

Over the last 20 years, brain imaging methods such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), have greatly contributed to our understanding of the perception and modulation of the pain experience (Irene and Mantyh 2007). It is now accepted that the central nervous system plays a crucial role in pain processing and many characteristics of chronic pain are caused by changes within the peripheral and central nervous system (Maihofner et al. 2004; Apkarian et al. 2005). Neuroimaging techniques provide a tool for understanding the mechanisms involved in generating and sustaining chronic pain. Moreover, neuroimaging can potentially be used as an objective and

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reliable method to assess the efficacy of analgesic drugs, and therefore help with the development of new treatment strategies.

The Role of Neuroimaging in Understanding Pain Processing

Most of our current knowledge of pain processing is based on acute pain or sensitization-model studies in healthy volunteers. These studies are easier to conduct for ethical reasons, and are easier to interpret as a standardized stimulus is used in a homogeneous population. From the brain imaging studies on experimental pain in healthy volunteers, we know that acute pain evokes a response in several brain regions including the primary and secondary somatosensory cortices, the insular cortex, the anterior cingulate gyrus, the prefrontal cortex and the thalamus. These structures were consistently reported to be activated by various painful stimuli, in a range of paradigms, in both fMRI and PET studies. Depending on the type of stimulation, pain-related activation was described in the posterior parietal cortex, the brainstem, the basal ganglia, the amygdala and the cerebellum (Irene and Mantyh 2007; Apkarian et al. 2005). It is clear that there is no single "pain center" in the brain, but rather there is an extensive, interconnected network of cortical and subcortical structures involved in the central processing of pain.

Pain is not a straightforward sensory process. Firstly, the pain experience does not linearly depend on the intensity of the nociceptive stimulus. As nociceptive information is transmitted along the sensory pathways it undergoes modulation, including both facilitation and inhibition, within the dorsal horn of the spinal cord. Secondly, pain is a multidimensional, unpleasant conscious experience strongly modulated by external (e.g., contextual) and internal (e.g., psychological, genetic) factors (Keefe et al. 2004; Porro 2003; Seminowicz and Davis 2006; Ochsner et al. 2006). Neuroimaging studies have demonstrated that negative emotions such as depression or anxiety augment the perceived pain intensity (Craig 2005) and increase the pain-related brain activation (Giesecke et al. 2004). How the pain is experienced is also affected by attention (Tracey et al. 2002; Eccleston and Crombez 2005), anticipation (Fairhurst et al. 2007) and pain memories (Gedney and Logan 2004). Perception of pain can change in pathological states such as inflammation or after lesion to the sensory nervous system (Irene and Mantyh 2007; Bingel et al. 2007).

Differences in Brain Activation in Response to Experimental and Clinical Pain

It is not certain whether the same brain regions that are associated with the experimental pain are also involved in pathological chronic pain syndromes (Xavier and Didier 2007).

In recent years, there have been an increasing number of clinical pain studies providing new insights into pathological mechanisms in chronic pain. The results

of the meta-analysis done by Apkarian et al. (2005) suggest that there are differences between pain processing in healthy volunteers versus chronic pain patients. The regions most frequently observed in connection with acute pain in healthy volunteers were the primary and secondary somatosensory cortices, the anterior cingulate cortex, the insular cortex, and the thalamus, whereas in the chronic pain patients activation in these regions was reported less often (activated in 82% of healthy volunteers' studies vs. 42% of patients' studies). The region that was most often reported to be activated in clinical pain studies was the prefrontal cortex (81% in patients vs. 55% in healthy controls) (Apkarian et al. 2005).

The prefrontal cortex is involved in pain control mechanisms, in processing the ascending input from the spinothalamic tract, and it is a source of descending modulation via its connections from the brainstem's descending pain modulatory system (Casey et al. 2003; Lorenz et al. 2003). This region plays an important role in processing of negative emotions, interoception, cognition, and detecting negative outcomes (Dolan 2002; Rushworth et al. 2005). Activation in the medial prefrontal cortex was reported during ongoing lower back pain, and correlated with the intensity of the pain (Baliki et al. 2006). The thalamus is another region affected by chronic pain. Several studies reported a decrease of cerebral blood flow and metabolic rate in the thalamus contralateral to the clinical pain (Di Piero et al. 1991; Hsieh et al. 1995; Pagni and Canavero 1995; Kupers et al. 2000). These changes normalize after successful pain treatment (Di Piero et al. 1991). Apkarian et al. (2005) suggest that, in patients there is a decrease in the sensory aspect of pain processing and an increase in affective and cognitive processing of pain. This may be a result of clinical pain having a stronger emotional value (Price 2000). Another possible explanation is that the ongoing pain leads to more generalized changes, affecting the baseline state and leading to an altered response to evoked pain (Apkarian et al. 2005). This explanation is congruent with a study by Baliki et al. (2008), who reported changes in the default brain activity in chronic pain patients in comparison to healthy controls. This disruption of the default state network may explain the different activation pattern observed in clinical pain studies than in healthy controls, as well as the cognitive and behavioral impairment reported in chronic pain patients (Apkarian et al. 2004b).

As mentioned earlier, pain is not passively transmitted to the brain, but rather the nociceptive inputs is changed and modulated at each level of the pain neuraxis. The inputs from the dorsal horn of the spinal cord are modulated (inhibited or facilitated) by the descending pain modulatory system. This system involves monoaminergic projections from the higher brain regions including the prefrontal cortex, the anterior cingulte cortex, the insular cortex, the amygdala, the hypothalamus, and the brainstem. It has been demonstrated, in human models of pain, that the pain modulatory system is critical for sustaining central sensitisation in menthol (Seifert and Maihoefner 2007) and capsaicin-evoked allodynia (Zambreanu et al. 2005; Iannetti et al. 2005; Caterina et al. 2007). In a study on capsaicin-evoked allodynia and its modulation by gabapentin, the brainstem was the main region where the effect of the drug on sensitization was the strongest (Iannetti et al. 2005). The pain modulatory system is also crucial for sensitisation and maintaining chronic pain in patients (Suzuki et al. 2004). Patients with chronic pain, either neuropathic or

inflammatory, have impaired endogenous control of pain with increased descending facilitation or impaired inhibition (Ren and Dubner 2002; Gebhart 2004).

Differences in Brain Activation in Response to Evoked and Spontaneous Pain in Chronic Pain Patients

In chronic pain patients, there are differences in the brain activation pattern in response to disease-related evoked (i.e., acute) pain in comparison to the ongoing, tonic pain. The evoked disease-related pain results in similar brain activation as the acute pain in healthy controls (Geha et al. 2008; Kulkarni et al. 2007; Baliki et al. 2005). Tonic clinical pain results in different brain activity pattern, than evoked clinical pain; for example dynamic mechanical allodynia in patients with postherpetic neuralgia results in different activation patterns than the ongoing pain (Geha et al. 2007, 2008). Also the evoked pain differs from the ongoing, tonic pain in patients with arthritis (Kulkarni et al. 2007). This observation is very important for treatment development as it highlights the fact that the evoked pain typically used in pain studies, even though it is disease-related, is not the same as the ongoing pain the patients experience during the course of their disease and often describe as their most problematic symptom.

It was demonstrated, in a study on chronic back pain, that ongoing, tonic pain correlates with stronger activation in the regions involved in emotion, cognition and motivational drive, such as, the medial prefrontal cortex, the rostral anterior cingulate cortex, as well as the thalamus and amygdala, whereas the increase of clinical pain correlates with activation in the regions observed during acute pain processing, i.e., the somatosensory cortices and the insula (Baliki et al. 2006). Kulkarni et al. also observed differences between ongoing arthritic pain and evoked pain. Both conditions activated several brain regions, sensory, affective and motivational, but the tonic pain was associated with activation in the brainstem and greater activation in the regions involved in affective processing of pain (Kulkarni et al. 2007). Successful treatment and reduction of tonic pain also correlates with changes in activation in the areas processing emotions and reward.

Towards Mechanism-Based Classification of Chronic Pain

Different types of allodynia result in distinct brain activation patterns, and this is congruent with the results of the psychophysical studies and the hypothesis that different types of allodynia have different pathophysiological mechanisms as has been suggested by a study on syringomyelia by Ducreux et al. (2006). They reported different results of psychophysical tests as well as distinct activation patterns between patients with cold allodynia, and patients with tactile allodynia. The only region that was consistently activated during both types of allodynia in this study

was the prefrontal cortex (Ducreux et al. 2006). Cold allodynia evokes responses in dorsolateral prefrontal cortex and brainstem (regions usually involved in sensitization), in addition to the regions activated in response to the noxious cold (Seifert and Maihoefner 2007). Activation in the prefrontal cortex was also found by Schweinhardt et al. (2006) in a study on neuropathic pain patients as well as in studies on capsaicin-evoked allodynia in healthy volunteers (Lorenz et al. 2002).

Neuroimaging in Primary Headaches

Headaches involve trigeminal rather than spinal nociceptive input and imaging studies' findings are different from the studies on other pain syndromes. Migraine attacks are typically associated with increased activation in the brainstem (Weiller et al. 1995; Bahra et al. 2001), but other brain regions such as the dorsal rostral pons and posterior hypothalamus were also reported to be active during the attacks, and remain active despite successful pain relief by sumatriptan injection (Denuelle et al. 2007). The results of the neuroimaging studies have demonstrated that the hypothalamus plays an important role in primary headaches (Philip and Goadsby 2007). The hypothalamus is involved not only in migraine attacks, but also in triggering attacks of trigeminal autonomic cephalalgias, such as cluster headache (Sprenger et al. 2004) or hemicrania continua (Matharu et al. 2004). Hypothalamus is interconnected with the brainstem nuclei and takes part in the descending inhibition of pain (Aimone et al. 1988) The results of morphometric studies of primary headaches are incongruent. It has been observed that migraine attacks are associated with structural changes within the trigeminal somatosensory system and brainstem but it is not clear whether this is a cause or a result of migraine (Cristina et al. 2006; DaSilva et al. 2007; Welch et al. 2001). The changes observed in periaqueductal gray matter are in line with the hypothesis of impaired pain modulation in primary headaches (Rocca et al. 2006; Welch et al. 2001). Other studies report a decrease in gray matter volume in several pain processing regions, such as the insular cortex and cingulate cortex (Schmidt-Wilcke et al. 2008) as well as the parietal, prefrontal and the orbitofrontal cortices (Kim et al. 2008), but not in the brainstem. The changes in all these regions correlated negatively with the headache duration and frequency of headaches, suggesting that these changes are secondary to frequent attacks of pain (Kim et al. 2008).

Structural Brain Changes as a Result Of Chronic Pain

The results of neuroimaging studies on brain structure or chemistry demonstrate, that chronic pain affects not only the function of the brain, but also leads to long lasting changes. Some of these changes seem to be reversible, when the pain is alleviated (Maihofner et al. 2004). However, the pain related-changes may reflect

neurodegeneration as well as neuronal reorganisation. (Apkarian et al. 2004a; Schmidt-Wilcke et al. 2005; Grachev et al. 2000). The changes were observed in several brain areas and may be an acceleration of age-related brain atrophy. Apkarian and his group demonstrated that in patients suffering from lower back pain, the density of the gray matter decreases in several cortical and subcortical areas, including the prefrontal cortex (Apkarian et al. 2004a). There was also a decrease of the N-acetylaspartate/creatine ratio, a marker of neuronal well-being, in the prefrontal cortex of these patients (Grachev et al. 2002). The reduction of gray matter has been described not only in lower back pain (Neilly et al. 2008; Schmidt-Wilcke et al. 2006), but also in several other chronic pain conditions such as migraine (Schmidt-Wilcke et al. 2008; Rocca et al. 2006), chronic tension headache (Schmidt-Wilcke et al. 2005), irritable bowel syndrome (Davis et al. 2008) and fibromyalgia (Anil et al. 2007; Schmidt-Wilcke et al. 2007). It remains to be determined whether these changes are due to chronic pain conditions itself, the drugs the patients are taking, the lifestyle changes due to disuse or a combination of these factors. Current work attempts to disentangle the causal nature of this degeneration.

Neuroimaging as a Tool in Studies on Analgesia

Neuroimaging can be used to objectively study pain by measuring brain activation. Pain is a highly subjective experience, difficult to assess in an objective way. Currently, in order to measure pain we have to rely on a patient's subjective report using unidimensional rating scales. These scales, although easy to use and accepted in clinical and research settings, are imprecise, relative, context-dependent and vary significantly between and within patients. Moreover, pain scales do not provide information about the underlying pain mechanisms (Chizh et al. 2008). Neuroimaging offers an objective and quantitative method to assess pain by measuring the magnitude of pain-related brain activation (Coghill et al. 2003). This method has its limitations, but it is a step forward from using the patients' report.

Neuroimaging is also promising as a tool to study the pathomechanisms of chronic pain. It is still poorly understood how chronic pain is maintained and why it persists. At the same time treatment depends on mechanisms rather than etiology (Sindrup and Jensen 1999). Understanding the neurophysiological mechanisms of pain would lead to optimisation of therapy, help to better identify patients who will respond to a treatment, and potentially identify new treatment strategies.

There are no good models for chronic pain, as models usually reflect a single mechanisms of pain whereas, in chronic pain conditions there is usually more than one mechanism driving the pain (Klein et al. 2005). Neuroimaging alone or with standardized psychophysical assessment such as quantitative sensory testing, may help to diagnose which mechanism is responsible for pain in a particular patient (Cruccu et al. 2004). For many years it has been known that there is a need for better characterisation and mechanism-based classification of pain (Woolf et al. 1998).

Neuroimaging may be used as a tool to assess the effects of peripherally and centrally acting analgesics. Neuroimaging studies are able to demonstrate effects of drugs on the central nervous system comparable with the behavioral measures. There were several studies published on remifentanil, a rapidly acting opioid receptor agonist and its effect on pain processing. fMRI has proven to be sensitive enough to detect changes in brain activity with increased concentration of the drug, and to demonstrate the correlation between the brain activation, and both drug dose and pain ratings (Wise and Tracey 2006; Wise et al. 2002; Tracey 2001). Functional neuroimaging can also be used to study the pharmacodynamics and pharmacokinetics of analgesic drugs (Wise et al. 2004). Imaging is a tool to better understand the mechanisms of action of approved analgesic drugs; for example, the study on capsaicin-evoked sensitisation model and gabapentin by Iannetti et al. (2005). Finally, neuroimaging is useful for translation and back translation between animal and human studies (Borsook and Becerra 2006).

There are, however, certain limitations in the application of functional imaging methods in clinical studies. First, the disease or medication may affect the neurophysiological process we are measuring to inquire about the brain activity; for example changes in neurovascular coupling or changes in metabolic activity. Therefore, it is important to control these effects while designing drug studies using neuroimaging methods. There are several ways of dealing with these limitations using control tasks to assess global haemodynamic effects, independently measure the baseline physiological state using arterial spin labeling methods, that directly and quantitatively measure regional blood flow changes or measure physiological parameters. Secondly, none of the functional imaging methods have both superior temporal and spatial resolution. Using multi-modal imaging, for example combining fMRI with electroencephalography or with magnetoencephalography, allows us to establish in which order brain regions become active in response to a painful stimulus. Combining fMRI with PET makes it feasible to study neurochemical changes such as decrease in opioid binding that normalizes after reduction of pain (Jones et al. 1994). Recently, it has become possible to collect whole brain data using arterial spin labeling rather than just a single slice. This opens new possibilities; for example, controlling for baseline blood flow in fMRI studies, comparing global changes before and after treatment or studying ongoing, continuous pain as this method allows quantitation of the blood flow and not just relative changes as fMRI.

Conclusions

There is a need for a better understanding of the mechanisms responsible for the generation and maintenance of chronic pain and for the development of new, effective therapies. Neuroimaging is a noninvasive method that allows for study of the neurophysiology of pain processing as well as the pathological changes that occur in chronic pain conditions. In the recent years, brain imaging studies contributed significantly to our understanding of the pathophysiology of chronic pain and the

better characterization of pain syndromes such as neuropathic pain or primary headaches. Brain imaging methods offer a tool to objectively assess pain and provide useful information about mechanisms that drive pain. Neuroimaging may potentially improve mechanism-based classification of pain and lead to better diagnostic accuracy and identification of patients that would respond to treatment.

From neuroimaging studies, we know that tonic, ongoing pain is processed differently from evoked pain, even if the evoked pain is clinically-relevant, which is important in drug development. Neuroimaging results made us realize that chronic pain, if left untreated, may actually have a damaging effect on the brain; disrupting its function and leading to structural changes.

Brain imaging techniques may be useful as a quantitative measure of pain, making it possible to assess pain without the need to rely on subjective ratings. Neuroimaging may improve drug development by making the evaluation of treatment efficacy easier and more objective, and by identifying new therapeutic targets.

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Neuroimaging Human Drug Addiction

P.A. Woicik, N. Alia-Klein, N.D. Volkow, and R.Z. Goldstein

Introduction

In this chapter, we explore the addiction syndrome by examining its emotional and cognitive-behavioral components while focusing on brain areas and circuits that subserve them. We begin with a brief description of neurobiological theories of addiction and then attempt to weave them into a unified brain-behavior theoretical approach. Our discussion focuses on the use of multimodal imaging, including positron emission tomography (PET), magnetic resonance imaging (MRI), and event related potentials (ERPs). PET has been used to quantify regional brain function such as glucose metabolism (with ^{18}F-fluorodeoxyglucose, FDG) and cerebral blood flow (CBF with oxygen-15) in drug-addicted individuals. The imaging of dopamine (DA) in vivo has also been accomplished by PET specifically through the use of radioactively labeled radiotracers such as [^{11}C]-raclopride and ^{18}F-desmethoxyfallypride (for imaging DA D2/D3 receptors), and [^{11}C]-cocaine, [^{11}C]-altropane, or I^{123}I]β-CIT (for imaging the DA transporters, DAT) which have proved invaluable for the assessment of stimulant drugs and nicotine (Brody et al. 2004; Brody et al. 2002; Montgomery et al. 2007; Barrett et al. 2004a; b). Functional magnetic resonance imaging (fMRI) has been especially well suited for the study of drug addiction phenomena as many effects tend to be of short duration, making this method ideal for distinguishing smaller cortical regions (Gatley et al. 2005) and tracking symptoms related to specific neurotransmitter dysregulation in drug addiction (Knutson and Gibbs 2007).

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Defining Drug Addiction

Characteristic Features of Drug Addiction

Although drug abuse often leads to drug addiction, it is not necessarily a precursor for the disorder. For example, NIDA reports that while over 50% of adolescents experiment with drugs, only 2–15% (depending on the drug) become addicted (American Psychiatric Association 2000; Anthony et al. 1994). Although the addictive potential of drugs may partly explain, in part, why some drug users are more susceptible to addiction, the interaction of several factors, including genetic, developmental, and environmental factors, predispose individuals to addiction. Of relevance here is recent neuroimaging research (PET) demonstrating the existence of a protective factor for drug addiction that relates to DA function (Chang and Haning 2006; De Wit and Wise 1977; Maldonado 1997; Maldonado et al. 1997; Volkow et al. 1999a; b; Volkow et al. 2002a). For instance, in non-alcoholic members of alcoholic families we found higher than normal DA D2 receptor availability in caudate and ventral striatum (Fig. 1) (Volkow et al. 2006a). These data suggest that greater DA D2 receptor availability may protect an otherwise vulnerable population from the onset of alcohol dependence.

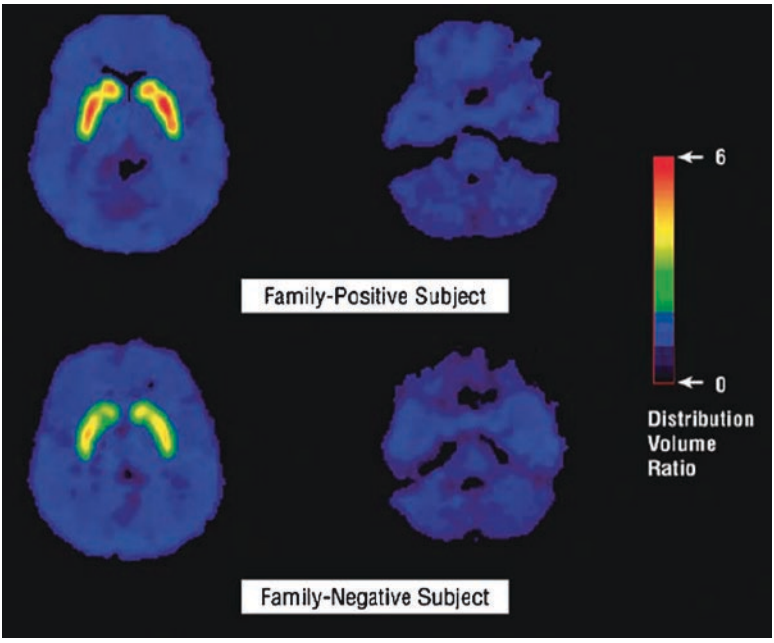

Fig. 1 Images for the distribution volume ratios of carbon 11 [^{11}C]-labeled raclopride showing higher dopamine D2 receptor availability in a family-positive than in a family-negative subject. Data from Volkow et al. 2006 (*Arch. Gen. Psychiatry*)

All drugs of abuse can be classified according to their psychoactive effects on the central nervous system (CNS), affecting behavior, cognition, and neurobiology. These substances are reinforcing, as they increase the probability of a response (i.e., drug consumption) by either increasing pleasure/reward (positive reinforcement) or decreasing displeasure or pain (negative reinforcement). With repeated administration they trigger conditioning progressing those who are susceptible to the disorder from drug abuse to drug addiction. Drug addiction can be characterized by a recurrent pattern of behaviors that include drug craving, intoxication, bingeing, and withdrawal with the cycle culminating in a preoccupation with obtaining, consuming, and recovering from the drug. Indeed, the cardinal symptom of drug dependence is the drive toward drug consumption despite marked reductions in the "high" from the drug and despite adverse personal, social, and occupational consequences. What remains a source of ongoing debate is whether the cognitive and emotional deficits that underlie these behaviors predate the onset of the disorder, or are result from chronic drug use. Current data suggest (Mackler and Eberwine 1991; Nestler 2000; Nestler 2001; Volkow et al. 2002b) that both predisposing factors (e.g., genetic markers, stressful environments) and chronic drug use contribute to addiction.

Neurobiological Models of Drug Addiction

Neurobiological models of drug addiction have traditionally focused on drug reinforcement and neuropsychological changes that occur with repeated drug use. For example, the opponent-process model suggests a shift in the type of reinforcement from a drug (from positive to negative reinforcement) as its administration becomes more frequent (Solomon and Corbit 1974). Other theories have focused on how chronic drug use induces alterations to endogenous processes that regulate reinforcement and stress reactivity (allostasis), resulting in the dysfunctional reward processing and compulsive behavior (Koob and Le Moal 2001). Others emphasize the importance of the brain circuitry underlying incentive motivation and reward (Robinson and Berridge 1993; Robinson and Berridge 2001) and similarly suggest that these regions become altered with chronic drug use, making the brain circuit "hypersensitive" (over-responsive) to the reinforcement provided by the drug (and drug cues). The hypersensitivity of this circuit is believed to increase the "wanting" of the drug without affecting the euphoric effects or "liking" of the drug (Goldstein et al in press; Robinson and Berridge 2001). This alteration may therefore make drug addicted individuals more susceptible to compulsive drug use and relapse.

These theories have been invaluable in guiding research on drug addiction. However, a remaining challenge is the development of a model that captures the neurobiology underlying the chronic nature of addiction with a putative description of why those affected by the disease fail to control disadvantageous drug use behavior

when faced with catastrophic consequences (Robinson and Berridge 2003; Shalev et al. 2002; Stewart and Wise 1992).

Neural Circuitry Underlying Drug Addiction

The characteristic behaviors associated with drug addiction result in part from a complex web of overlapping neural circuits comprised of multiple brain regions. The interaction among three main neural circuits, the mesolimbic, mesocortical, and nigrostriatal circuits, is believed to underlie the phenomenology of drug addiction. At the center of the mesolimbic system or the "reward pathway" are DA fibers, which originate in the ventral tegmentum area (VTA) and terminate in the ventral striatum including the nucleus accumbens (NAc), the amygdala, and the hippocampus (Everitt et al. 2001). This ancient circuit is essential for learning and survival and was first discovered in animals that were found to press a lever for electrical stimulation to the NAc.

The response of the DA mesolimbic circuit to drugs of abuse is considered crucial for drug reinforcement (particularly NAc), drug related memory (hippocampus), and conditioning (amygdala) (Koob and Bloom 1988). It has also been linked to drug craving, and the emotional and motivational changes observed in drug abusers during withdrawal. Because the reward circuit is also activated by drugs in nonaddicted individuals, it cannot fully explain the progression to drug addiction. Thus, addiction cannot be fully explained by reward processes and evidently includes neuroadaptive changes that result in conditioned responses.

The nigrostriatal circuit originates in the substantia nigra and terminates in the striatum. Recent studies have suggested a strong role for dopaminergic transmission in this pathway in regulating habit formation (Faure et al. 2005) as well as sustaining various cognitive and motor processes that include executive control mechanisms. This pathway has been implicated in chronic drug use (White 1996) specifically when drug use shifts from voluntary use (involving prefrontal cortical control) to habitual use (involving striatal control). Underlying this shift may also be a transition at the neural level from ventral to dorsal areas of the striatum (Everitt et al. 2008; Ito et al. 2002).

The mesocortical circuit encompasses the anterior cingulate gyrus (ACG), and orbitofrontal cortex (OFC), and prefrontal cortex (PFC) regions. These regions interact to regulate higher order, supervisory, cognitive-behavioral functions such as the attribution of emotional salience to stimuli in the environment and the adaptive control of behavior. The circuit is also likely to include the insula, which is involved in the awareness of internal sensations from the body such as drug craving. Dysfunction of this circuit is associated with specific aspects of drug addiction such as compulsive drug administration and poor inhibitory control (Volkow and Fowler 2000). Stimulation of these circuits by a drug can occur directly by triggering DA action or indirectly, by modulating other neurotransmitters. In turn, activation of these prefrontal regions has been shown to trigger DA cell firing in anticipation of receiving drug rewards (You et al. 2007).

The Impaired Response Inhibition and Salience Attribution (I-RISA) Model of Addiction

The I-RISA model of addiction highlights the role of the PFC as underlying the core neuropsychological (cognitive, emotional, and behavioral) impairments in drug addiction. Specifically, the model maps neurochemistry and functional neuroanatomy onto four distinct clusters of behavior associated with drug addiction (intoxication, craving, bingeing, and withdrawal) with a focus on the compromised supervisory functions of the frontal cortex (Goldstein and Volkow 2002) (Fig. 2). These cortical regions are activated during intoxication, craving, and bingeing and deactivated during withdrawal. They are also involved in

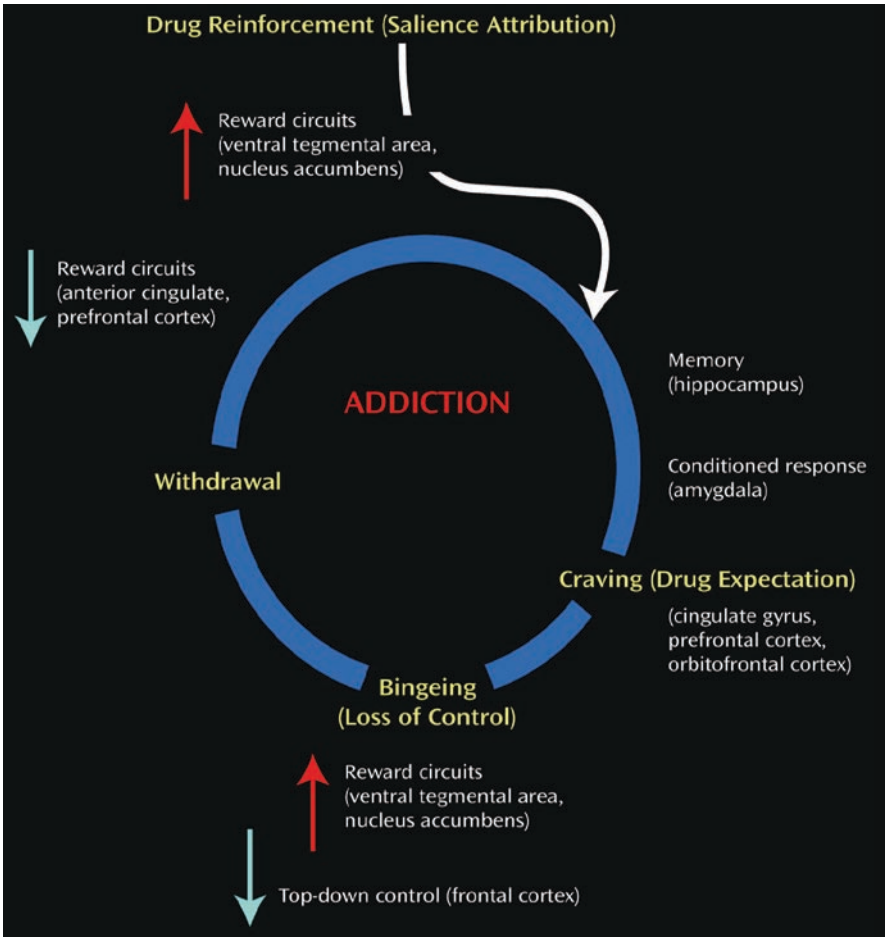

Fig. 2 The impaired response inhibition and salience attribution (I-RISA) model of drug addiction: An integrative model of brain and behavior. From Goldstein et al. 2002 (*Am J Psychiatry*)

higher order cognitive and motivational functions that are uniquely associated with the behavioral clusters; *salience attribution* with intoxication, *expectancy* with craving, and *inhibitory control* with bingeing and withdrawal. The interplay among the neural circuits described above is hypothesized to contribute to the shift from self-willed behavior to drug-driven behavior and highlights the tendency for drug addicted individuals to simultaneously attribute extreme value (salience) toward the drug at the expense of other potentially rewarding alternatives. Ultimately, these processes promote an inability to inhibit maladaptive drug use (impaired response inhibition). For example, consumption of a drug activates cortical circuits (the OFC and ACG) in proportion to the DA stimulation in mesolimbic regions by favoring a target response (salience or importance attributed to the drug) and decreasing nontarget-related background activity (Kiyatkin and Rebec 1996).

Core Features of Human Drug Addiction

Drug Intoxication

The Role of Dopamine

Intoxication occurs when an individual consumes a drug dose large enough to produce significant behavioral, physiological, or cognitive impairments. Neuroimaging studies assessing the effects of acute drug administration have traditionally relied on single drug exposure. This process of short-term drug administration to induce a "high" or "rush" has been traditionally associated with increases in extracellular DA in limbic brain regions, particularly the NAc; however, there is also evidence of increased DA concentrations in frontal regions. Stimulant drugs such as cocaine and methylphenidate (MPH) increase DA by blocking dopamine transporters (DAT), the main mechanism for recycling DA back into the nerve terminals. The "high" associated with stimulant intoxication is positively related to the level of DAT blockade (Volkow et al. 1997a) and drug-induced increases in DA (Volkow et al. 1999c; Volkow et al. 1999d). In fact, DA enhancing effects are directly associated with the reinforcing effects of cocaine, MPH, and amphetamine (Laruelle et al. 1995; Volkow et al. 2002a). This enhancement is different from natural reinforcers for which increases in DA appear to occur mostly for the prediction of reward rather than to the reward itself (Koob and Bloom 1988; Pontieri et al. 1996; Pontieri et al. 1998; Schultz et al. 2000). These studies suggest that along with mesolimbic DA, subcortical brain areas, PFC regions are involved in the intoxication process and their response to drugs is in part related to previous drug experiences. Other factors that affect the extent of the "high" from a drug are the rate of drug delivery and clearance to and from the brain (Volkow et al. 1997a). It is important to note that the magnitude of the increase in DA is reduced with the progression from drug abuse to drug dependence (Volkow et al. 2002a).

PET studies have revealed that drug intoxication is also generally associated with reduced glucose utilization throughout the brain including the frontal cortex (London et al. 1990a; b; Volkow et al. 1990). However, there are some exceptions. For example, acute administration of MPH, a stimulant drug that shares the same mechanism of action as cocaine, has been found to increase levels of glucose metabolism in the PFC, OFC, and striatum, in active cocaine abusers with low D2 receptor availability (Ritz et al. 1987; Volkow et al. 1999a). Studies utilizing CBF and blood oxygenation level dependent (BOLD) methods generally have shown activations during drug intoxication (Adams et al. 1998; Ingvar et al. 1998; Mathew et al. 1992; Nakamura et al. 2000; Tiihonen et al. 1994; Volkow et al. 1988a) with exceptions for cocaine intoxication?? which is found to lower CBF throughout the brain, including the frontal cortex (an effect considered to result from cocaine's vasoconstricting effects) (Wallace et al. 1996). fMRI studies indicate BOLD activations in the frontal cortex (as measured by self-reported "high") across several drugs (Breiter et al. 1997; Kufahl et al. 2005; Stein et al. 1998;). Some studies have linked the pleasurable experience during drug intoxication with subcortical striatal function after acute cocaine and alcohol administration (Breiter et al. 1997; Gilman et al. 2008). Including one recent study demonstrating a relationship between alcohol-induced striatal activations and self-reported anxiolytic effects from alcohol (Gilman et al. 2008).

The above studies implicate a role of DA in PFC and striatal function that is specifically associated with drug use experience. However, the existence of a primary DA dysfunction and where it might originate (either from striatal DA mechanisms or PFC regions, or their interplay) are still in question.

Drug Craving

The pharmacological effects of a drug are modulated by non-pharmacological contextual factors (e.g., places, people, or paraphernalia associated with drug intake). As these factors are consistently paired with the pharmacological effects of the drug they are integrated into the pleasurable, intense experience associated with drug use, becoming "motivational magnets" or "drug cues" through Pavlovian conditioning (Berridge 2007; Berridge et al. 2008). This conditioning shapes an individual's expectations of the effects of a drug and, in turn, modifies the neural and behavioral responses to the drug so that when drug cues are encountered, an urgent desire to consume the drug develops. For this reason, drug cues are central to addiction providing a potent context for a craving state, particularly when excessive salience attribution (a value representation assigned to a stimulus) is given to drugs and drug cues. Therefore, acute drug administration on its own, is not necessary for brain activation in individuals with prior exposure to drugs. With repeated drug intoxication, attention, learning, and memory circuits set the expectations for and prepare the drug addicted individual for the drug's acute effects (e.g., Johanson et al. 2006).

In laboratory settings, a craving state is usually achieved by exposing subjects to images depicting drug-related stimuli. For example, we found that a cocaine cue video elicited a significant increase in the release of DA in the dorsal striatum in cocaine-addicted individuals. This increase in DA was positively associated with self-reported drug craving with the largest effect occurring in the most severely addicted subjects (Volkow et al. 2006b). Recently, we demonstrated that in cocaine abusers increases in striatal DA via oral MPH (which similarly to cocaine increases DA by blocking DA transporters but possessing a longer half-life) induced craving only when subjects were exposed to cocaine cues (as compared to a condition where cocaine cues were absent). These findings suggest that DA increases to cocaine cues reflect fast stimulation of DA cells and highlight the context dependency of MPH's effects.

Other brain imaging studies (indexed by CBF, glucose metabolism, or BOLD) of drug craving conducted on several drug-addicted samples have shown that drug cues elicit craving and this process is associated with activations in the perigenual and ventral ACG (Brody et al. 2002; Brody et al. 2004; Childress et al. 1999; Daglish et al. 2003; Grusser et al. 2004; Kilts et al. 2001; Maas et al. 1998; McClernon et al. 2005; Myrick et al. 2004; Tapert et al. 2003; Tapert et al. 2004; Wexler et al. 2001; Wilson et al. 2005), medial PFC, (Grusser et al. 2004; Heinz et al. 2004; Tapert et al. 2004; Wilson et al. 2005), OFC (Bonson et al. 2002; Brody et al. 2002; Daglish et al. 2003; Grant et al. 1996; Maas et al. 1998; Myrick et al. 2004; Sell et al. 2000; Tapert et al. 2003; Tapert et al. 2004; Wrase et al. 2002) bilateral insula (Brody et al. 2002; Daglish et al. 2003; Kilts et al. 2001; Sell et al. 2000; Tapert et al. 2004; Wang et al. 1999), and ventral tegmental area (Due et al. 2002; Sell et al. 1999; Smolka et al. 2006). Brain regions that are involved with memory processing and retrieval are also activated during craving, including the amygdala (Bonson et al. 2002; Childress et al. 1999; Due et al. 2002; Grant et al. 1996; Kilts et al. 2001; Schneider et al. 2001), hippocampus, and brainstem (Daglish et al. 2003). Of note is evidence showing that these effects are observed even when controlling for the effects of pharmacological withdrawal (Franklin et al. 2007).

In general, findings from craving studies suggest that mesocortical, PFC, OFC, and ACG activation in drug abusers is biased toward the processing of drug cues and expectation plays a significant role in this process. Such evidence in part explains the difficulty for drug abusers to focus on other reward cues in the environment.

Compulsive Drug Administration (Bingeing)

Bingeing refers to a discrete period of time when an individual experiences an extreme loss of control and engages in repeated consumption of the substance. Bingeing episodes are sometimes referred to as "runs" where the drug is consumed for longer periods or in greater amounts than intended often at the expense of survival behaviors such as eating, sleeping, and maintaining physical safety.

These periods usually discontinue when the individual is severely exhausted and/or unable to procure any more of the drug.

Animal models of addiction have provided important clues about the underlying neurobiology of bingeing behavior (Deroche-Gamonet et al. 2004; Vanderschuren and Everitt 2004) showing that these behaviors involve DA, serotonergic, and glutamatergic circuits (Cornish et al. 1999; Loh and Roberts 1990). In humans, the thalamo-OFC circuit and the anterior cingulate cortex (ACC) are probably involved. Indeed, there is evidence showing that these regions, particularly the OFC, are critical in other disorders involving compulsive behaviors such as obsessive-compulsive disorder (Chamberlain et al. 2008; Yoo et al. 2008; Zald and Kim 1996) (see also reviews Menzies et al. 2007; Rotge et al. 2008).

It is difficult to test compulsive drug self-administration in humans, as it requires an examination of unrestricted drug use. However, clever laboratory designs have overcome some of the practical constraints encountered when studying bingeing in humans. For example, in a recent fMRI study, non-treatment-seeking cocaine-dependent subjects were permitted to choose when and how often they would self-administer intravenous cocaine within a supervised one-hour session. Repeated self-administered-induced-high was negatively correlated with activity in limbic, paralimbic, and mesocortical regions including the OFC and ACC. Craving, on the other hand, was positively correlated with activity in these regions (Risinger et al. 2005) (also see (Foltin et al. 2003). Simulating compulsive drug self-administration vis-à-vis paradigms of other compulsive behavior (such as gambling when it is clearly no longer beneficial), may offer invaluable insight into the circuits underlying loss of control in addictive disorders.

Drug Withdrawal and Relapse

Drug withdrawal refers to a variety of symptoms including fatigue, irritability, anxiety, and anhedonia that appear when a drug that causes physical dependence is suddenly terminated (Gawin and Kleber 1986). These symptoms can vary depending on the type of drug and the length of abstinence from last drug use and are often distinguished as “early” vs. “protracted” withdrawal symptoms.

In general, PET studies of drug-addicted individuals suggest durable drug-related adjustments (mostly reduced sensitivity) in regional neural responsiveness during withdrawal. We demonstrated that the relative CBF values for the PFC and the left lateral frontal cortex were significantly lower in regular cocaine users during withdrawal (10 days) from cocaine than in healthy controls (Volkow et al. 1988b). CBF has also been assessed via MR dynamic susceptibility contrast after overnight withdrawal from nicotine, as well as after nicotine replacement. Results of this analysis showed a reduction in thalamic CBF during withdrawal but increased CBF in the ventral striatum with nicotine replacement (Tanabe et al. 2008). Studies of glucose metabolism have shown similar deficits during drug withdrawal with lower metabolic activity in these regions during protracted withdrawal (1–6 weeks since last

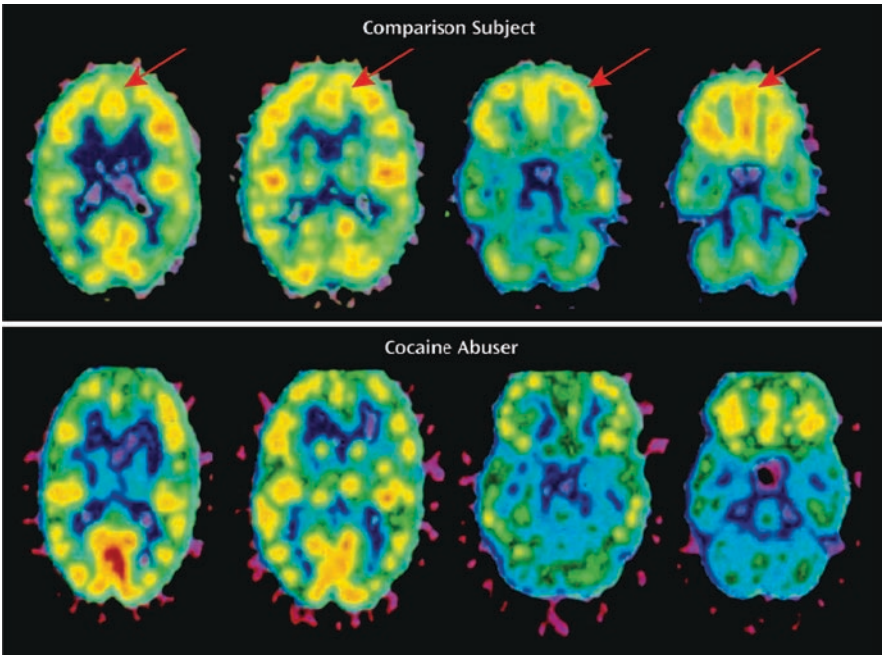

Fig. 3 Lower relative glucose metabolism in the prefrontal cortex and anterior cingulate gyrus of a cocaine abuser during protracted withdrawal (1–6 weeks) than in a normal comparison subject. Data from Volkow et al. 1992 (*Synapse*)

use), especially in the PFC (Volkow et al. 1992a) as compared to control subjects (Fig. 3), but greater in some regions (e.g., the OFC, basal ganglia) during early withdrawal (within one week of abstinence) (Volkow et al. 1991). This latter effect has been linked with subjective craving in cocaine abusers. These effects are consistent with recent findings showing pronounced neuropsychological deficits (e.g. verbal memory, executive function, and attention) in cocaine addicted individuals during protracted withdrawal as compared to those in early withdrawal (Woicik et al. 2009). Metabolic activity during alcohol withdrawal is lower throughout the striatal-thalamo-OFC circuit during early detoxification but predominantly lower in the OFC during protracted alcohol withdrawal (Volkow et al. 1992b; Volkow et al. 1993; Volkow et al. 1994; Volkow et al. 1997b). Other studies have reported similar results (Catafau et al. 1999; Volkow et al. 1997c).

Some studies exploring the neurotransmission underlying withdrawal suggest serotonergic involvement in these abnormalities showing less sensitivity in the striatal-thalamo-OFC circuit to a serotonin agonist (*m*-chlorophenylpiperazine) in alcoholic subjects (Hommer et al. 1997). Others highlight the role of DA during withdrawal observing that striatal DA response and/or receptor availability is significantly lower in drug (cocaine) abusers during early and protracted withdrawal. Lower striatal DAD2 receptor binding during withdrawal has also been found in heroin (Wang et al. 1997), methamphetamine (Volkow et al. 2001), alcohol (Volkow et al. 1996), and nicotine dependent individuals (Fehr et al. 2008). This effect was

associated with lower metabolism in the OFC and ACC in cocaine addicted subjects and alcoholics and exclusively in the OFC in methamphetamine subjects (Volkow et al. 2007a; Volkow et al. 2009). In addition, lower availability of D2-like receptors in the ventral striatum has been positively associated with alcohol craving and cue-induced activation of the medial PFC and ACC (as assessed with fMRI) in detoxified alcoholics (Heinz et al. 2004).

Dysphoria

Dysphoria is a core symptom of drug withdrawal that is characterized by a persistent inability to derive pleasure from common non-drug related rewards (e.g., food, personal relationships). This anhedonic state might possibly reflect an adaptive response to repeated DA enhancement by drugs of abuse in the reward circuit rendering the reward system less sensitive to natural reinforcers (Barr and Phillips 1999; Barr et al. 1999; Cassens et al. 1981). This adaptive DA-induced response may compromise the function of the PFC, OFC, and ACC in drug-addicted individuals promoting deficits that appear similar to that of non-drug-addicted depressed patients. Indeed, abnormalities in the dorsolateral, ventrolateral, and medial aspects of the PFC including ACC and OFC have been found in studies of clinically (non-drug-addicted) depressed patients (Elliott et al. 1998; Mayberg et al. 1999) that indicate these regions as compromised during cognitive (e.g., planning tasks) and pharmacological challenges. It is therefore possible that drug induced alterations to the function of the PFC, ACC, and OFC may impair the ability to regulate emotions (Payer et al. 2008). This is significant because emotional regulation is important for coping with stress, a factor that is a strong predictor of relapse (Goeders 2003) and possibly anhedonia (Greicius et al. 2007). Therefore, more research in this area should prove valuable, given that these as well as striatal and insula regions are associated to relapse outcomes (see review by Sinha and Li 2007).

Neurocognitive Mechanisms

Salience Attribution

Salience attribution, a major component of I-RISA, is an emotive/cognitive factor that is at the root of reward/punishment processing (O'Doherty 2004). It is considered a value representation assigned to a stimulus that is used to help guide behavior (i.e., seek out reward/avoid punishment). Results from animal and human studies have demonstrated that both subcortical (including the ventral striatum/NAc, thalamus, and cerebellum) and cortical regions (PFC including the OFC and ACG, and the inferior frontal gyrus) are involved in processing salient stimuli (Elliott et al. 2000; Francis et al. 1999; George et al. 1993; Goldstein et al. 2006; Lane et al. 1997; Pardo et al. 1993; Rainville et al. 1997; Sprengelmeyer et al. 1998). Neuroimaging

studies of drug addiction suggest that drug-induced increases in DA, which are associated with the perception of pleasure from a drug, are initially responsible for flagging the drug as highly salient. This event innately motivates drug seeking behavior regardless of whether the individual consciously recognizes the drug as being pleasurable (Volkow et al. 2008). In addition, the motivation to procure drugs overrides the motivational value of nondrug rewards (Goldstein and Volkow 2002).

In a recent fMRI study we examined salience attribution in cocaine addicted individuals (vs. healthy controls) using monetary reward for performance on a sustained attention task (Goldstein et al. 2007). Our results suggest that cocaine addicted subjects may have compromised PFC sensitivity (encompassing the OFC, dorsolateral PFC, and ACC) to monetary reward. Moreover, we found that sensitivity to money in the dorsolateral PFC and ACC was positively correlated with self-reported motivation in healthy control subjects but not in cocaine abusers. In another fMRI study, alcoholic subjects exhibited reduced activation in the ventral striatum to monetary reward but increased activation in this area to alcohol cues. The increase in striatal activation to alcohol cues was positively associated with subjective craving for alcohol (Wrase et al. 2007). Together these findings suggest that drug addicted individuals exhibit low sensitivity and motivation toward non-drug reinforcers and that mesocorticolimbic activation is biased towards the processing of more immediate drug related cues or the drug itself. Indeed, other studies have highlighted the role of the PFC in regulating the value of rewards in drug addiction by modulating the magnitude of DA increases in the ventral striatum (Volkow et al. 2007a) possibly via DA 1 receptors (Brenhouse et al. 2008; Rebec and Sun 2005; Sun and Rebec 2005; Sun and Rebec 2006).

Impaired Response Inhibition

Another integral component of I-RISA is the compromised ability to control behavior. Response inhibition refers to the ability to suppress response to prepotent or competing stimuli (Garavan and Hester 2007). It involves several cognitive components (e.g., attention, memory, behavioral monitoring (including error detection)) and is typically assessed in humans with objective measures (e.g., stop signal reaction time tasks, go/no-go tasks, and Stroop interference tasks) that probe these processes. Frontal mechanisms play a significant role in response inhibition as they ultimately control prepotent responses presumably so that slower decision making can influence behavior (Friedman and Miyake 2004).

Drug addicted individuals exhibit impairment in response inhibition especially during periods of bingeing and relapse but the relationship between drug dependence and impaired response inhibition is currently considered bidirectional (i.e., studies indicate that chronic drug use can impair response inhibition (Roesch et al. 2007; Simon et al. 2007), but impulsivity may also predispose to drug addiction (Dom et al. 2006a; b; Everitt et al. 2008; Ivanov et al. 2008; Tarter et al. 2003; Zilberman et al. 2007).

Neuroimaging studies of drug addiction have linked impulsive responding with alterations (hypoactivations) in the PFC (including the OFC, ACG) in current,

abstinent, and recovering drug abusers (Bolla et al. 2004; Eldreth et al. 2004; Forman et al. 2004; Fu et al. 2008; Gruber and Yurgelun-Todd 2005; Hester and Garavan 2004; London et al. 2004; Kaufman et al. 2003) (also see reviews Aron and Paulus 2007; Garavan and Hester 2007). Specifically, the OFC response represents the effort exerted to inhibit the response whereas the ACG response reflects error detection and response conflict. These prefrontal reductions (with the exception of the ACG) during inhibition are best observed when tasks demand a significant working memory load (Hester et al. 2004). In addition to frontal mechanisms, the insula and cerebellum may also have a role in response inhibition; hypoactivations in the insula have been found in stimulant abusers (see reviews by Aron and Paulus 2007; Garavan and Hester 2007) and an over-reliance on the left cerebellum has been found in alcohol addicted subjects (Hester and Garavan 2004). A more recent trend in this line of research has been to classify the neural mechanisms associated with various components of response inhibition by examining the role of reflective impulsivity (Clark et al. 2006; Clark et al. 2009; Ersche et al. 2008) vs. motor impulsivity (Everitt et al. 2008; Passetti et al. 2008; Stoffel and Cunningham 2008) vs. "waiting impulsivity" (i.e., the inability to tolerate delays of reinforcement) (Belin et al. 2008) to account for individual differences within this broad cognitive-behavioral construct.

Similar to state measures, trait measures of inhibitory control (e.g., impulsivity, harm avoidance (low), self control (low)) are strongly linked to drug addiction particularly stimulant addiction (Conrod et al. 2000; Goldstein et al. 2002; Goldstein et al. 2007). Drug addicted individuals with low inhibitory control typically meet criteria for other psychopathology that are characterized by poor impulse control (e.g., antisocial personality disorder, Conrod et al. 2000, and/or attention deficit disorder, Kollins 2008a; Kollins 2008b). What remain unclear are the neural mechanisms that underlie trait impulsivity as it relates to drug addiction. Dalley and colleagues created an animal model of trait impulsivity to investigate the predictive relationship between D2 receptor availability in the ventral striatum (an individual difference factor predictive of cocaine self administration and "high" from cocaine) and trait impulsivity. Lower D2 receptor levels in the ventral striatum correlated with higher cocaine administration in trait impulsive vs. non-impulsive rats (Dalley et al. 2007). This finding suggests a predisposing neurobiological marker (D2 receptor level in the ventral striatum) for drug addicted individuals. In human stimulant abusers, we have observed that lower glucose metabolism in the DA-innervated OFC correlates with self reported approach tendency (low harm avoidance) (Goldstein et al. 2002). These findings corroborate results from a previous study, where higher relative OFC glucose metabolism at rest correlated with better behavioral control in drug-addicted subjects (Goldstein et al. 2001). We also found reduced inhibitory control (as measured by the self control scale of the Multidimensional Personality Questionnaire) in cocaine abusers to be associated with less BOLD activation in the lateral PFC in response to monetary reward. Interestingly, we did not find this relationship in healthy control subjects (instead, the PFC activation to monetary reward was exclusively and positively associated with the level of activation of the OFC in control subjects) (Goldstein et al. 2007) (Fig. 4). Together these results suggest that prefrontal impairment, particularly in OFC, underlies trait impulsivity.

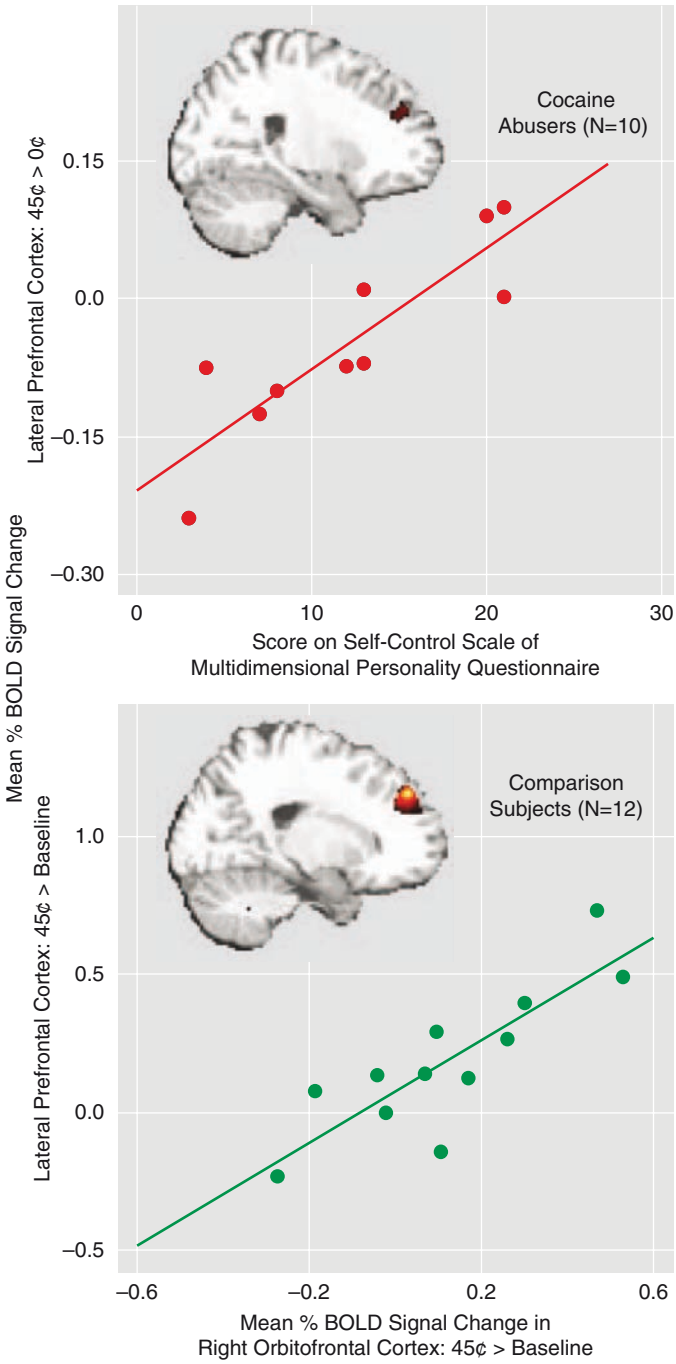

Fig. 4 Upper panel = Correlation between the lateral prefrontal cortex and inhibitory control in 16 cocaine abusers; Lower panel = Correlation between the lateral prefrontal cortex and orbitolfrontal cortex in 12 comparison subjects. Data from Goldstein et al. 2007 (Am J Psychiatry)

Such evidence is supported by recent data indicating that, at the molecular level, chronic stimulant use induces the transcription of Delta FosB in the OFC and that this process plays a role in the disruption of behavioral control (Winstanley et al. 2008). Moreover, in non-alcoholic subjects with high familial risk for alcoholism, increases in D2 receptors were associated with enhanced metabolism in OFC and cingulate gyrus, which we postulated may have been the mechanisms that protected them against alcohol abuse (Volkow et al. 2006a).

Neuroimaging studies show that deficits in inhibitory control may continue for months into abstinence (Fu et al. 2008; Tapert et al. 2007); however there is recent evidence indicating improvement in the ability to inhibit behavior appropriately when environmental cues are explicit or when the timing of cues are varied (i.e., advanced warning) (Leland et al. 2008). Such evidence is promising and supports treatment approaches that emphasize resistance to immediate drug use urges (Leland et al. 2008).

Deficits in Decision Making

Decision making is represented by a conflict between potential rewards and potential risks within which individuals must appraise the status of their needs. Drug addicted individuals tend to make disadvantageous decision making (Bartzokis et al. 2000; Bechara and Damasio 2002; Bechara et al. 2002; Clark et al. 2006; Grant et al. 2000; Mazas et al. 2000; Paulus et al. 2005; Paulus 2007; Paulus et al. 2008; Petry et al. 1998), with the severity of this deficit varying as a function of the type of drug abused (Bechara and Martin 2004), and the length of abstinence from that drug (Bartzokis et al. 2000; Paulus et al. 2002); see also (Bolla et al. 1999). A widely used paradigm for assessing decision making processes is the delayed discounting task in which subjects are provided with two options: small immediate rewards that are associated with long-term gains and better decision making ability, or large immediate rewards that are associated with long-term losses and poor decision making (e.g., Bechara et al. 1997; Iowa Gambling Task Bechara et al. 2002). Studies utilizing these tasks show that drug addicted individuals fail to consider future outcomes and therefore discount delayed reward (Coffey et al. 2003; Petry 2003; Petry et al. 1998; Petry and Casarella 1999; Madden et al. 1999;). This decision making deficit is associated with frontal (OFC, ACC, insula, and PFC) as well as subcortical regions (cerebellum) (Bolla et al. 2003; Bolla et al. 2005; Ersche et al. 2006; Monterosso et al. 2007; Paulus et al. 2002; Paulus et al. 2005) (see also reviews by Aron and Paulus 2007; Garavan and Hester 2007; Paulus 2007). For example, poor decision making is associated with activations in the OFC, the dorsolateral PFC, ACC, and parietal cortex in methamphetamine addicted individuals (Paulus et al. 2002). Cocaine abusers also show greater activations in the right OFC; however, unlike methamphetamine-addicted subjects, they show lower activation of the right dorsolateral and left medial PFC (possibly reflecting response to expectation of reward or planning) (Bolla et al. 2003). Activation of the insular cortex has also

been associated with decision-making deficits in stimulant abusers. This activation was correlated with the number of errors made during the task and therefore may suggest the inability of stimulant abusers to switch behavior between winning and losing on the basis of the extent of error evaluation (Paulus et al. 2008). In general, hyper/hypoactivations in these regions vary with abstinence from the drug (Bolla et al. 2003; Bolla et al. 2005; Paulus et al. 2002) (see also reviews by Aron and Paulus 2007 Garavan and Hester 2007). Finally, the possibility that decision making impairments result from dysfunctional dopaminergic function is probable, given that DA function is an integral component for distinguishing the mismatch between what is expected, and what is observed.

We recently showed that a subgroup of cocaine addicted subjects cannot distinguish between gradients of reward and thus equating moderate and low reward with high rewards (Goldstein et al. 2008). Such a deficit is likely to contribute to the impairment in delayed reward that characterizes addicted individuals as there is no emotional advantage for the subject to delay for a bigger reward if its saliency is equivalent to that of a moderate or smaller reward. Note too the possibility of a similar deficit underlying decision-making related to negative reinforcers (punishments), which could translate to a disregard for threat that would otherwise inhibit behavior.

A related field of study pertains to the ability to contemplate regret. Studies examining counterfactual thinking or regret, where the outcome of a decision is compared with alternative outcomes, suggest a critical role of the OFC in mediating the experience of regret (Camille et al. 2004; Coricelli et al. 2007). While there is no direct evidence of the extent regret affects decision-making capacity in drug-addicted individuals, this area of study warrants further exploration.

Expectation

Essential for the development of drug addiction is the expectation of a drug's effects. Early neuroimaging studies have examined expectation within the context of pain anticipation in healthy control subjects and have shown that the expectation of pain activates more anterior regions of the frontal cortex than the experience of pain (Ploghaus et al. 1999). These and other studies indicate activations of the OFC and/or the ventral cingulate gyrus in particular, for expectation of a variety of pleasant and unpleasant stimuli (Chua et al. 1999; Koyama et al. 2005; O'Doherty et al. 2002; Petrovic et al. 2005; Ploghaus et al. 1999; Ploghaus et al. 2003; Sarinopoulos et al. 2006; Volkow et al. 1991; Wager et al. 2004). We demonstrated that expectation plays a significant role in drug addiction. Specifically, we used PET to measure glucose metabolism and manipulated expectation by administering oral MPH vs. a placebo. In cocaine abusers we found MPH-induced metabolic increases in the cerebellum, occipital cortex and thalamus when MPH was expected (Volkow et al. 2003); however, in another study with drug naive subjects these activations were not present (Volkow et al. 2006c).

These findings suggest that activation in cerebellum, occipital cortex, and thalamus regions may reflect conditioned responses in cocaine abusers.

In a recent fMRI study of detoxified alcoholics, reduced activation of the ventral striatum was found during expectation of a monetary gain, but increased activation in this area was observed following exposure to alcohol cues. Moreover, the activation to alcohol cues was positively correlated with subjective craving (Wrase et al. 2007). Finally, there is evidence that brain response to drug cues (i.e., smoking cues) may be strongly modulated by drug availability. Perhaps not surprisingly, when the ability to acquire and use a drug (nicotine in this case) is near, brain areas associated with arousal, attention and cognitive control (thalamus as well as PFC regions such as the dorsal lateral and medial PFC, OFC, and ACC) are activated. However, these regions are not activated when drug addicted individuals are aware that there is no possibility to smoke. Interestingly, self reported craving in this study was unchanged across availability contexts (McBride et al. 2006).

In general, certain cortical (PFC, OFC, and ACC) and mesocortical brain regions have a significant role in the relationship between drug cues and drug availability. However, activation of PFC regions may be contingent upon perceived drug availability.

Clinical Applications and Future Research Directions

Neuroimaging technology has had a tremendous impact on the basic knowledge of addiction-related brain circuits. It has identified cortically regulated cognitive and emotional processes which result in the overvaluing of drug reinforcers, the undervaluing of alternative reinforcers, and deficits in inhibitory control. These changes in addiction as represented in the I-RISA model expand the traditional concepts of emphasized limbic-regulated responses to reward by providing evidence for the involvement of the frontal cortex throughout the addiction cycle.

It is now conceivable that interventions designed to strengthen and remediate brain areas affected by chronic drug use via cognitive-behavioral interventions and pharmaceuticals may be highly beneficial to drug addicted individuals just as they have been for other disorders (e.g., Papanicolaou et al. 2003; Volkow et al. 2007b). As the long-term goal of clinical research is to use reliable and valid scientific results for the development of effective intervention and prevention efforts, neurobiological and neurobehavioral information about the drug-addicted individual's I-RISA, can help guide the development of treatment for drug dependence. Future studies should focus on cognitive-behavioral rehabilitation efforts geared towards improving inhibitory control functions (i.e., gaining control over cognitive functions to suppress craving and other automatic processes, see (Beck 1993), and lessening the value attributed to the drugs while increasing the value attributed to nondrug reinforcers. Indeed, strategies that interfere with conditioned responses (Childress et al. 1987; Franken et al. 1999; McClernon et al. 2007; O'Brien et al. 1990; Rose et al. 2007) and that probe brain regions that are

compromised in drug addiction, should prove promising. Albeit, it is reasonable to predict that medications could facilitate these processes by impeding the underlying brain circuits.

Future investigations should also be directed toward a better understanding of the rate and amount of recovery of brain structure (e.g., gray and white matter abnormalities), DA function, and glucose metabolism in addicted individuals as reductions to all have been documented in drug addicted individuals (Bae et al. 2006; Bartzokis et al. 1999; Berman et al. 2008; Harris et al. 2008; Lim et al. 2002; Lyoo et al. 2004; Moeller et al. 2005; O'Neill et al. 2001; Tang et al. 2004; Thompson et al. 2004; Wang et al. 2004; Volkow et al. 1988c; Volkow and Fowler 2000). Most importantly, these investigations should focus on the extent that this recovery directly translates into better cognition or behavior (Yeh et al. 2007). Another aspect that requires more clarity is whether these abnormalities predate the development of the addictive disorder, are precipitated by the disease, or are an interaction of the two. Also, as we gain knowledge of the genes that are involved in the vulnerability to addiction the use of brain imaging will enable us to understand how genes affect brain function and behavior.

The overarching goal of brain imaging studies is to offer biomarkers for particular outcomes in those who are susceptible to drug dependence. Although, recent evidence highlights the role of specific brain regions and neurotransmitters in drug-related behaviors, these studies also offer insight into the neurobiology underlying broader human phenomenology that encompass motivation and free-will.

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Anxiety: Uncover Roles of Stress Related Genes by Imaging Genetics

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Introduction

Anxiety is a subjectively-experienced setpoint that determines a range of normal behavioral responses, as well pathological variation. Anxiety and mood disorders may result from failure in individual adaptive responses to fear and stress (McEwen, 2000), where the normal homeostatic setpoint of mood is replaced by an allostatic state marked by chronic anxiety and dysphoria, and vulnerability to stress-induced relapse (McEwen and Stellar, 1993). In humans, anxiety and emotionality (Neuroticism) are moderately to highly heritable traits (0.4–0.6) but also strongly influenced by stress exposures in a pattern consistent with gene x environment interaction (Caspi et al., 2002). These stress-induced changes are superimposed on genetic differences and are probably dependent on genetic variation in resiliency. While the detection of gene effects on psychiatric measures of complex behaviors such as trait anxiety would require substantially large sample size, it has been noted that allelic effects on fear and stress responses are more readily detectable in relatively small sample size via the changes of the conserved neural components accessed by brain imaging (Hariri et al., 2002; Heinz et al., 2005; Pezawas et al., 2005). Because of this, the functional and structural changes in the components of neural circuitry observed with brain imaging are important intermediate phenotypes and have become effective handles in the studies of the genetics of anxiety and mood disorders.

Anxiety and Stress Responses Accessed by Brain Imaging

The neural circuitry of fear and stress responses involves the prefrontal cortex, amygdala, the nucleus accumbens, hypothalamus, and other parts of the limbic system. Access via brain imaging to these highly conserved neural components has

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greatly promoted our understanding in mood and anxiety related neurobiology. While some of the neuroimaging approaches used in the studies of anxiety disorders measure structural (morphometric) changes (Bremner et al. 1995; Pederson et al. 2004), others have focused on the functional (metabolic) changes of the brain regions such as blood flow measured by functional magnetic resonance imaging (fMRI) and turnover of radioactive tracer measured by positron emission tomography (PET) (Hariri et al. 2002; Rauch et al. 1997; Shin et al. 2004). Stress exposures can lead to long-lasting and permanent changes in brain structure, as shown for the reduction of hippocampal volume in adults with chronic, long-last post-traumatic stress disorder (PTSD) (Bremner et al. 1995; Gurvits et al. 1996; Villarreal et al. 2002). Functional imaging studies have also revealed the important roles of amygdala in individual fear and stress responses. Increased amygdala activation was observed with fMRI in individuals with social phobia when they were exposed to slides of neutral faces (Birbaumer et al. 1998). Altered functions in the prefrontal cortex and other parts of the limbic system has also been observed in individuals with PTSD and other anxiety disorders (Bremner et al. 1997; Carrion et al. 2001; Nordahl et al. 1990).

A body of evidence has shed light on the important roles of several genetic loci in influencing individual stress vulnerability and the level of anxiety. One of the loci is the serotonin transporter promoter. The serotonin transporter promoter polymorphism (5-HTTLPR), a tri-allelic variation consisting of two variable repeat sequence alleles, short (s) and long (l), and an additional single base variation in the l alleles (la/lg), is modestly predictive of the trait, anxiety (Lesch et al. 1996), with each copy of the reduction-of-function s allele contributing approximately 0.1 SD in trait anxiety (Sen et al. 2004). Gene x stress interaction was also found to play a major role in determining the effect of the s allele to increase dysphoric symptoms such as depressive ideation and suicidality (Caspi et al. 2003). While this observation was also replicated in several other studies including in healthy controls (Kendler et al. 2005) and in substance abusers at high risk of suicidality (Roy et al. 2007), the genetic association of HTTLPR with trait anxiety, a measure of complex behavior, was not always evident (Munafo et al. 2008). However, brain intermediate phenotypes via neuroimaging accessing the neurobiology of anxiety and mood have revealed much stronger effects for serotonin transporter promoter polymorphism. These include the robust association of the s allele to greater amygdala activation in response to fearful stimuli, measured by blood oxygen level-dependent (BOLD) fMRI (Hariri et al. 2002). Morphometric analysis also showed s allele-predicted volume reduction of limbic regions critical for processing of negative emotion, particularly perigenual cingulate and amygdala (Pezawas et al. 2005). A relationship of the s allele to lower frontal/amygdala connectivity was also observed by functional analysis, which may help explain failure to down-modulate amygdala responses, and appears predictive of depression (Pezawas et al. 2005).

Study of the brain responses to pain stress is another example of the imaging genetics of stress resiliency. Responses to pain, a stressor that frequently leads to chronic anxiety and mood disorder, are modulated by the same limbic emotional circuitry. The ability to activate endogenous opioid release in amygdala, nucleus

accumbens and other components of the limbic system in response to sustained pain, as measured by PET and the μ-opioid receptor-selective radiotracer – [^{11}C] carfentanil, is strongly predictive of the resilience against pain stress, both in terms of higher pain threshold and diminished affective response to pain (Zubieta et al. 2001). The functional Val158Met missense variant of catechol-*O*-methyltransferase (COMT), of which the substitution of valine with methione at codon 158 was shown to affect the thermostability of the enzyme involved in catecholamine metabolism and result in three to fourfold reduction of the enzyme activity (Lotta et al. 1995), was implicated in trait anxiety, accounting for a modest part of the trait variation (Enoch et al. 2003). This COMT genetic polymorphism was also found to be linked to individual pain stress responses by brain imaging with individuals carrying met/met homozygous alleles showing impaired region-specific μ-opioid receptor activation. This finding was also accompanied by strong parallel predictions of reduced pain threshold, and more affective response to pain (Zubieta et al. 2003a). The diminished capacity to activate the endogenous opioid system under pain stress in individuals with met/met alleles of COMT was presumably the result of the perturbation of dopaminergic neurotransmission. This hypothesis was supported by evidence gathered in animal studies where chronic activation of dopaminergic system reduced the neuronal content of enkephaline peptides and compensatory elevation of μ-opioid receptors in multiple brain regions (Chen et al. 1993; George and Kertesz 1987; Unterwald et al. 1994).

Genetic Variation of *NPY* Gene Affects Emotion and Stress Response Demonstrated by Brain Imaging

Neuropeptide Y (NPY), a 36 amino-acid peptide neurotransmitter, is an evolutionarily highly conserved molecular component of brain systems processing stress and emotion. Using a multilevel approach including genetic imaging analysis, we recently investigated effects of haplotype-based *NPY* genetic variation on individual stress responses and emotion (Zhou et al. 2008). Haplotype-driven *NPY* expression was shown to predict brain response to emotion and stress challenges accessed via neuroimaging as well as trait anxiety, which is consistent with the function of NPY as an anxiolytic neuropeptide and underlines its role in determining inter-individual variation in stress resiliency.

NPY is abundantly expressed in the regions of mammalian brain, including the prefrontal cortex, amygdala, the nucleus accumbens, hypothalamus, and other parts of the limbic system (Adrian et al. 1983; Allen et al. 1983; Tatemoto et al. 1982). NPY was known to have diverse functions both in the central nervous system and the peripheral tissues, including regulation of food intake(Clark et al. 1984; Stanley and Leibowitz 1985), fat metabolism and energy balance (Billington et al. 1991; Kuo et al. 2007), cardiovascular functions (Jacques et al. 2006), circadian rhythms (Antonijevic et al. 2000), and reward (Josselyn and Beninger 1993). In human studies, both cerebrospinal fluid and plasma NPY levels was found to correlate with anxiety

and stress levels (Boulenger et al. 1996; Irwin et al. 1991; Widerlov et al. 1988). The more direct evidence of anxiolytic-like effects of NPY was notably obtained in a wide range of pharmacologically validated animal models (Heilig et al. 1989, 1993; Broqua et al. 1995; Sajdyk et al. 1999) and NPY release was profoundly induced by stress (Thorsell et al. 1999), suggesting a role for endogenous NPY in the control of stress- and anxiety-related behaviors.

We conducted a haplotype-based analysis of *NPY* genetic variation in order to capture effects of unknown loci and locus combination. To determine NPY haplotype composition, which represents allelic combination of multiple genetic loci in the *NPY* region in each of the chromosome pair, six single nucleotide polymorphisms (SNPs) and a two-nucleotide insertion/deletion genetic variation were genotyped in a sample ($n=516$) of unrelated Finnish Caucasians. The seven common polymorphisms (minor allele frequency >0.05) covered *NPY* from one kb upstream of the transcription start site to beyond the 3′ UTR, spanning around 8 kb on chromosome 7, and captured common haplotypes and linkage disequilibrium (LD) features observed in the International Hapmap Project (http://www.hapmap.org). A block of strong pairwise LD was observed for the six markers at the 5′ end, and this block encompasses at least 70% of *NPY* from the 5′ upstream region to exon 3. Five common haplotypes (frequency >0.01) account for 93.8% of haplotypes in this block.

Haplotype-based *NPY* expression variation was observed in both postmortem brain tissues and lymphoblastoid cell lines. Two approaches were used for the analysis. In a separate sample of 28 Caucasian postmortem brain tissues, differential allelic expression assay using a common *NPY* coding SNP (cSNP, rs5574) as a haplotype tag was employed to determine relative mRNA expression levels of the common haplotypes. A 1.2- to 3.3-fold expression variation was observed for 16 (57%) of the samples. The haplotype-based expression variation was also consistent with their cladistical clustering, indicating that expression variation is linked to gene ancestry. The differences in haplotype-driven expression were also verified in lymphoblastoid cell lines, which also naturally express NPY at the mRNA level, by reverse transcription polymerase chain reaction (RT-PCR). Forty-seven cell lines derived from healthy Finnish individuals were selected to represent the six common diplotypes consisting of the three major haplotypes and the results were consistent with the brain haplotype effects detected by differential allelic expression. Based on the brain and lymphoblast mRNA expression data, the six common *NPY* diplotypes which account for 72% of all diplotypes were functionally classified into three expression groups: low (two copies of low expression haplotypes), intermediate (one copy each of low/high expression haplotype), and high (two copies of high expression haplotypes). This expression level-based grouping of *NPY* diplotypes were subsequently used as the genetic basis for the analysis of anxiety and stress related complex behaviors and intermediate phenotypes, including two brain imaging modalities. A significant correlation of the *NPY* diplotype-predicted expression and individual plasma NPY peptide levels was also observed in a sample of both healthy individuals ($n=24$) and alcoholic patients ($n=18$). In both groups, individuals with the low expression diplotypes had significantly lower NPY levels than the

ones with high expression diplotypes. Plasma NPY levels were intermediate in individuals with the intermediate expression diplotypes.

To assess the effect of genetic variation of *NPY* on individual emotional and stress response, a well-characterized BOLD fMRI based brain imaging analysis was employed. This fMIR paradigm was previously used to measure amygdala activation during emotion task challenges with fear and anger-related face viewing interleaved with sensorimotor task (Hariri et al. 2002; Tessitore et al. 2002). Amygdala activation in response to provocative stimuli, such as threat-related facial expressions, predicts affective arousal, including anxiety responses (Haas et al. 2007; Somerville et al. 2004), and greater amygdala reactivity in individuals possessing the lower-transcribing allele (s allele) of the serotonin transporter promoter polymorphism was previously observed (Hariri et al. 2002, 2005). We compared amygdala responses to emotional challenges based on diplotype-predicted *NPY* expression in a sample of 71 individuals, all of whom were participants in a parent study, the Adult Health and Behavior (AHAB) project, at the University of Pittsburgh. As shown in Fig.1, amygdala activation in individuals with the low *NPY* expression diplotype (LL) was significantly higher than those with the high expression diplotype (HH). In addition, *NPY* haplotypes appeared to predict amygdala reactivity in an allele-dosage fashion, with heterozygous individuals (LH) being intermediate in activation. *NPY* diplotypes accounted for 9% of the variance in amygdala metabolic response to emotional challenge. Importantly, the magnitude of amygdala activation predicts measures of temperamental anxiety in this sample. Together, the results suggest that NPY effects on temperamental anxiety are mediated in part through biased amygdala reactivity. In addition to these effects, task-related hippocampal activation was predicted by *NPY* diplotypes in a similar allele-dosage fashion. This finding is of interest because the functional interactions of the amygdala and hippocampus are critical for emotional memories, and long-lasting changes in hippocampal architecture are induced by stress (McEwen 1999).

Using molecular imaging with positron emission tomography (PET) and [^{11}C] carfentanil, a selective μ-opioid receptor radiotracer, we also tested the effect of *NPY* diplotype on brain responses to a pain stressor and found a coherent effect of the low expression diplotype to predict greater stress vulnerability in a sample of 35 healthy volunteers who were participants of a University of Michigan study of stress responses. The stress model employed consisted of moderate levels of pain induced by the administration of small amounts of 5% hypertonic saline in the masseter muscle, maintained over 20 min and standardized between individuals (Zubieta et al. 2001; Zubieta et al. 2002). This stimulus activates endogenous opioid neurotransmission by activating μ-opioid receptors in multiple brain regions, including the prefrontal, cingulate and insular cortices, medial and lateral thalamus, hypothalamus, nucleus accumbens, amygdala and periaqueductal gray (Zubieta et al. 2001). Using this methodology the anxiety-associated COMT Met158 allele was previously linked to reduced pain thresholds, more negative emotional state, and diminished opioid system responses (Zubieta et al. 2003a). Here, we found significantly higher levels of μ-opioid system activation in prefrontal cortex, posterior insula, medial and lateral thalamus, ventral basal ganglia (ventral caudate, ventral

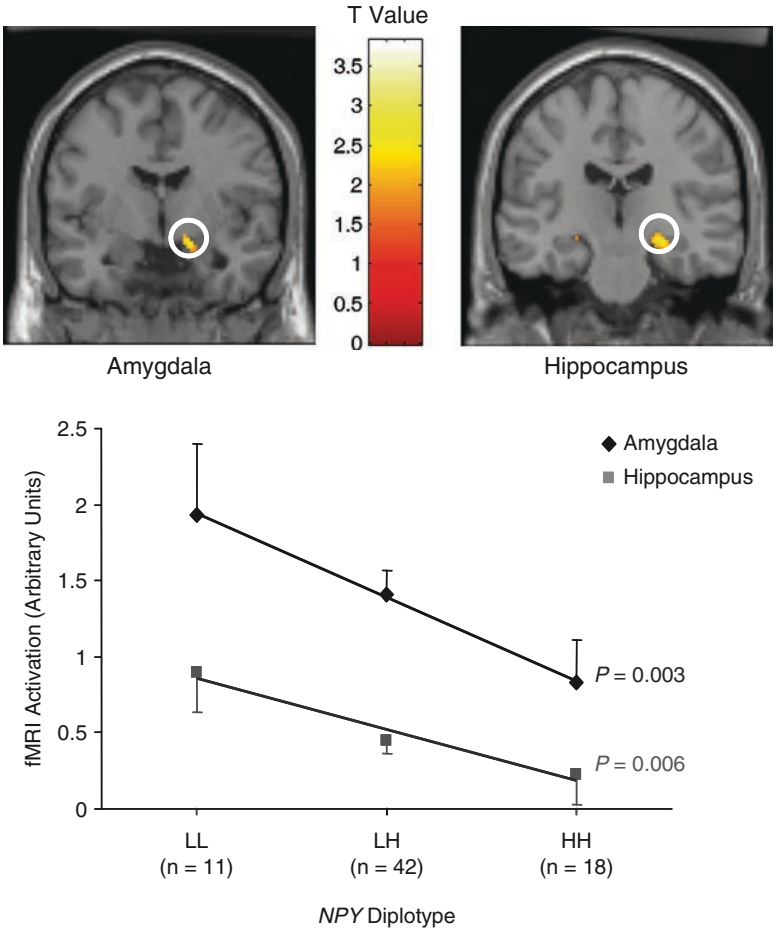
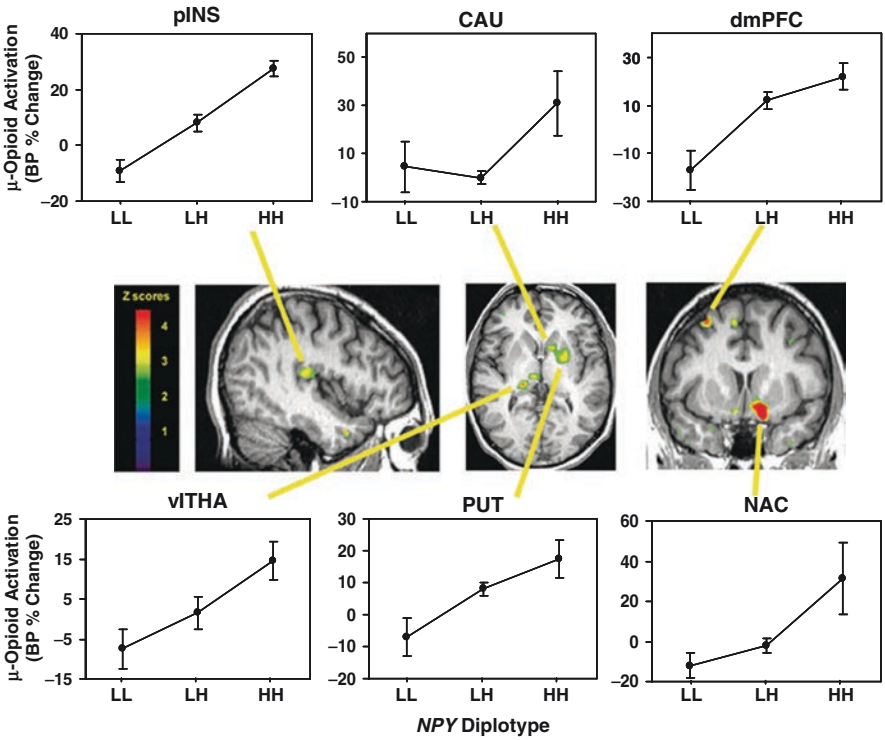


Fig. 1 Effect of diplotype-predicted *NPY* mRNA expression on fMRI measured amygdala and hippocampal activation in response to threatrelated facial expressions. Top: statistical parametric maps representing *NPY* diplotype-biased (LL>LH>HH) mean right dorsal amygdale ($x=16$, $y=-8$, $z=-14$, 64 voxels, $t=2.82$, $P=0.003$) and right hippocampal ($x=24$, $y=-20$, $z=-12$, 132 voxels, $t=2.56$, $P=0.006$) activation is shown overlaid on an average sagittal and coronal MRI. Bottom: right amygdala (black diamonds) and hippocampal (gray squares) activities (means and s.e.m.) from clusters grouped by *NPY* diplotypes

putamen and nucleus accumbens and amygdala) in individuals who carry high expression *NPY* diplotypes (Fig. 2). *NPY* diplotype-predicted expression variation accounted for 13% of the variance in the activation of μ-opioid neurotransmission in the amygdala, but for as much as 18–35% of the variance in prefrontal cortex, thalamus, and nucleus accumbens, and 37% in the posterior insular cortex. The ability of these high *NPY* expression individuals to better activate the endogenous opioid system is consistent with the well established role of this neurotransmitter system in the regulation of stress (Watkins and Mayer 1982; Akil et al. 1984)



Region	x y z coordinates (mm)	F Score	Cluster p-value (corrected)	Cluster size (mm^3)
L NAC	-11 7 -11	33.6	$p < 0.0005$	478
R CAU	13 1 -6	20.2	$p < 0.0005$	571
L dmPFC	-25 20 45	16.3	$p < 0.0005$	500
L PUT	-23 2 3	12.8	$p < 0.0005$	262
R AMY	27 0 -28	11.3	$p < 0.0005$	96
R vlTHA	12 -17 1	10.9	$p < 0.0005$	276
L pINS	-42 -24 18	6.6	$p < 0.005$	204

Fig. 2 Effect of diplotype-predicted *NPY* mRNA expression on pain/stress induced μ-opioid system activation. Activation (mean ± S.E.) measured by the change of the μ-opioid receptor binding potential (BP % change) among the *NPY* diplotypes (LL, $n=8$; LH, $n=21$; HH, $n=6$) are shown in the dorsomedial prefrontal (dmPFC) and posterior insular (pINS) cortices, ventrolateral thalamus (vlTHA), ventral putamen (PUT) and caudate (CAU) nuclei, nucleus accumbens (NAC) and ventral amygdala (AMY, see the table). Z-scores represented by the pseudocolor scale (left) are superimposed over an anatomically standardized MRI image. The table shows the localization using International Consortium for Brain Mapping (ICBM) stereotactic atlas coordinates and ANOVA F scores and multiple comparison-corrected levels of significance. The correction for multiple comparisons took into account the size of the region under consideration, using SPM2 cluster threshold corrections

and anxiety-like responses in animal models (Sora et al. 1997; Moles et al. 2004) and humans (Zubieta et al. 2001, 2003b; Smith et al. 2006). Compared to its effects on these intermediate phenotypes, *NPY* explained less of the variance in more complex,

self-rated pain and affective response phenotypes measured by the McGill Pain Questionnaire (MPQ) sensory subscale scores and the Positive and Negative Affect Scale (PANAS) negative affect ratings. *NPY* diplotypes accounted for 3% and 5% of the variance in the individual pain stress experience, respectively.

The effects of *NPY* diplotype-predicted expression variation on emotion and stress responses observed by brain imaging analysis were also consistent with our findings of genetic effects of *NPY* on trait anxiety – a complex behavioral phenotype. *NPY* diplotype-predicted expression was found to be inversely correlated with trait anxiety, measured with the Tridimensional Personality Questionnaire (TPQ) Harm Avoidance subscales HA1 (Fear of Uncertainty) and HA2 (Anticipatory Worry), in a relatively modest sample of 137 healthy Finnish Caucasian individuals. In contrast to the larger effects of *NPY* diplotype on brain functional responses measured by neuroimaging, the effects of *NPY* on trait anxiety were modest, accounting for 3.3% of the variance in HA1 and 3.4% of the variance in HA2. In addition, compared to the same 137 healthy controls, lower Diplotype-predicted *NPY* mRNA expression was also observed in a small number of individuals with clinical anxiety disorders ($n=18$) diagnosed with the Structured Clinical Interview for DSM-III-R (SCID). No correlation of *NPY* diplotype-predicted expression to the HA subscale scores was observed in relatively small samples of subjects with SCID-diagnosed alcoholism, drug addiction, anxiety disorders, and major depression, indicating possible dysregulation of the stress axis in these patients. A relatively small effect of genetic variation on complex behavior was also observed in other studies (Sen et al. 2004), and the gene effects on these types of complex behaviors may be masked by environmental factors (Caspi et al. 2002, 2003). In order to consistently detect gene effects on a complex behavior such as trait anxiety, very large study samples may be required.

The haplotype-based *NPY* expression variation, correlated with brain imaging findings of individual emotional and stress responses, was also found to be partially contributed by a genetic variation in the *NPY* promoter region. Using reporter gene (DsRed) constructs containing DNA fragments of *NPY* promoter and upstream regulatory region with all known naturally occurring and moderately common sequence variants (allele frequency >0.05) and in vitro transfection analysis in a neuronal cell line, RN46A, a sequence change from T to C at −399 base pair (bp) of the *NPY* promoter region was found to cause 30% reduction of the promoter activity. The allelic effect of this variant is consistent with the directions of haplotype-based expression variation. However, *NPY* haplotypes have greater predictive value for expression level than the −399 bp variant, indicating that in addition to this sequence variant, other unidentified locus (loci) contained in the haplotype block may also be involved in the regulatory effects on *NPY* expression.

The *NPY* haplotype-predicted amygdala and hippocampal activation to emotional stimulus examined by BOLD-fMRI and brain responses to pain stress challenge accessed by PET demonstrated important roles of NPY in modulating individual emotional response and anxiety state. Along with other studies, such as the effects of 5-HTTLPR and COMT genetic polymorphism on emotional and stress responses (Hariri et al. 2002; Zubieta et al. 2003a), it also illustrates that brain

responses to emotional and stress challenges measured by neuroimaging are powerful intermediate phenotypes and they provides valuable tools for the analysis of genetic effects on anxiety.

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Imaging CNS Disease States: Alzheimer's Disease

Bradford C. Dickerson

Introduction

Alzheimer's disease (AD) is the most common cause of dementia (Kukull and Bowen 2002), and is the fourth leading cause of death in the United States. In the vast majority of individuals, symptoms begin after the sixth decade of life. The disease typically starts with mild memory difficulties and progresses insidiously, leading eventually to cognitive impairment that interferes with complex activities of daily life and ultimately results in the loss of independent function. Current treatments are primarily symptomatic, as clinical trials demonstrate a short-term improvement in cognitive function but not an obvious slowing of the rate of decline (Cummings 2004). Increasing emphasis is being placed on the development of disease-modifying therapies – drugs that impede the underlying pathophysiologic process of neurodegeneration in AD and thereby slow the rate of cognitive decline. Extensive efforts are being directed toward the identification of candidate molecules and testing in animal models of AD, and several clinical trials of putative disease-modifying therapies are now underway. At present, the potential efficacy of disease-modifying therapies is evaluated primarily using clinical measures of cognition and behavior. In animal models, traditional behavioral assessments are often used, such as the rate at which rodents learn to navigate a maze. In clinical trials, outcome measures are typically performance-based cognitive instruments, such as the Alzheimer's Disease Assessment Scale (ADAS-Cog) (Rosen et al. 1984), or structured surveys of clinician/caregiver impression of change (Schneider et al. 1997).

Although the efficacy of disease-modifying treatments for AD must ultimately be demonstrated using clinically meaningful outcome measures such as the slowing of decline in cognitive function, these trials will require a study on hundreds of patients for several years. Thus, surrogate markers of efficacy with less variability

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than clinical assessments are desperately needed to reduce the number of subjects. These markers may also prove to be particularly valuable in the early phases of drug development to detect a preliminary "signal of efficacy" over a shorter time period.

Since the pathophysiologic process underlying the cognitive decline in AD involves the progressive neurodegeneration of particular brain regions, repeatable in vivo neuroimaging measures of brain anatomy, chemistry, physiology, and pathology form an important class of potential biomarkers, in addition to markers derived from cerebrospinal fluid or blood. A growing body of data indicates that the natural history of gradually progressive cognitive decline in AD can be reliably related to changes in such imaging measures. Furthermore, regionally specific changes in brain anatomy, chemistry, physiology, and the presence of pathology can be detected by imaging prior to the point at which the disease is symptomatic enough to make a typical clinical diagnosis. Finally, evidence that alterations in synaptic function are present very early in the disease process, possibly long before the development of clinical symptoms and significant cell loss, which may relate closely to symptomatic progression in manifesting disease, have been collected(Selkoe 2002; Coleman et al. 2004). Thus, potential disease-modifying therapies may act by impeding the accumulation of neuropathology, slowing the loss of neurons, altering neurochemistry, or preserving synaptic function; neuroimaging modalities exist to measure each of these putative therapeutic goals.

The pathophysiologic process of AD is considered to take place over years, possibly decades, prior to the development of dementia. Currently, however, the clinical diagnosis of AD is made after a patient has developed impairment in multiple cognitive domains that is substantial enough to interfere with routine social and/or occupational function (dementia). It is only after this point that FDA-approved medications are currently indicated – that is, clinically probable AD. By this time, substantial neuronal loss and neuropathologic change damaged many brain regions. Although data from animal models suggest that it may be possible to impede this process as it is developing (Schenk et al. 1999; Weiner et al. 2000), and potentially reverse some aspects of it (Lombardo et al. 2003), it is not clear whether the pathology typically present when patients are clinically diagnosed with AD can be reversed. Thus, it would be ideal to initiate treatment with neuroprotective medications at a time when – or even before – AD is mildly symptomatic (DeKosky and Marek 2003).

To achieve this goal, we need to improve our capability to identify individuals with prodromal AD – the earliest symptomatic phase of AD prior to dementia. Currently, individuals are categorized as having mild cognitive impairment (MCI) when symptoms suggestive of AD are present but mild enough that traditional diagnostic criteria (which require functional impairment consistent with dementia) are not fulfilled. This gradual transitional state may last for a number of years. Diagnostic criteria for MCI have been developed (Petersen et al. 1999) and operationalized (Grundman et al. 2004) in a manner that suggests that cohorts of such individuals can be reliably identified for clinical trials. If the pathophysiologic

process of AD can be slowed at this stage of the disease, then it may be possible to preserve cognitive function and delay the ultimate development of dementia for a period of time, which is clearly clinically meaningful. Therefore, MCI patients present an excellent target population for clinical trials of disease-modifying therapies. However, it is important to recognize that many patients with MCI have underlying pathologies other than AD. Thus, greater specificity is required if we are to identify the subset of individuals with MCI who are exhibit early symptoms of AD. There has been a recent international effort to revise the diagnostic criteria for AD to enable a diagnosis to be made of typical amnesic AD prior to dementia – these criteria include specific clinical and psychometric criteria along with sensitive and specific biomarkers (Dubois et al. 2007). Although the field will likely move in this direction, studies investigating the overall sensitivity and specificity of these diagnostic criteria are needed.

Finally, presymptomatic AD is the phase of the disease when pathologic alterations develop but cognitive impairment is not apparent. This is probably best studied through the identification of cohorts with particular risk factors, such as genetic determinants (e.g., amyloid precursor protein (APP) or presenilin mutations, Down syndrome) or susceptibility factors (e.g., apolipoprotein E (APOE)-ε4). Ideally, it would ultimately be possible to initiate disease-modifying therapies at this point based on the presence of risk factors, much as is done in the case of primary preventive measures for cerebrovascular disease. However, given that some of these therapies may not be benign, it would be best to have a panel of biomarkers that could be used to help guide the timing of such therapies, such that individuals at elevated risk for AD could be followed over time. When changes in biomarkers indicate the earliest phase of active pathophysiology, treatment could be initiated.

A variety of measure of brain structure, function, and pathology are excellent candidate biomarkers.

Imaging Biomarkers of AD-Related Alterations in Brain Anatomy

Neuroimaging techniques that provide measures of brain structure have been applied to the diagnosis of AD for decades. AD involves a selective degeneration of neuronal populations in specific brain regions – starting initially in the medial temporal lobe (MTL) – which is marked macroscopically by volume loss in these regions (Fig. 1). Therefore, in diagnosis or monitoring of progression, neuroimaging measures can be useful in identifying individuals with changes in brain structure consistent with AD (Scheltens et al. 2002) and in following the degenerative changes in those regions over time. If a drug is able to impede the degenerative process of the disease, it would theoretically slow the rate of atrophy and other changes in brain structure that are hallmarks of the progression of AD.

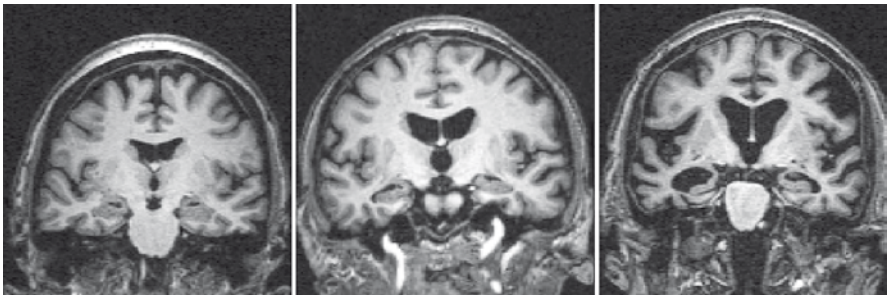

Fig. 1 Coronal MRI sections from individual subjects (Control, MCI, and AD), illustrating a mild degree of atrophy in MCI and greater atrophy in mild AD compared to age-matched control

As MRI and computational technologies have matured, it has become possible to perform increasingly sophisticated investigations of brain morphometry. In vivo structural neuroanatomic data can be routinely acquired for clinical purposes at a resolution of 1 cubic millimeter. In addition, new techniques for the coregistration of one scan to another have improved methods for quantifying the structural change over time within individuals (Fox and Schott 2004). Despite the caveat that subtle longitudinal changes in neurodegenerative diseases can be challenging to detect, longitudinal MRI measures of changes in brain structure have been successfully used as outcome measures in clinical trials of disease-modifying therapies for multiple sclerosis (Filippi et al. 2002).

Methodological advances in both theoretical and practical aspects of computational neuroanatomy have led to the development of sophisticated software to semi-automate the analysis of MR image data (Ashburner et al. 2003). To date, many of these methods provide information on differences in global or large-scale brain structure between groups of individuals or within individuals over time. One such approach is the voxel-based morphometry, which involves the spatial normalization of each subject's brain image volume into a common space (Ashburner and Friston 2000). Results with this technique have suggested that normal aging is associated with a linear decline in grey matter with relative sparing of MTL structures (Good et al. 2001), but that AD patients have widespread atrophy (Karas et al. 2003). Another approach, called the cortical pattern matching, involves warping individual scan data into a common space, manually delineating major sulcal and gyral landmarks, and then computing statistics that indicate the discrepancy between the cortical patterns of a study group and those of a control population. This approach and a related hippocampal surface-based method have shown robust capacity to differentiate AD patients from controls, and to quantify change in brain structure over time (Thompson et al. 1998; Csernansky et al. 2000; Thompson et al. 2001). Finally, one method that has been applied to the development of surrogate imaging markers for clinical trials is the brain boundary shift integral (BBSI) approach (Fox et al. 2000). This semi-automated method involves the rigid coregistration of one scan (baseline) and another (follow-up), and essentially

subtracts the follow-up data from the baseline data to calculate the positional shift in global tissue boundaries, thus enabling the quantization of tissue loss at brain-CSF edges over time.

Annual whole brain atrophy rates measured by BBSI are 2–3% in AD patients, whereas they are 0.2–0.5% for normal aging (Fox and Schott 2004). Rates of whole brain atrophy relate to the global decline in cognitive function and cortical neurofibrillary tangles during post-mortem examination (Silbert et al. 2003).

Quantitative ROI-based MRI methods reliably detect AD-related neuroanatomic abnormalities, with diminished hippocampal volume being the most consistent finding. Reliable protocols have also been developed to detect atrophy of entorhinal cortex and other regions involved in early AD (Killiany et al. 2000; Goncharova et al. 2001). Hippocampal volume derived from MRI correlates strongly with histological HF volume and neuronal loss (Bobinski et al. 2000) and severity of AD pathology (Jack et al. 2002; Gosche et al. 2002), as well as memory impairment (De Leon et al. 1997; de Toledo-Morrell et al. 2000). These measures are also useful for the identification of subgroups of individuals with mild memory impairment, those who progress to a clinical diagnosis of AD within a few years (Killiany et al. 2000; Jack et al. 1999; Visser et al. 1999; Convit et al. 2000; Dickerson et al. 2001; Mungas et al. 2002). Furthermore, it is possible to detect atrophy of these regions up to 5 years before the expression of clinical symptoms in individuals with APP mutations (Schott et al. 2003).

Manual ROI-based longitudinal MRI studies of atrophy rates in AD have focused primarily on the MTL. The annual rate of hippocampal volume loss is reported to be two to three times greater in mild AD patients than in controls, ranging from 4 to 8% per year; rates of volumetric change in the temporal lobe and temporal horn of the lateral ventricle also differ significantly between AD patients and controls (Jack et al. 2000; Laakso et al. 2000). In a longitudinal study of initially asymptomatic individuals harboring an APP genetic mutation, a hippocampal atrophy rate similar to that of AD patients was detected over 2 years during which symptoms first appeared (Fox et al. 1996).

Although hippocampal volume appears to be a reliable and valid surrogate measure in AD clinical drug trials (Jack et al. 2003), questions remain due to data from a clinical trial in AD patients using active immunization with beta amyloid showing an *increase* in atrophy in the patients who developed a significant antibody response, raising the question as to whether a therapeutic response to disease-modifying agents will always parallel reduced rates of atrophy (Fox et al. 2005). The explanation for these paradoxical results remains to be elucidated, and may ultimately provide valuable information about the relationship of changes measured using structural imaging modalities to the pathophysiology of AD.

Further insights into questions regarding the best analytic approaches to answer specific questions from structural data will be gained from ongoing large-scale multicenter studies that involve serial MRI scans, such as the Alzheimer's Disease Cooperative Study trial of MCI and the ADNI. These efforts will be supported by the development of collaborative infrastructure through groups such as the Biomedical Informatics Research Network (Martone et al. 2004).

Imaging Biomarkers of AD-Related Alterations in Brain Chemistry

Proton magnetic resonance spectroscopic imaging (MRS) is a technique that enables the quantization of particular neurochemical constituents of brain tissue. Metabolic components that are commonly measured include N-acetyl aspartate (NAA) and myoinositol (mI), which are considered to represent the density of living neurons and glial cells, respectively, and choline (Cho), which is a marker of cell membrane turnover. In AD patients, decreases in NAA are found in MTL, posterior cingulate, and other regions typically affected by neurofibrillary pathology early in the disease (Schuff et al. 2002; Jessen et al. 2000; Kantarci et al. 2000). Levels of mI tend to be increased in AD, and Cho may be increased or unchanged. In individuals with MCI, MRS measures are different from normal aging (Kantarci et al. 2000) and relate to the memory performance (Kantarci et al. 2002); these cross-sectional studies suggest that mI may increase prior to decreases in NAA and Cho. The combination of volumetric and spectroscopic MR measures appears to provide better diagnostic sensitivity and specificity for AD (vs. controls) than either measure alone (Schuff et al. 1997).

Several longitudinal clinical-imaging studies have examined changes in MRS with progression of AD. Over the course of a year, total grey-matter NAA declined to a greater degree in AD patients (12.36%) than controls (0.94%), correlated with decline in the clinical measure of global impairment, and was more sensitive to change in the total grey-matter volume (Adalsteinsson et al. 2000). In AD patients, 2-year decline in MTL NAA correlated with decline in MMSE (Jessen et al. 2001); a similar though non-significant finding was observed after 1 year (Dixon et al. 2002). Longitudinal changes in MRS measures have also been evaluated in AD patients during therapeutic trials with cholinergic agents (Satlin et al. 1997; Frederick et al. 2002; Krishnan et al. 2003). Changes in both NAA and Cho correlate with changes in cognitive function. These findings not only support the feasibility of MRS measures in AD clinical trials, but also indicate that AD-related changes detected by MRS may be reversible, and may reflect aspects of neuronal integrity or function. Thus, spectroscopic measures in AD may provide a bridge between traditional measures of brain structure and function.

Imaging Biomarkers of AD-Related Alterations in Brain Function

Techniques to measure aspects of brain function in vivo have begun to provide revolutionary insights into cerebral activity at rest, during task performance, and the alterations that occur in individuals with neurodegenerative disease. Since functional neuroimaging tools assess inherently dynamic processes that may change over short time intervals in relation to a host of factors, these measures have unique

characteristics that may offer both strengths and weaknesses as potential biomarkers for neurodegenerative disease. Resting measures of metabolism and perfusion, discussed first, are probably closest to use in clinical trials, while measures of task performance-related brain activity are likely from practical use at this point.

Brain Metabolism and Perfusion at Rest: FDG PET and SPECT

Functional tomographic techniques detect signals related to the functional properties of brain regions three-dimensionally using radiolabeled compounds. The two major techniques that have been applied to AD are positron emission tomography (PET) and single photon emission computed tomography (SPECT). Using PET, the regional cerebral metabolic rate of glucose can be measured (with fluorodeoxyglucose, or FDG). With SPECT, cerebral blood flow rates (perfusion) can be measured. For over 20 years, these techniques have been applied to the study of dementia, and one of the most consistent findings in AD is a reduction at rest of metabolism and perfusion in posterior temporoparietal, posterior cingulate, and frontal regions, and sparingly in primary somatomotor cortices (Fig. 2). Animal model studies have also suggested that posterior cingulate hypometabolism may be an early feature of the disease (Reiman et al. 2000), and demonstrated that it occurs after entorhinal lesions, possibly as a result of disconnection (Meguro et al. 1999). This "functional signature" of AD has been studied extensively as a potential marker of disease state – a diagnostic marker to differentiate AD from normal aging and other neurodegenerative diseases, and can do so in the proper clinical context with relatively high sensitivity and specificity when compared with clinical diagnoses (Jagust 2000). In PET or SPECT studies of AD patients followed by autopsy, the in vivo resting functional findings have also demonstrated relatively high sensitivity to detect post-mortem AD neuropathology, but rather lower specificity (Mega et al. 1999; Mega et al. 1997; Bradley et al. 2002). In addition, multicenter studies have demonstrated that PET data acquired using different instruments can be pooled in a manner that minimizes the site-related variance and enables the detection of disease effects (Herholz et al. 1999; Herholz et al. 1993).

Longitudinal studies have shown that baseline PET and SPECT measures are useful in the prediction of future cognitive decline in AD patients (Wolfe et al. 1995; Jagust et al. 1996) and the early detection of disease state in individuals with MCI (Herholz et al. 1999; Johnson et al. 1998; Arnaiz et al. 2001; Chetelat et al. 2003). Serial functional imaging studies have demonstrated that progressive metabolic decline correlates with cognitive decline in AD patients (Jagust et al. 1988; Haxby et al. 1990). Progressive metabolic abnormalities parallel cognitive decline in both older cognitively intact individuals (de Leon et al. 2001) and subjects with mild memory impairment who carry the APOE-ε4 allele (Small et al. 2000). In individuals in their fifties without cognitive decline, progressive metabolic decline has been observed in ε4 carriers after 2 years (Reiman et al. 2001).

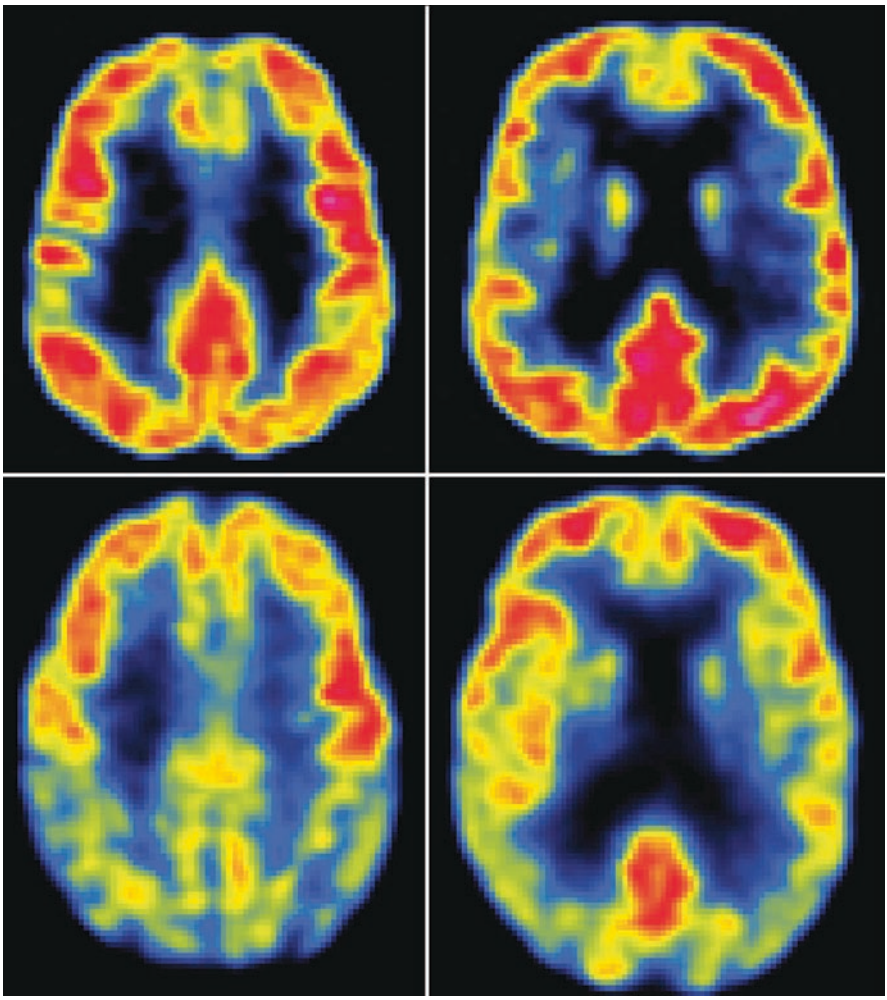

Fig. 2 FDG-PET data from an older control subject (*top*) and a patient with probable Alzheimer's disease (*bottom*), illustrating prominent temporoparietal hypometabolism. Figure courtesy of Keith Johnson, M.D

Finally, PET and SPECT measures of resting brain function appear to be sensitive to medication effects in clinical drug trials and relate to clinical measures in a manner that suggests their potential utility as surrogate markers. In four studies of cerebral metabolism or perfusion in AD patients given cholinesterase inhibitors, these functional brain measures parallel clinical measures in demonstrating stability or improvement in treated vs. placebo groups or in predicting response in treated patients (Mega et al. 2001; Nakano et al. 2001; Nobili et al. 2002; Tune et al. 2003).

Task-Related Brain Hemodynamics and Metabolism: fMRI and FDG PET

Functional neuroimaging techniques can also be used to measure regional brain "activation" during the performance of cognitive tasks. Most of these techniques, including FDG-PET, O-15-PET, and functional MRI (fMRI), measure task-related changes in regional brain metabolism, blood flow, or deoxyhemoglobin concentrations, which are considered to reflect neuronal activity. Functional neuroimaging studies have shown that AD patients differ on these measures of regional brain activation from controls during the performance of a variety of tasks. In fMRI studies of memory task performance in patients diagnosed with AD, hippocampal and parahippocampal activation is consistently decreased in comparison to older controls (Small et al. 1999; Rombouts et al. 2000; Kato et al. 2001; Sperling et al. 2003; Machulda et al. 2003). While memory-task related fMRI data regarding MTL activation in individuals with MCI are less consistent, with reports of both decreased and increased activation (Small et al. 1999; Machulda et al. 2003; Dickerson et al. 2004), they do indicate that differences are present in comparison to older controls. Some of the variability in fMRI data on MTL activation appears to relate to the degree of impairment along the MCI spectrum, suggesting that fMRI may be sensitive to relatively subtle clinical differences (Dickerson et al. 2004). Moreover, differences in memory-related MTL activation are associated with the likelihood of subsequent cognitive decline (Dickerson et al. 2004), which implies that fMRI may be a sensitive technique for prediction of future clinical status.

FMRI may be particularly valuable in evaluating acute and subacute effects of medications on neural activity that may have both symptomatic and disease-modifying properties. Recent animal studies have suggested that anti-amyloid strategies may result in acute changes in synaptic function and behavior, in addition to altering amyloid plaque formation (Dodart et al. 2002; Walsh and Selkoe 2004). FMRI studies have demonstrated significant alterations in memory-related activation with the administration of pharmacologic agents known to impair memory (Sperling et al. 2002). Finally, fMRI investigations of the effects of cognitive enhancing drugs on brain activation during cognitive task performance have shown that changes can be detected after administration of cholinesterase inhibitors in patients with AD (Rombouts et al. 2002) and MCI (Saykin et al. 2004).

Imaging Biomarkers of AD-Related Brain Pathology

Our understanding of the basic mechanisms involved in the pathogensis of AD and other neurodegenerative diseases has rapidly advanced (Walsh and Selkoe 2004), and there exist specific disease-modifying strategies that target brain amyloid formation (Schenk et al. 1999). These advances, including preliminary evidence suggesting that such agents may be effective, have motivated intense research

efforts in developing a means to accurately identify plaque pathology in vivo with neuroimaging.

Radiochemistry research has led to the development of a class of tracers that specifically bind to amyloid plaques in mouse models of AD (Mathis et al. 2002), cross the blood-brain barrier, and label plaques in vivo in humans. One such compound, known as Pittsburgh Compound B (PiB), has been tested in animal models of AD, in human AD post-mortem brain tissue, in living healthy elderly subjects, and in patients with a clinical diagnosis of AD (Klunk et al. 2004). Another agent labels both fibrillar amyloid and tau (Small et al. 2006).

PiB has recently been tested in living human subjects, including normal elderly and patients with the clinical diagnosis of mild probable AD (Fig. 3). The absolute level of PiB retention was approximately the same in the cerebellum and white matter of AD patients and normal control subjects, brain areas known to lack substantial deposits of fibrillar amyloid. In contrast, PiB retention was very high in AD patients in frontal, temporal and parietal neocortical regions (Klunk et al. 2004; Nordberg 2007). As expected, PiB binding similar to AD is observed in some subjects with MCI (Forsberg et al. 2008), reflecting the presence of significant pathology even at this mild level of clinical impairment, as has also been observed

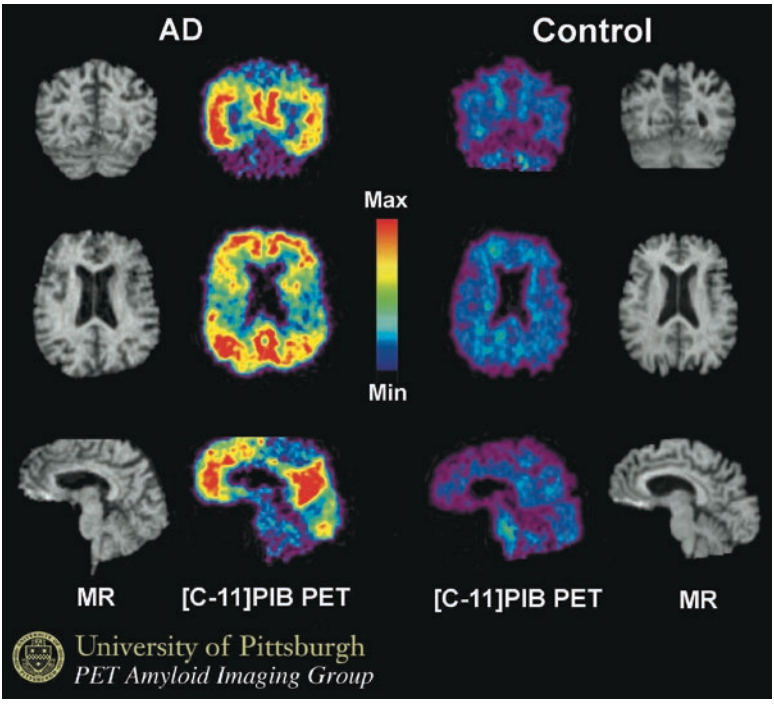

Fig. 3 In vivo PET-based detection of beta amyloid. Increased retention of the Pittsburgh Compound-B (PIB) is found in the frontal and temporoparietal regions in patients with clinical AD. Figure courtesy of William E. Klunk, M.D., Ph.D

in pathologic studies. PiB imaging has also confirmed what has long been known from post-mortem studies (Tomlinson et al. 1968): many elderly cognitively intact individuals carry a substantial burden of AD neuropathology (Mintun et al. 2006). Yet brain amyloid levels may plateau and remain fairly stable longitudinally in AD dementia (Engler et al. 2006). Thus, PET pathology markers may assist in the diagnosis, but PET metabolic markers or MRI atrophy markers will likely be important for measuring changes in the rate of disease progression. Whether these findings will be useful in the prediction of the eventual development of AD dementia in non-demented individuals remains a topic of intense investigation.

The successful visualization of direct markers of AD neuropathology in living humans is a major step forward in the field, and suggests that more specific in vivo diagnostic and monitoring capabilities may be on the horizon. Furthermore, initial studies comparing AD and FTD patients suggest that it may be possible to differentiate neurodegenerative diseases using specific tracers that bind to pathologic proteins (Rabinovici et al. 2007), rather than indirectly through the effects of the diseases on brain function and structure. In addition, these approaches may be very useful in the burgeoning efforts to improve translational research between animal models and humans. However, a number of issues will need to be studied as part of the validation of these methods as surrogate markers. While visualization of a "signal of pathology" has been demonstrated, work is still in progress to refine quantitative metrics and determine the specificity of these measures. Finally, it is still unclear how early in prodromal or presymptomatic AD these imaging pathologic signals will be detectable.

Conclusions

As a variety of imaging biomarkers of anatomy, chemistry, physiology, and pathology in AD become available, these tools may be employed in a targeted manner in the putative neurotherapeutics study. Substantial progress has already been made in validating a number of imaging biomarkers of AD against clinical and pathologic data, and several potential roles for imaging markers in drug development are emerging. Preliminary comparisons of imaging measures to standard cognitive or behavioral measures in clinical trials suggest that at least some types of imaging measures show changes that are expected in AD over time more consistently than behavioral measures (Grundman et al. 2004; Jack et al. 2003). Therefore, multicenter Phase 3 studies of drug efficacy, the use of an imaging-based outcome measure may be more reliable than the standard clinical or cognitive outcome measures, thereby increasing the power to detect a small effect and reducing the sample size. Conversely, an imaging-based measure (e.g., hippocampal atrophy or temporoparietal hypoperfusion) could be used along with clinical and psychometric measures as inclusion criteria to reduce heterogeneity of subjects and select a "leveraged cohort" of individuals who have a greater likelihood of a given clinical outcome, such as cognitive decline or "conversion" from MCI to AD within a few years

(e.g., high risk of imminent diagnosis of clinical AD due to genetic risk or clinical characteristics *plus* hippocampal atrophy or temporoparietal hypofunction). Such applications of imaging-based biomarkers in large-scale multicenter clinical trials with hundreds of subjects may necessitate "high throughput" markers – those that can be derived from standardized, efficient data acquisition and processing tools. In early phase studies, in which a "go ahead" or "kill" decision for a new compound hinges on a pivotal, proof-of-concept trial, it may be acceptable to use a relatively novel, more labor-intensive, less widely-available, or less cost-effective imaging-based measure if it has the capacity to detect evidence of the presence or absence of a disease-modifying effect in a short timeframe or with few subjects. For example, studies of AD animal models suggest that acute treatment with beta-amyloid anti-aggregating compounds or monoclonal antibodies may rapidly "rescue" long-term potentiation (Walsh and Selkoe 2004), and functional neuroimaging techniques can clearly reveal changes in brain activity associated with the acute administration of psychopharmacologic agents (Sperling et al. 2002; Rombouts et al. 2002). It is intriguing to imagine a potential mouse-to-man translational drug development pipeline in which drugs that show promise of safety and efficacy in animal models are considered for proof-of-concept human trials. Such trials may involve the use of a battery of neuroimaging markers as part of the inclusion criteria to select asymptomatic or mildly symptomatic patients with a particular level of AD pathology.

Finally, imaging measures also hold promise for predicting future changes in clinical status or cognitive performance, and for detecting abnormalities in brain structure or function in cognitively intact individuals prior to symptom onset. Thus, imaging biomarkers may take on even greater importance as potential surrogate markers if treatments are initiated earlier in the disease, when symptoms are very mild or absent and thus difficult to use as an outcome measure. If we are to achieve the goal of identifying disease-modifying therapies to delay the clinical symptoms of AD in cognitively intact individuals at elevated risk for the disease, we will need validated surrogate markers of disease pathophysiology. The rapid pace of developments in the field of imaging biomarkers and their preliminary use in clinical trials provides reason for optimism that progress is being made on both fronts.

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Brain Development and CNS Plasticity

Damien A. Fair and Bradley L. Schlaggar

Introduction

> One of the strong claims of developmental cognitive neuroscience is that a comprehensive understanding of mature cognition cannot be attained without understanding both normal and abnormal development of the human brain… we cannot understand how the mature system works until we understand how it is constructed in development, and we cannot fully understand that process of normal construction without understanding how development can go awry (Johnson and Pennington 1999)

Over the last 15 years there has been an almost logarithmic growth of investigations regarding the functional development of the human brain. Not surprisingly this escalation has coincided with the first developmental studies using functional magnetic resonance imaging (fMRI) (Casey et al. 1995; Hertz-Pannier et al. 1997). Since this time there has been significant progress in understanding the maturation of brain function and how this maturation might map onto behavior.

In this chapter we review basic structural and functional developmental phenomena of the human brain. We point out that much of our knowledge concerning typical development comes from studies examining neuroplasticity. We will also discuss theories of how these phenomena interact over time to yield adult function. We emphasize how such knowledge is not only directly related to our understanding of neurological and psychiatric disorders of development, but provides insight into the nature of disorders in adulthood as well.

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Structural Brain Development

The development of the mammalian nervous system encompasses a wide variety of structural and functional phenomena that extends over a protracted period of time between birth and maturity. This prolonged developmental span, thought to allow for a relatively lengthened period of juvenile learning, is also an extension for substantial brain growth, circuit organization, and myelin formation. Very little information is available detailing these events in the human or even non-human primate (Levitt 2003), but the rise of non-invasive neuroimaging in humans, discussed throughout this chapter, is beginning to change this trend.

It is now largely accepted that the mammalian cerebral cortex is composed of numerous morphologically and functionally distinct areas. At birth an initial parcellation of these areas has likely taken place; however, certain anatomical and functional areal properties change over the course of postnatal development. These changes are routinely characterized as either "progressive" or "regressive" maturational events. The following sections will summarize some of these basic developmental mechanisms that occur in the postnatal period in order to provide a framework for a discussion on neuroplasticity and its relation to developmental disorders. We note that the focus here is on those events most commonly referenced in cognitive neuroscience and psychology, but by no means do these represent the entire collection that occur throughout maturity (Cowan et al. 1984; Lidow et al. 1991; Levitt 2003).

Progressive Maturational Events

General Brain Growth

Humans exhibit substantial brain growth between birth and adulthood. Adult brains are approximately four times larger than that of infants. This brain growth is not linear. Maximal growth rates occur at full term birth, and by 6 years of age the brain is approximately 95% of the adult size. By 10 years, intracranial volumes have reached adult dimensions (Pfefferbaum et al. 1994). The bulk of this early growth comes from a variety of sources including increases in axon collaterals, dendritic arbors, synapses and dendrites (neuropil), as well as myelination. Importantly, the production of new neurons does not contribute to general brain growth postnatally (see (Kornack and Rakic 2001; Rakic 2002, 2006; Levitt 2003; Fair and Schlaggar 2008)).

Myelination

Myelination is the most commonly referenced progressive neuroanatomical process that occurs postnatally. Increased myelination proceeds from primary sensory and motor regions to association areas (Flechsig 1920; Brody et al. 1987; Paus et al. 2001; Sowell et al. 2002), roughly following the hierarchical organization introduced by

Felleman and Van Essen 1991. The current descriptions of white matter development with non-invasive techniques such as MRI mostly agree with the limited results from earlier histological work (Yakovlev and Lecours 1967; Benes et al. 1994; Paus 2005; Lenroot and Giedd 2006; Toga et al. 2006). The most consistent finding in white matter maturity is the linear protracted development, which advances into young adulthood (Pfefferbaum et al. 1994; Giedd et al. 1999a; Casey et al. 2005; Paus 2005; Toga et al. 2006).

Axonal Growth and Synaptic Exuberance

By approximately 9 months of age nearly all short and long-range axonal connections between brain regions are thought to be complete (Conel 1939–1963). However, the development of large fiber bundles must be distinguished from general synapse formation. From approximately 30 weeks gestation through the first two postnatal years there is substantial growth in the number of synaptic contacts throughout the cortex (Levitt 2003). The relative region-wise timing of this synaptic exuberance continues to be debated. Bourgeois, Rakic, and colleagues (Rakic et al. 1986; Bourgeois et al. 1994) suggest that synaptic numbers increase relatively uniformly throughout the cortex during this period, whereas Huttenlocher, Dabholkar and colleagues (Huttenlocher et al. 1982; Huttenlocher 1990; Huttenlocher and Dabholkar 1997) suggest that synaptic numbers follow the myelination pattern described above, where synaptic numbers in primary sensory areas increase prior to association areas. The accumulative synapse remodeling that occurs throughout development results in the maximum number of synapses produced in a child as being approximately 140–150% of that in the adult (Elman et al. 1996; Levitt 2003; Innocenti and Price 2005).

Regressive Anatomical Maturational Events

Pruning of Exuberant Axons and Synapses

Cell death, and consequently some axonal retraction, continues throughout development and aging (Luo and O'Leary 2005) (of note: axonal retraction also can and does occur independently of cell death, via elimination of axon collaterals (O'Leary 1989; Luo and O'Leary 2005)). Additionally, the proliferation of synapses observed early in the development mentioned above is followed by a protracted period of synaptic pruning that reaches adult levels in the late second decade of life (Huttenlocher 1979; Huttenlocher et al. 1982; Elman et al. 1996; Huttenlocher and Dabholkar 1997; Casey et al. 2005). These regressive processes are not random. They are selective and reduce connectivity between specific regions. They also span a protracted period of time, are in large part activity dependent, and result in distinct functional areas (Ebbesson 1980; Greenough et al. 1987; Luo and O'Leary 2005).

The gray matter signal revealed with MRI most likely represents a collection of cell bodies, axons, dendrites, glia and blood vessels (Gogtay et al. 2004; Paus 2005). Signal changes measured after late childhood likely reflect synapse reduction (Paus 2005). Unlike myelination, gray matter changes are mostly non-linear. Studies differ on the details (Giedd et al. 1999a; Sowell et al. 2001, 2003; Gogtay et al. 2004; Paus 2005; Toga et al. 2006), but, in general, there appears to be a differential peak in gray matter volume (or density) between childhood and early adolescence that begins to decline first in sensorimotor regions and later rostrally in dorsolateral prefrontal regions and caudally/laterally in the parietal and temporal cortex (Sowell et al. 2001; Gogtay et al. 2004; Paus 2005). Also see Toga et al. 2006 for a detailed review.

Functional Brain Development

While there are other techniques that contribute to our knowledge of functional brain development (e.g., EEG/ERP), the emphasis in this section will be on functional MRI. fMRI allows one to relate changes in task-driven activation patterns over development. It can be used to study neurophysiologic changes that accompany changes in brain anatomy and connectivity to inform us of how complex behavior develops from birth into adulthood. Since early developmental fMRI studies (Casey et al. 1995) the number of investigations studying functional brain development with fMRI has substantially increased. A detailed assessment of all of these studies is beyond the scope of this discussion, but some general developmental trends are beginning to emerge.

Recent evidence suggests that the general rules of structural maturation discussed above (e.g., primary sensory and motor cortex development followed by association cortex for myelination) do not necessarily hold true for "functional" maturation. In children, association cortex, despite its structural immaturity with regard to myelination and synaptic pruning, is not sitting dormant. Regions in association cortex are actively involved in information processing in childhood, and often activate in the same way as would be observed in the adult. For example, Brown et al. 2005, using a large cross-sectional dataset of subjects ages 7–35 years performing a set of lexical processing tasks, found a number of regions in association cortex that were equally active in children and adults (see Fig. 1c). In contrast, many early processing (i.e., primary sensory and motor) regions, thought to mature first with regard to myelination and pruning, showed significant functional changes over age (see Fig. 1c). Work by several others has shown similar types of dynamics in different tasks (Mills and Neville 1997; de Haan 1998; Luna et al. 2001; Turkeltaub et al. 2003; Johnson et al. 2005). In addition, these types of findings are not specific to fMRI, as other techniques such as EEG/ERP have shown adult-like activations in regions typically viewed as structurally immature (Johnson et al. 2005).

Together, these data suggest that a region's structural maturity is not a sine qua non of functional maturity and a given region's functional maturity does not indicate whether, by structural indices, it is mature. In fMRI, for any given task, there is a natural

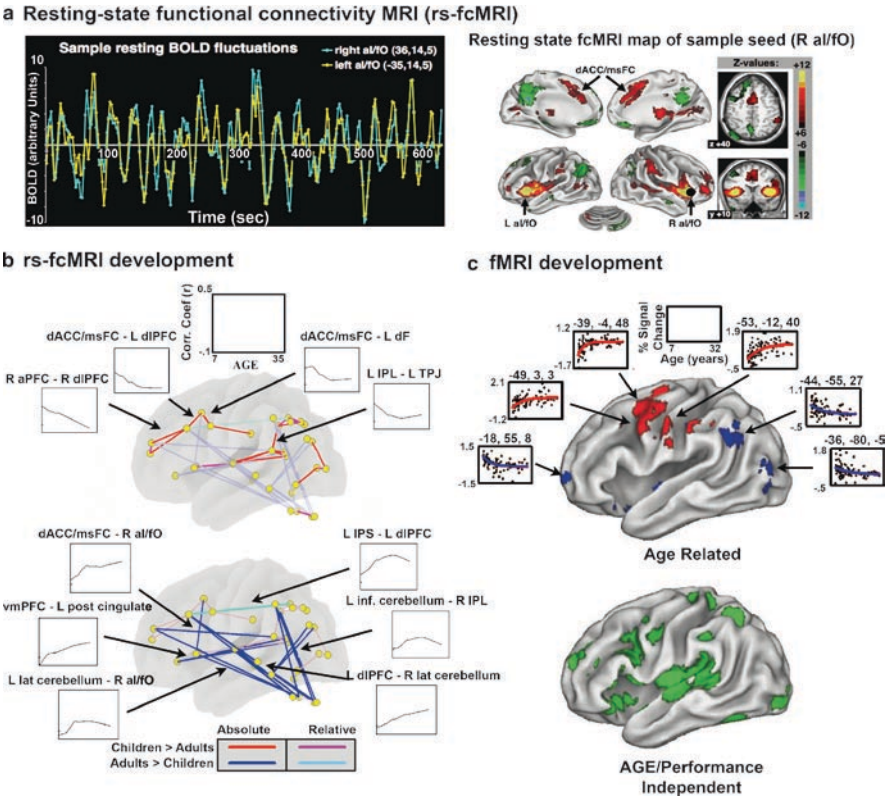

Fig. 1 Both the development of spontaneous brain activity and functional brain activity show multiple types of changes over age. (**a**) rs-fcMRI is measured by calculating the correlations in spontaneous BOLD fluctuations between brain regions. Spontaneous resting state BOLD fluctuations for two sample regions (right aI/fO and left aI/fO), measured in a single subject are shown to the *left*. To the *right* a voxelwise rs-fcMRI map is shown, which depicts all brain voxels whose spontaneous BOLD fluctuations correlate with the sample seed region, right aI/fO (4, 16, 36). (**b**) The natural trajectory over development of correlated spontaneous activity (i.e., rs-fcMRI) shows an increase in long-range functional connections over age and a decreased short-range connectivity with age. Regions involved with task level control in the left- and right-hemisphere are placed on a transparent brain to aid with visualization. *Red* and *blue lines* highlight significant differences for connections between a group of children (7–9 years) and adults (21–31 years). *Light blue* and *pink lines* highlight connections present in both children and adults, but that still differed significantly in connection strength between groups. Selected LOWESS curves reveal how correlations for the selected connection change over age. (**c**) The natural trajectory of functional activity (i.e., fMRI) over development for three controlled lexical association tasks shows regions that "grow-up," regions that "grow-down," and regions that stay the same. Age/Performance independent regions (or regions that do not change activity over age) are shown in *green*. Regions that "grow up" over age are shown in *red*, while regions that "grow down" over age are shown in *blue*. For selected regions, non-linear regression curves reveals peak brain activity plotted as a function of age. *BOLD* blood oxygen level dependent signal; *aI/fO* anterior insula/frontal operculum; *dACC/msFC* dorsal anterior cingulated/medial superior frontal cortex; *dlPFC* dorsolateral prefrontal cortex; *dF* dorsal frontal; *aPFC* anterior prefrontal cortex; *IPL* inferior parietal lobule; *IPS* intraparietal sulcus; *mC* mid cingulated; *vmPFC* ventromedial prefrontal cortex; *TPJ* temporoparietal junction. (**a**) Adapted from Dosenbach et al. (2007). (**b**) Adapted from Fair et al. (2007a). (**c**) Adapted from Brown et al. (2005) with permission

trajectory of progressive and regressive functional changes, as well as maintenance of function, that do not necessarily map onto structural development. The implications for these general observations will be discussed in more detail below.

Development of Spontaneous Neural Activity

As early as 1875, spontaneous synchronized neural activity has been used to study various aspects of adult brain organization (Caton 1875; Berger 1929, 1933; Swartz and Goldensohn 1998). However, despite the passing of over 130 years since its recognition, there remains uncertainty as to the role of intrinsic spontaneous brain activity toward brain function. In adults, spontaneous correlated activity has been suggested to be important for gating information flow (Varela et al. 2001), building internal representations (Varela et al. 2001; Buckner and Vincent 2007; Raichle and Snyder 2007), and maintaining mature network relationships (Varela et al. 2001; Buckner and Vincent 2007; Raichle and Snyder 2007). Much less work has been done in regards to development, but there are suggestions that spontaneous activity is important for the establishment of early cortical patterns (e.g., ocular dominance columns or ODC) (Sur and Leamey 2001; Katz and Crowley 2002; Luo and O'Leary 2005; Price et al. 2006) and may represent (in a Hebbian sense) a history of repeated co-activation between regions (Bi and Poo 1999; Miltner et al. 1999; Dosenbach et al. 2007; Fair et al. 2007a, 2008; Seeley et al. 2007; Kelly et al. 2009).

While the formation of ODC occurs fairly early after birth, little work has been done concerning the development of spontaneous activity in later postnatal years. With that said, the relatively new and increasingly utilized technique of resting state functional connectivity MRI (rs-fcMRI) is quickly changing this trend (Fig. 1a). Resting state fcMRI is based on the discovery that spontaneous low-frequency (< ~0.1 Hz) blood oxygen level dependent (BOLD) signal fluctuations in sometimes distant, but functionally related gray matter regions, show strong correlations at rest (Biswal et al. 1995). These low frequency BOLD fluctuations appear to relate to spontaneous neural activity (Biswal et al. 1995; Leopold et al. 2003; Nir et al. 2006). In effect, rs-fcMRI evaluates regional interactions that occur when a subject is not performing an explicit task (i.e., subjects are "at rest") (Biswal et al. 1995; Lowe et al. 1998; Greicius et al. 2003; Beckmann et al. 2005; Fox et al. 2005; Salvador et al. 2005; Achard et al. 2006; Damoiseaux et al. 2006; Nir et al. 2006; Dosenbach et al. 2007; Fair et al. 2007a, b). Because rs-fcMRI does not require active engagement in a behavioral task, and it is unburdened by experimental design, subject compliance, or training demands, it is becoming a frequently used tool for examining changes in network structure in disease (Greicius et al. 2004; Just et al. 2007; Castellanos et al. 2008; Church et al. 2009), in aging (Greicius et al. 2004; Andrews-Hanna et al. 2007), and across development (Fair et al. 2007a, 2008; Fransson et al. 2007; Kelly et al. 2009; Lin et al. 2008).

Rs-fcMRI has proven very effective for the study of the maturation of cortical networks (Fair et al. 2007a, 2008; Fransson et al. 2007; Kelly et al. 2009).

In a study examining the development of two brain networks important for top-down control (Dosenbach et al. 2006, 2007, 2008), Fair et al, found that the strength of correlation coefficients (i.e., functional connections) *between* the two control networks is greater in children, and declines over age, while the strength of correlation coefficients linking regions *within* each of the networks increases over age. These results were contextualized with findings that suggested that children have a greater proportion of short-range functional connections (i.e., connections between regions close in space) that tend to decrease in strength over age, and in contrast, long-range functional connections (i.e., connections between regions more distant in space) that tend to increase in strength over age. The trend towards an increase in distant, long-range connections and the decrease in local, short-range connections (see Fig. 1b) results in specialized functional networks that have been thoroughly described in the adult literature and have been implicated in several disease processes (Raichle et al. 2001; Fox et al. 2005; Fransson 2005; Dosenbach et al. 2006, 2007, 2008; Fair et al. 2007a, 2008).

There are several aspects of these findings that deserve further discourse. As with the fMRI work presented above, these findings provide insight regarding the trajectory of regional brain interactions over age. In addition, the results not only supply a context with which to consider atypical brain development (discussed below), but also a context for considering the nature of mature function as well. For example, Brown et al. (discussed above) examined how brain activation changed over age in relation to the development of controlled lexical processing (Fig. 1c). Multiple sorts of regional developmental trajectories were observed throughout the cortex, including regions that "grew up," regions that "grew down," and many regions that were active but did not change at all with age (or with performance). To highlight how rs-fcMRI can inform discussion regarding the function of any given region, consider a region that has received a great deal of attention in the literature regarding executive functions, dorsal the anterior cingulate cortex (dACC). In Brown et al, the dACC was an age/performance independent region, meaning that for the particular set of tasks its activation profile showed equal activity in children and adults, independent of accuracy or reaction time (see Fig. 2). A reasonable conclusion to draw from these results is that for this task the dACC functions similarly in children and adults. However, if one observes the context of this activity in relation to how this region is functionally connected in children compared to adults, the consideration becomes more complex (Fig. 2). As noted above, the dACC has a strikingly different functional connectivity profile and regional relationships in children compared to adults (Fig. 2), suggesting that perhaps despite equivalent activity over age for this region, its functional role may be different. Because network relationships change, activity in many regions may "mean" something different in a child compared to an adult.

This idea is consistent with findings regarding perinatal ischemic stroke and neuroplasticity (which will be discussed in more detail below). Evidence suggests that while language function eventually develops normally (or near normally) in children with perinatal ischemic stroke, the developmentally transient deficits that are observed in children as a consequence of the stroke manifest quite differently

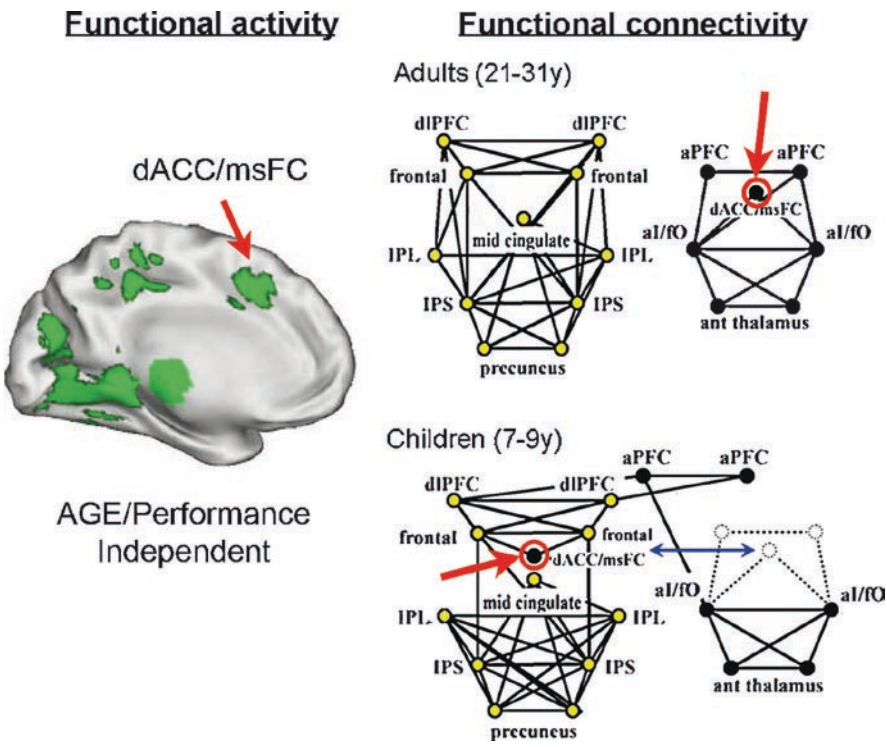

Fig. 2 Same activity, different context. Because network relationships change, activity in many regions may "mean" something different in a child compared to an adult. For the lexical association tasks from Brown et al. 2005, the anterior cingulate (dACC/msFC) is an age/performance independent region, meaning that for this particular set of tasks its magnitude of activity is the same in children and adults. However, the dACC/msFC has a strikingly different resting functional connectivity profile and regional relationships in children compared to adults. These data suggest that perhaps despite equivalent activity over age for this region, its functional role may be different. Because network relationships change, activity in many regions may "mean" something different in a child compared to an adult. Adapted from Fair et al. (2007a) with permission and Brown et al. (2005) with permission

than comparably placed lesions in the adult brain. For example, Thal and Bates (Thal et al. 1991) found that early delays with word comprehension were more common with *right* temporal lesions, rather than *left* temporal (wernicke's area) lesions as commonly seen in adult populations. Left temporal "Wernicke" type lesions in children resulted in expressive delays, a symptom most commonly observed with left inferior frontal gyrus (Broca's) lesions in adult patients. The fact that adults who had acquired unilateral brain injuries in language centers during childhood do not manifest the same deficits as an adult who acquired a similar lesion in adulthood indeed suggests that the same brain regions in children may have different functions than in the adult.

Theories of Functional Brain Development

The majority of work that has attempted to link functional, anatomical, and behavioral development in humans has come from a maturational perspective (Atkinson 1984; Diamond and Goldman-Rakic 1989; Sowell et al. 1999; Pujol et al. 2006). The maturational viewpoint presumes a latent adult functional organization and posits that the anatomical or functional maturation of any specific brain region will relate directly to the emergence of newly acquired motor, sensory, and cognitive skills (Johnson 2001). While this viewpoint often overwhelms the field, it is now becoming clear that brain development is dynamic. Interactions between brain regions are constantly changing over age, leading to an assortment of adjustments with regard to the contribution of a brain region to any particular task.

One view of normal functional brain development advanced by Johnson and colleagues, termed interactive specialization, considers the dynamic interplay between structural and functional changes observed over development (Johnson 2000a, 2000b, 2001; Johnson and Munakata 2005). Johnson points out that although cortical regions and pathways have biased information processing properties at birth due to anatomical connectivity, they are much less selective than in adults (i.e., they are "broadly tuned"). Interactive specialization predicts that shortly after birth, large sets of regions and pathways will be partially active during specific task conditions; however, as these pathways interact and compete with each other throughout development, selected regions will come online, be maintained, or become selectively activated or "tuned" as particular pathways dominate for specific task demands. Thus, regional specialization relies on the evolving and continuous interactions with other brain regions over development.

Developmental Neuroplasticity

The term neuroplasticity is used in a variety of contexts but often refers to the brain's ability to organize (or reorganize) in a novel way in response to some perturbation. Perturbations can come in the form of experimental circumstances, natural experience (e.g., extended piano training), sensory loss (e.g., blindness), or intrinsic conditions (e.g., infarction). However, circumstances that drive neuroplasticity are not limited to aberrant or clinical situations. In fact, much work regarding the nature of neuroplasticity has come from studies of learning (Buonomano and Merzenich 1998)

While the specific plastic mechanisms responsible for translating electrical activity into functional and structural changes at the synapse is beyond the scope of this discussion (Buonomano and Merzenich 1998; Sur and Leamey 2001; Majewska and Sur 2006), what will hopefully become clear in the following sections is that the plastic processes that respond to atypical circumstances are very likely the same ones that guide the trajectory of the typical developing brain.

The classic studies of developmental plasticity investigated the consequences of peripheral receptor deprivation on the development of central structures. Nobel Prize winning work by Hubel and Wiesel in the 1960s, in kittens, demonstrated that monocular deprivation during the critical period reveals plastic mechanisms in which the size of ocular dominance column representation corresponding to the sutured eye is significantly reduced. Conversely, layer IV representation of the unaffected eye increases in size.

In the rodent somatosensory system, a comparable line of investigation emerged in the 1970s spearheaded by Tom Woolsey and Herb Killackey (Van der Loos and Woolsey 1973; Kaas et al. 1983; Schlaggar and O'Leary 1993). These investigators demonstrated that the typical differentiated structural units of rodent somatosensory cortex (i.e., barrels) corresponds to a large mystacial vibrissae on the contralateral face of the rodent, and that this organization is dependent upon an intact sensory periphery during a critical period. Lesions to the infraorbital branch of the trigeminal nerve during development wipe out the so-called barrel field. Likewise, removal of a row of whiskers during development prevents the differentiation of the corresponding row of barrels in the contralateral somatosensory cortex.

These early animal studies of the 1960s and 1970s undoubtedly changed the discourse regarding the nature of the development of cortical organization and the differentiation of cortical area-specific features. These studies not only provided insight regarding the brain's capabilities to organize or reorganize in response to drastic disturbances (i.e., neuroplasticity) but also gave us a keen awareness regarding the role of afferent-mediated, activity-dependent mechanisms in the typically developing brain. These studies generally focused on a particular sensory system but had implications for general principles of how the mammalian cortex is divided up or parcellated into specific areas.

The topic of areal differentiation and the relative contributions of intrinsic (i.e., genetic) and extrinsic (i.e., afferent-mediated) mechanisms toward parcellation sparked intense debate at one point (Rakic 1988, O'Leary 1989). This debate has largely settled and it is now generally agreed that an interplay between intrinsic and extrinsic mechanisms account for typical development and plastic response to perturbation. As mentioned above, there is considerable evidence to suggest that the initial broad parcellation of cortex is regulated by molecular determinants intrinsic to the developing cortex (Sur and Leamey 2001); however, this initial parcellation is subsequently refined and can even be altered once thalamic innervation takes place (Sur and Leamey 2001; Lopez-Bendito and Molnar 2003; Sur and Rubenstein 2005).

For example, Schlaggar and O'Leary showed that embryonic rodent visual cortex when transplanted to parietal somatosensory cortex in newborn rats can develop architectural and connectional features unique to somatosensory cortex (Schlaggar and O'Leary 1991). In another example, Sur et al. (Sur et al. 1988; Sur and Leamey 2001) have shown that in ferrets, when normal visual cortical targets and normal auditory inputs are experimentally lesioned during development, visual thalamic afferents redirected to innervate the auditory cortex can produce physiological function in the auditory cortex appropriate to vision (Sur et al. 1988 ; Sur and Leamey 2001). These rewired ferrets perceive visual cues as visual even though the

auditory cortex is being activated by the stimuli and despite the maintenance of some aspects of the natural auditory cortical organization. Other experiments have demonstrated that thalamic innervation can modify initially broad gene expression such that the zone of expression will match that of the thalamic innervation (Gitton et al. 1999). While work on areal development in humans and non-human primates has been limited, the potential may be on the horizon using a rs-fcMRI technique to non-invasively identify functional areas in primates (Cohen et al. 2008).

The types of brain changes and functional responses to the manipulations described in animal models above are not limited to experimental conditions. Naturally occurring perturbations also give insight into the capacity of the brain to organize itself in novel ways. In humans the two most featured examples are early blindness and perinatal stroke. Sensory deprivation observed in the early blind leads to the visual cortex becoming functionally active with the tactile sensations associated with Braille reading (Sadato et al. 1996; Buchel 1998; Burton et al. 2002b). Functional neuroimaging has suggested that this activity may not only be associated with tactile sensation per se, but also with complex linguistic operations (Burton et al. 2002a).

As mentioned above, children with perinatal stroke are capable of acquiring relatively normal cognitive functions, such as language, after experiencing a cortical insult that in adults would likely lead to devastating lifetime disabilities (Bates 1999). The fact that persistent language deficits often do not ensue in children with extensive left hemisphere lesions (Hecaen 1976 ; Vargha-Khadem et al. 1985) or, in the most extreme case, left hemispherectomies (Strauss and Verity 1983; Vargha-Khadem and Polkey 1992) only underscores the brain's robust adaptive capabilities. The phenomenon of this developmental plasticity has long been accepted, but its underlying neurobiological mechanisms are not well characterized. Functional neuroimaging has provided mixed results, with some investigations suggesting that the development of normal language in the presence of an early lesion is accomplished by the functional reorganization of homotopic regions in the contralateral hemisphere (Guzzetta et al. 2008; Tillema et al. 2008). Other reports suggest that intrahemispheric organization (Liegeois et al. 2004) supports normal development, while still other work suggests that an alternate functional organization and developmental time course ensues, which is variable and largely depends on the timing, location, and size of the stroke (Fair et al. 2006). Several factors may be contributing to these discrepancies including the sampled populations, as well as several methodological differences (Fair et al. 2006).

With this said, work conducted by Webster, Ungerleider, and Bachevalier in non-human primates provides a potential mechanism that accounts for the robust adaptive capabilities after perinatal stroke (Webster et al. 1994, 1995a, b). They have shown that in normally behaving adult Macaques, visual recognition memory requires the interaction of inferior temporal lobe (area TE) and the medial temporal lobe (entorhinal and perirhinal areas). While there is considerable overlap between adult and infant anatomical connections of the inferior temporal cortex, there is substantial elimination and refinement of initially widespread projections that eventually defines the adult connectional relationships between the inferior and

medial temporal lobe structures. For example, while area TE projects to the medial temporal lobe in infants and adults, an adjacent region, area TEO, projects to the medial lobe structures only in infant monkeys – suggesting elimination of transient TEO projections (Webster et al. 1991a, 1991b, 1994, 1995b; Rodman and Consuelos 1994). As opposed to lesions of TE in the adult animal, TE lesions in infant monkeys results in the sparing of visual recognition memory. This ability, it appears, is partially subserved by the maintenance of the normally transient TEO projections.

The combination of these studies pertaining to neuroplasticity suggests that the activity dependent "plastic" mechanisms that drive the characterization of the cortex in atypical circumstances are likely the same as those driving cortical characterization in normal circumstances as well.

Neuroplasticity Is Not Always Beneficial and Often Insufficient

Although mostly advantageous to the developing organism, plastic changes are not always beneficial and often times are insufficient to result in clinically satisfactory recovery of function. In perinatal ischemic stroke, motor recovery, although more favorable than that seen in adults, does not recover to the same degree as language. Similar observations have been reported with regard to spatial attention (Stiles et al. 2003a). Visuo-spatial deficits resulting from perinatal stroke, although more mild than when the injury occurs in adulthood, persist beyond early childhood and, in contradistinction to the situation for language, are associated with similarly located lesions observed to produce visuospatial neglect in adults (Stiles et al. 2003b).

Other conditions exist where extensive neuroplastic mechanisms are detrimental to function. For instance, plasticity associated with repeated movements can lead to focal hand dystonia (Bara-Jimenez et al. 1998). The pain associated with phantom limb syndrome is also believed to be associated with cortical reorganization following amputation (Flor et al. 1995). It has been suggested, at least in adults, that altered brain activity measured with fMRI in the setting of stroke can impair performance (Martin et al. 2004; Naeser et al. 2005a, b).

Neuroplasticity and Its Relationship to Neuropsychiatric Disorders

Due to the rise of non-invasive neuroimaging there continues to be a growing number of investigations regarding the functional neuroanatomy of several developmental neuropsychiatric disorders. Most of these investigations using fMRI (or structural MRI) attempt to identify an abnormal region or set of regions that corresponds to the illness in question. While these investigations continue to provide insight regarding the functional neuroanatomy of several disease states, they also provide a salient example of where neuroplasticity appears to be insufficient to support normal function. It is unclear why the plastic mechanisms that help sup-

port relatively normal cognitive functions after disturbances, such as stroke, are unable to compensate for the presumed "abnormal" region (or regions) responsible for a particular neuropsychiatric disorder. This question has recently led several investigators to suggest that many developmental neuropsychiatric disorders and even those that manifest in adulthood, may be the result of abnormal plastic mechanisms themselves (Agid et al. 2007). While a functional imaging study may reveal an abnormally behaving brain region for any particular task, abnormal neuroplasticity may alter the typical trajectory of many developmental interactions across broad cortical and subcortical networks. As stated by Anthony Grace, "many, if not most, psychiatric disorders emerge as a developmental consequence of an underlying genetic predisposition interacting with environmental factors. The fact that the pathology is probably present from birth, but does not become expressed until late adolescence or early adulthood, suggests that the disorder is not a direct result of a lesion, but instead is due to a series of alterations…" (Agid et al. 2007).

There is growing evidence supporting this idea. PTSD, Schizophrenia, ADHD, Bipolar disorder, and Parkinson's disease have all been linked to abnormal plastic mechanisms (Giedd et al. 1999a, b; Rapoport et al. 1999; Saugstad 2001; Rauch et al. 2006; Agid et al. 2007; Rapoport and Gogtay 2008). In addition, there are several neuroimaging reports that suggest many of these psychiatric illnesses have their roots in early development. Recent work in autistic patients using rs-fcMRI has shown decreased integration between frontal and parietal brain regions (Just et al. 2007) that typically increase their interactions over age (Fair et al. 2007a). Similar findings have been reported for ADHD (Castellanos et al. 2008), which has also been related to a delayed trajectory of cortical maturation (Shaw et al. 2007). Lastly, recent work in Tourette syndrome, using rs-fcMRI and the typical developmental trajectories shown in Fig. 1b as context (Fair et al. 2007a), has shown "developmentally delayed" architecture across multiple functional networks (Church et al. 2009).

Chapter Review and Clinical Implications

Throughout this chapter we have laid out a number of related developmental phenomena (i.e., myelination, pruning, spontaneous activity, neuroplasticity, etc), which interact to produce a common trajectory for the typically developing brain. We have also pointed out how it is likely that the same neuroplastic mechanisms that support normal development continue to interact despite, and with, perturbations to yield an alternative functional neuroanatomy capable of supporting normal cognitive functions. In addition, we highlight the growing number of investigators who believe that abnormal neuroplasticity may contribute to many neuropsychiatric disorders by altering typical developmental trajectories of many brain interactions across broad cortical and subcortical networks. This may be true for disorders of development and those illnesses that manifest in adulthood as well.

The implication here, as paraphrased by Anthony Grace, is that if such disorders that manifest in childhood, adolescence, or even adulthood are indeed due to altered plasticity during maturation, then it might be that the most effective means to address the pathology is through medicinal or behavioral interventions that target such plasticity and do so well before the onset of symptoms (Agid et al. 2007). The idea is parallel to a concept referred to by Susan Anderson as "neuronal imprinting," where a drug administered early in development may not appear to have any effect at the time, but later in life, well after the initial exposure, drug effects emerge (Hess 1972; Andersen and Navalta 2004). Neuronal imprinting is typically discussed in the context of the long-term side effects of prescribing medications to children (Andersen and Navalta 2004); however, it is not clear why "neuronal imprinting" should always result in adverse effects. To the contrary, it may be possible to actually "correct" an abnormal developmental trajectory with behavioral or medicinal interventions. Evidence for this theory may exist in the ADHD literature, where the decline of ADHD symptoms in adulthood (Klein and Mannuzza 1991; Teicher et al. 1997) may have a relationship to stimulant treatment in childhood that corrects an abnormal developmental trajectory (Andersen and Navalta 2004).

Exploring the validity of these ideas will be challenging. It will likely require continued advancement of techniques that are capable of non-invasively measuring developmental interactions and trajectories equally and comparably in both human and animal models. MRI technologies including fMRI and rs-fcMRI will undoubtedly assist in these efforts. Irrespective of the challenges, the evidence linking many childhood, adolescent, and even adult CNS disorders to developmental phenomena, may suggest that the most effective means of treatment for many neurologic and psychiatric illnesses moving forward will require a deep understanding of typical and atypical brain development.

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Imaging in CNS Disease States: PTSD

J. Douglas Bremner

Lasting Effects of Posttraumatic Stress Disorder

Posttraumatic stress disorder (PTSD) affects about 8% of Americans at some time in their lives (Kessler et al. 1995). For many trauma victims, PTSD can be a lifelong problem (Saigh and Bremner 1999). The development of effective treatments is limited by gaps in knowledge about the underlying neurobiological mechanisms that mediate symptoms of PTSD. Until 12 years ago, no brain imaging studies had ever been performed in patients with PTSD or other stress related psychiatric disorders. The past decade has seen an explosion of research using brain imaging to assess changes in the brain in PTSD (Bremner 2005). These studies have implicated the amygdala, hippocampus, and the medial prefrontal cortex (including the anterior cingulate) in PTSD and other stress related psychiatric disorders. This chapter reviews brain imaging studies in the field of PTSD, and integrates them with the basic science findings on the neuroscience of stress.

Neural Circuits of PTSD

PTSD is characterized by specific symptoms, including intrusive thoughts, hyperarousal, flashbacks, nightmares, and sleep disturbances, changes in memory and concentration, and startle responses. Symptoms of PTSD are hypothesized to represent the behavioral manifestation of stress-induced changes in brain structure and function. Stress results in acute and chronic changes in the neurochemical systems and specific brain regions, which result in long-term changes in brain "circuits," involved in the stress response (Vermetten and Bremner 2002a, b; Bremner 2002a; Pitman 2001).

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The brain regions that are felt to play an important role in PTSD include the hippocampus, the amygdala, and the medial prefrontal cortex.

Preclinical and clinical studies have shown alterations in memory function in PTSD patients (Elzinga and Bremner 2002), as well as changes in a circuit of brain areas, including the hippocampus, the amygdala, and the medial prefrontal cortex, that mediate alterations in memory (Bremner 2003). The hippocampus, a brain area involved in verbal declarative memory, is very sensitive to the effects of stress. Stress in animals was associated with damage to the neurons in the CA3 region of the hippocampus (which may be mediated by hypercortisolemia, decreased brain derived neurotrophic factor, and/or elevated glutamate levels) and inhibition of neurogenesis (Gould et al. 1998; Magarinos et al. 1996; McEwen et al. 1992; Nibuya et al. 1995; Sapolsky et al. 1990, 1996).

Antidepressant treatments were shown to block the effects of stress and/or promote neurogenesis (Nibuya et al. 1995; Malberg et al. 2000; Czeh et al. 2001; Santarelli et al. 2003; Lucassen et al. 2004). Animal studies have demonstrated several agents with potentially beneficial effects on stress-induced hippocampal damage. It has been found that phenytoin blocks the effects of stress on the hippocampus, probably through modulation of excitatory amino acid induced neurotoxicity.(Watanabe et al. 1992a) Other agents, including tianeptine, dihydroepiandosterone (DHEA), and fluoxetine have similar effects (Malberg et al. 2000; Czeh et al. 2001; Lucassen et al. 2004; Garcia 2002; D'Sa and Duman 2002; Duman et al. 1997, 2001; Duman 2004; McEwen and Chattarji 2004). These medications may share a common mechanism of action through upregulation of the cAMP response element binding protein (CREB) that leads to regulation of the expression of specific target genes involved in structural modeling of the hippocampus. Such treatment effects on BDNF and trkB mRNA, can have long-term effects on brain structure and function. There is new evidence that neurogenesis is necessary for the behavioral effects of antidepressants (Santarelli et al. 2003; Watanabe et al. 1992b) although this continues to be a source of debate (Duman 2004; Henn and Vollmayr 2004).

In addition to the hippocampus, other brain structures including the amygdale and prefrontal cortex have been implicated in a neural circuitry of stress. The amygdala is involved in memory for the emotional valence of events, and plays a critical role in the acquisition of fear responses (Davis 1992). The medial prefrontal cortex includes the anterior cingulate gyrus (Brodmann's area 32) and the subcallosal gyrus (area 25), as well as orbitofrontal cortex. Lesion studies have demonstrated that the medial prefrontal cortex modulates emotional responsiveness through the inhibition of amygdala function (Morgan et al. 1993). Studies show that the neurons of the medial prefrontal cortex play an active role in the inhibition of fear responses that are mediated by the amygdala (Milad and Quirk 2002; Milad et al. 2006). Conditioned fear responses are extinguished following repeated exposure to the conditioned stimulus in the absence of the unconditioned (aversive, e.g., electric shock) stimulus. This inhibition appears to be mediated by the medial prefrontal cortical inhibition of amygdala responsiveness. Animal studies also show that early stress is associated with a decrease in the branching of neurons in the medial prefrontal cortex (Radley et al. 2004).

Changes in Brain Structure in PTSD

Studies in PTSD are consistent in the changes in cognition and brain structure. Multiple studies have demonstrated verbal declarative memory deficits in PTSD (Elzinga and Bremner 2002; Buckley et al. 2000; Brewin 2001; Golier and Yehuda 1998). Patients with PTSD secondary to combat (Vasterling et al. 1998; Bremner et al. 1993; Golier et al. 1997; Yehuda et al. 1995; Uddo et al. 1993) and childhood abuse (Bremner et al. 1995a, 2004a) were found to have deficits in verbal declarative memory function based on neuropsychological testing. Studies using a variety of measures (including the Wechsler Memory Scale, the visual and verbal components of the Selective Reminding Test, the Auditory Verbal Learning Test, Paired Associate Recall, the California Verbal New Learning Test, and the Rivermead Behavioral Memory Test), found specific deficits in verbal declarative memory function, with a relative sparing of visual memory and IQ (Vasterling et al. 1998, 2002; Bremner et al. 1993, 1995a; Golier et al. 1997; Yehuda et al. 1995; Uddo et al. 1993; Gilbertson et al. 2001; Jenkins et al. 1998; Moradi et al. 1999; Roca and Freeman 2001; Barrett et al. 1996; Gil et al. 1990; Sachinvala et al. 2000). These studies have been conducted both in patients with PTSD related to Vietnam combat (Vasterling et al. 1998, 2002; Bremner et al. 1993; Golier et al. 1997; Yehuda et al. 1995; Uddo et al. 1993; Gilbertson et al. 2001; Roca and Freeman 2001; Barrett et al. 1996; Sachinvala et al. 2000), rape (Jenkins et al. 1998) the Holocaust (Golier et al. 2002, Yehuda et al. 2005a, b) adults with early childhood abuse (Bremner et al. 1995a), and traumatized children (Moradi et al. 1999). Returning Iraq soldiers were shown to have decreases in verbal memory performance compared to their pre-deployment baselines, with greater verbal memory deficits in veterans with high levels of PTSD symptoms (Vasterling et al. 2006). These studies suggest that traumas such as early abuse with associated PTSD result in deficits in the verbal declarative memory.

Studies have also shown changes in hippocampal volume. Vietnam veterans with PTSD were originally shown to have 8% smaller right hippocampal volume based on MRI relative to controls matched for a variety of factors such as alcohol abuse and education ($p<0.05$); smaller volume was correlated with deficits in verbal declarative memory function as measured with the WMS (Bremner et al. 1995b). A second study from our group showed a 12% reduction in the left hippocampal volume in 17 patients with childhood abuse-related PTSD compared to 17 case-matched controls; this was significant after controlling for confounding factors (Bremner et al. 1997a). Smaller hippocampal volume was shown to be specific to PTSD within the anxiety disorders, and was not seen in panic disorder (Narayan et al. 1999). Gurvits et al. 1996 showed bilateral hippocampal volume reductions in combat-related PTSD compared to combat veterans without PTSD and normal controls. Combat severity was correlated with volume reduction. Stein et al. 1997 found a 5% reduction in the left hippocampal volume. Other studies in PTSD have found smaller hippocampal volume and/or reductions in NAA, a marker of neuronal integrity (Lindauer et al. 2005, 2006, 2004a; Bremner et al. 2003a; Freeman et al.

1998; Gilbertson et al. 2002; Schuff et al. 2001; Villarreal et al. 2002; Shin et al. 2004a; Emdad et al. 2006; Mahmutyazicioglu et al. 2005; Irle et al. 2005; Li et al. 2006; Hedges et al. 2003). Some studies have found smaller hippocampal volume in PTSD subjects compared to trauma exposed non PTSD subjects (Bremner et al. 2003a) while others have not, finding reductions in both trauma exposed non PTSD and trauma exposed PTSD relative to non trauma exposed non PTSD subjects (Winter and Irle 2004). Studies in childhood (De Bellis et al. 1999, 20001; Carrion et al. 2001) PTSD did not find hippocampal volume reduction, although reduced NAA (indicating loss of neuronal integrity) was found in the medial prefrontal cortex in childhood PTSD (De Bellis et al. 2000). Some studies of new onset or recent PTSD did not find changes in hippocampal volume (Bonne et al. 2001; Notestine et al. 2002), while others showed a reduction (Wignall et al. 2004). In a recent meta-analysis, we pooled data from all of the published studies and found smaller hippocampal volume for both the left and the right sides, equally in adult men and women with chronic PTSD, and no change in children (Kitayama et al. 2005). Another recent meta-analysis had similar findings (Smith 2005). More recent studies of holocaust survivors with PTSD did not find a reduction in hippocampal volume (Golier et al. 2005) although PTSD patients who developed PTSD in response to an initial trauma had smaller hippocampal volume compared to those who developed PTSD after repeated trauma, suggesting a possible vulnerability of smaller hippocampal volume (Yehuda et al. 2007). Several studies have shown that PTSD patients have deficits in hippocampal activation while performing a verbal declarative memory task (Bremner et al. 2003a; Shin et al. 2004a) or a virtual water maze task (Astur et al. 2006). Both hippocampal atrophy and hippocampal-based memory deficits reversed with treatment with the SSRI, paroxetine, which has been shown to promote neurogenesis (the growth of neurons) in the hippocampus, in preclinical studies (Vermetten et al. 2003). We hypothesize that stress-induced hippocampal dysfunction may mediate many of the symptoms of PTSD which are related to memory dysregulation, including both explicit memory deficits as well as fragmentation of memory in abuse survivors. It is unclear at the current time whether these changes are specific to PTSD, whether certain common environmental events (e.g., stress) in different disorders lead to similar brain changes, or whether common genetic traits lead to similar outcomes.

In addition to the hippocampus, other brain structures have been implicated in a neural circuitry of stress, including the amygdala and the prefrontal cortex. The amygdala is involved in memory for the emotional valence of events, and plays a critical role in the acquisition of fear responses. The medial prefrontal cortex includes the anterior cingulate gyrus (Brodmann's area 32) and the subcallosal gyrus (area 25), as well as the orbitofrontal cortex. Lesion studies have demonstrated that the medial prefrontal cortex modulates emotional responsiveness through the inhibition of amygdala function. Conditioned fear responses are extinguished following repeated exposure to the conditioned stimulus (in the absence of the unconditioned (aversive, e.g., electric shock) stimulus. This inhibition appears to be mediated by the medial prefrontal cortical inhibition of amygdala responsiveness. The insula plays a critical role in integrating the physiological stress response.

Animal studies also show that early stress is associated with a decrease in the branching of neurons in the medial prefrontal cortex (Radley et al. 2004). Several studies have found smaller anterior cingulate volume based on MRI measurements in PTSD (Rauch et al. 2003; Yamasue et al. 2003; Woodward et al. 2006), including women with abuse and PTSD (Kitayama et al. 2005). One study found a reduction in NAA/Cr measured with MRS (Mahmutyazicioglu et al. 2005), while another found a decrease in gray matter density (Corbo et al. 2005). An important question is whether these effects are reversible with treatment. Other findings related to volumetrics include smaller volumes of the corpus callosum in neglected children (Teicher et al. 2004) and adults with PTSD (Villarreal et al. 2004). One study showed a smaller volume of the insula with voxel based morphometry (Chen et al. 2006). A study in twins found smaller volume of the cavum septum pellucidum (May et al. 2004).

Functional Neuroimaging Studies in PTSD

Imaging studies of brain function in PTSD are consistent with dysfunction of the medial prefrontal cortex, the amygdala, and the hippocampus (Pitman 2001; Liberzon and Phan 2003; Liberzon and Martis 2006; Liberzon et al. 2003; Bremner 1998; Bremner 2002b; Rauch et al. 2006; Cannistraro and Rauch 2003). The methodology of imaging studies in PTSD is outlined in Table 1 and a summary of findings by the author, and brain region in Table 2. Studies of resting blood flow or metabolism with PET and SPECT showed alterations at rest in the medial prefrontal, temporal, and dorsolateral prefrontal cortex, the cerebellum, and the amygdala (Bonne et al. 2003; Chung et al. 2006; Bremner et al. 1997b). Stimulation of the noradrenergic system with yohimbine resulted in a failure of activation in the dorsolateral prefrontal, temporal, parietal and orbitofrontal cortex, and decreased function in the hippocampus (Bremner et al. 1997b). Exposure to traumatic reminders in the form of traumatic slides and/or sounds or traumatic scripts was associated with an increase in PTSD symptoms, decreased blood flow and/or failure of activation in the medial prefrontal cortex/anterior cingulate, including Brodmann's area 25, or subcallosal gyrus, area 32 and 24, as measured with PET, SPECT or fMRI (Britton et al. 2005; Yang et al. 2004; Bremner et al. 1999a, b; Lanius et al. 2001, 2003; Liberzon et al. 1999; Shin et al. 1999, 1997, 2001, 2004b, 2005; Semple et al. 2000; Lindauer et al. 2004b; Phan et al. 2006) (Fig. 1). Other findings in studies of traumatic reminder exposure include decreased function in the hippocampus (Bremner et al. 1999b), the thalamus (Lanius et al. 2001, 2003), the visual association cortex (Lanius et al. 2003; Bremner et al. 1999b; Shin et al. 1997, 2004b), the parietal cortex (Bremner et al. 1999b; Shin et al. 1997, 1999; Rauch et al. 1996; Sakamoto et al. 2005), and the inferior frontal gyrus (Lanius et al. 2003; Bremner et al. 1999b; Shin et al. 1997, 1999, 2001; Rauch et al. 1996; Sakamoto et al. 2005), and increased function in the amygdala (Liberzon et al. 1999; Shin et al. 2004b; wv2001; Shin et al. 1997), and the parahippocampal gyrus (Bremner et al. 1999a, b;

Table 1 Published functional imaging studies in PTSD-methods

Authors	Study population	Sample size	Control group	Sample size	Imaging methods	Active condition	Control
Rauch et al. (1996)	Mixed PTSD	8	None	0	PET O-15	Combat scripts	Neutral scripts
Semple (1996)	Combat PTSD+SA	6	Healthy subjects	6	PET FDG	Continuous performance test	Rest
Bremner et al. (1997a, b)	Combat-related PTSD	10	Healthy subjects	10	PET FDG	Yohimbine	Placebo
Shin et al. (1997)	Combat-related PTSD	7	Combat veterans without PTSD	7	PET O-15	Trauma imagery/ perception	Neg/neutral image/ perception
Bremner et al. (1999a)	Combat-related PTSD	10	Combat veterans without PTSD	10	PET O-15	Combat slides/sounds	Neutral slides/ sounds
Bremner et al. (1999b)	Women with abuse-related PTSD	10	Abused women without PTSD	12	PET O-15	Abuse scripts	Neutral scripts
Shin et al. (1999)	Women with abuse-related PTSD	8	Abused women without PTSD	8	PET O-15	Abuse scripts	Neutral scripts
Liberzon et al. (1999)	Combat-related PTSD	14	Healthy subjects/ combat controls	14/11	SPECT HMPAO	Combat sounds	White noise
Zubieta (1999)	Combat-related PTSD	12	Combat veterans without PTSD, healthy subjects	11/12	SPECT HMPAO	Combat sounds	White noise
Rauch et al. (2000)	Combat-related PTSD	8	Combat veterans without PTSD	8	fMRI	Masked fearful faces	Masked happy faces
Semple et al. (2000)	Combat PTSD+SA	6	Healthy subjects	7	PET O-15	Continuous performance test	Rest
Shin et al. (2001)	Combat-related PTSD	8	Combat veterans without PTSD	8	fMRI	Counting stroop-combat	Stroop general negative

Lanius et al. (2001)	Mixed civilian (SA or MVA)	9	Traumatized non-PTSD	9	fMRI	Traumatic scripts	Resting state
Pissiota et al. (2002)	Combat PTSD	7	None	0	PET	Traumatic sounds	Neutral sounds
Lanius et al. (2003)	Mixed civilian (SA or MVA)	10	Traumatized non-PTSD	10	fMRI	Sad, anxious, trauma script	Resting state
Bremner et al. (2003a)	Women with abuse-related PTSD	10	Healthy women	11	PET O-15	Trauma related word recall	Shallow encoding
Bremner et al. (2003b)	Women with abuse-related PTSD	10	Women with abuse without PTSD	12	PET O-15	Memory task	Shallow encoding
Clark et al. (2003)	Civilian PTSD	10	Healthy subjects	10	PET O-15	Working memory task	Fixed target
Bonne et al. (2003)	Civilian PTSD	11	Trauma controls/ healthy controls	17/11	SPECT HMPAO	Resting state	
Bremner et al. (2005a, b)	Women with abuse-related PTSD	8	Healthy subjects	11	PET O-15	Fear conditioning	Unpaired CS-US
Bremner et al. (2004a, b, c)	Women with abuse-related PTSD	12	Women with abuse without PTSD	9	PET O-15	Emotional stroop	Neutral stroop
Shin et al. (2004a, b)	Vietnam combat related PTSD	17	Vietnam veterans without PTSD	19	PET O-15	Traumatic scripts	Neutral scripts
Shin et al. (2004b)	Firefighters with PTSD	8	Firefighters without PTSD	8	PET O-15	Memory task	Shallow encoding
Lindauer et al. (2004a, b)	Policemen with PTSD	15	Policemen without PTSD	15	SPECT HMPAO	Traumatic scripts	Neutral scripts

(continued)

Table 1 (continued)

Authors	Study population	Sample size	Control group	Sample size	Imaging methods	Active condition	Control
Yang et al. (2004)	Children – earthquake related PTS	5	Children – earthquake – non-PTSD	6	fMRI	Earthquake pictures/ images	Neutral pictures/ imagery
Shin et al. (2005)	Firefighters+VN combat with PTS	13	Trauma exposed without PTSD	13	fMRI	Overt fearful faces	Neutral overt faces
Armony et al. (2005)	Acute PTSD – MVA	13	None	0	fMRI	Masked fearful faces	Masked happy faces
Sakamoto et al. (2005)	Mixed civilian PTSD	16	Healthy subjects	16	fMRI	Masked traumatic images	Masked neutral images
Protopopescu et al. (2005)	Sexual/physical abuse PTSD	11	Healthy subjects	21	fMRI	Traumatic word recall	Neutral word recall
Bryant et al. (2005)	Civilian PTSD	14	Healthy controls	14	fMRI	Oddball working memory	
Britton et al. (2005)	Combat PTSD	16	Combat controls/ Healthy controls	15/14	PET O-15	Traumatic scripts	Neutral scripts
Chung et al. (2006)	Civilian PTSD	23	Healthy controls	46	SPECT HMPAO	Resting state	None
Phan et al. (2006)	Vietnam combat related PTSD	16	Combat controls/ healthy subjects	15/15	PET	Negative pictures	Control pictures
Astur et al. (2006)	Civilian PTSD	12	Healthy controls	12	fMRI	Virtual water maze	Visual condition

Table 2 A summary of results of published functional imaging studies of the neural circuitry of PTSD

Authors	Hippocampus	Parahippocampus	Amygdala	mPFC AC (32/24/25)	mPFC OBF (11)	Anteromedial	Dorsolateral PFC (MFG 6)	Dorsolater PFC (IFG)	Posterior cingulate	Sup.temp (2)	Middletemp (21)	Inf. tempfusiform	Insula	Motor cortex	Sensor cortex	Visual association	Precune cuneus	Parietal (IPL)	ParietalSMG (40)	Cerebellum	Thalamus
Rauch et al. (1996)			↑	↑	↑			↓			↓		↑					↓			
Semple (1996)																		↓			
Bremner et al.																					
(1997a, b) (baseline)	NC	NC	NC	NC	NC		↓			↓				NC	NC	NC		NC		NC	NC
(activation)	↓	NC	NC	NC	↓		↓			↓				NC	NC	NC		↓		NC	NC
Shin et al. (1997)																					
(perc v neg)								↓L	↑								↑		↓R		
(imagery v neg)						↓		↓			↑					↓	↓			↑	
(perc v neu)				↓			↓														
(imagery v neu)				↑						↓								↓			
Bremner et al. (1999a)		↑		↓					↑		↓			↑				↑		↑	
Bremner et al. (1999b)	↓R	↑		↓	↓	↑	↑	↓R	↑	↑		↓R	↓R	↑	↓	↓			↓R	↓	
Shin et al. (1999)		↓L		↓	↑	↓	↓	↓	↓	↑								↓R			
Liberzon et al. (1999)			↑																		
Zubieta (1999)				NC		↑														NC	NC
Rauch et al. (2000)			↑	NC								NC									
Semple et al. (2000)		↑	↑	↓				↓								↑				↓	
Shin et al. (2001)	↑	↑		↓			↓	↓	↑	↑		↓	↑				↑	↑			
Lanius et al. (2001)				↓		↓															↓

(continued)

Table 2 (continued)

Authors	Hippocampus	Parahippocampus	Amygdala	mPFC AC (32/24/25)	mPFC OBF (11)	Anteromedial	Dorsolateral PFC (MFG 6)	Dorsolater PFC (IFG)	Posterior cingulate	Sup.temp (2)	Middletemp (21)	Inf. temptusiform	Insula	Motor cortex	Sensor cortex	Visual association	Precune cuneus	Parietal (IPL)	ParietalSMG (40)	Cerebellum	Thalamus
Pissiota et al. (2002)			→	→			→	→						←						→	←
Lanius et al. (2003)				→			→		←			→		→		→	→				
Bremner et al. (2003a, b)	→				→		→		→			→		→		→		→			
Bremner et al. (2003b)	→																				
Clark et al. (2003)							→					→						→			
Bonne et al. (2003)			→	→				→	→	→		→	→	→	→	→	→	→		→	
Bremner et al. (2005a, b)		→	→	→				→	→	→			→	→	→	→		→		→	
Bremner et al. (2004a, b, c)				→			→														
Shin et al. (2004a, b)				→	→					→		→				→				→	
Shin et al. (2004b)	→				→							→					→				
Lindauer et al. (2004a, b)				→																	
Yang et al. (2004)		→		→												→				→	
Shin et al. (2005)			←	→																	
Armony et al. (2005)			→																		
Sakamoto et al. (2005)							→							→						→	

Protopopescu et al. (2005)			↑																		
Bryant et al. (2005)			↑	↑				↑							↑				↑	↓	
Britton et al. (2005)			↓	↓									↓								
Chung et al. (2006)	↑	↑	↑				↓			↓								↓			
Phan et al. (2006)			↓	↓																	
Astur et al. (2006)	↓																				
Decreased Function	6	1	2	16	4	3	10	8	1	4	2	3	3	3	1	5	3	5	2	4	2
Increased Function	2	7	11	3	2	2	2	2	6	6	1	1	3	4	2	3	3	3	1	6	0

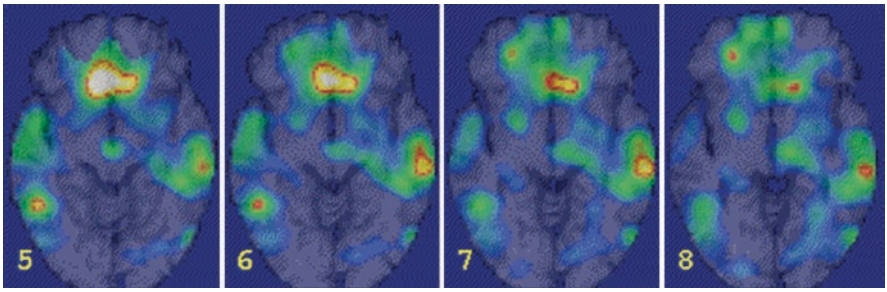

Fig. 1 Hippocampal volume on MRI in PTSD. There was smaller hippocampal volume in a representative patient with PTSD (*right*) relative to a non PTSD subject (*left*)

Liberzon et al. 1999). Shin et al. 2004b found a correlation between the increased amygdala function and the decreased medial prefrontal function with traumatic reminders, indicating that a failure of inhibition of the amygdala by the medial prefrontal cortex could account for increased PTSD symptoms with traumatic reminders. Other studies found increased amygdala and parahippocampal function and decreased medial prefrontal function during performance of an attention task (Semple et al. 2000), and increased amygdala function at rest (Chung et al. 2006), during a working memory task (Bryant et al. 2005), during recall of traumatic words (Protopopescu et al. 2005), with exposure to masked fearful faces (Rauch et al. 2000; Armony et al. 2005), overt fearful faces (Shin et al. 2005), traumatic sounds (Liberzon et al. 1999; Pissiota et al. 2002), and traumatic scripts (Rauch et al. 1996).

Several studies have examined neural correlates of cognitive tasks in PTSD. During working memory tasks, patients showed decreased inferior frontal (Clark et al. 2003) and parietal function (Bryant et al. 2005; Clark et al. 2003). Retrieval of emotionally valenced words (Bremner et al. 2001) (e.g., "rape-mutilate") in women with PTSD from early abuse resulted in decreases in blood flow in an extensive area which included the orbitofrontal cortex, the anterior cingulate, and the medial prefrontal cortex (Brodmann's areas 25, 32, 9), the left hippocampus, and the fusiform gyrus/inferior temporal gyrus, with increased activation in the posterior cingulate, the left inferior parietal cortex, the left middle frontal gyrus, and the visual association and motor cortex (Bremner et al. 2003b). Another study found a failure of the medial prefrontal cortical/anterior cingulate activation, and decreased visual association and parietal cortex function in women with abuse and PTSD, relative to women with abuse without PTSD, during performance of the emotional Stroop task (i.e., naming the color of a word such as "rape") (Bremner et al. 2004b). Shin et al. 2001 showed an increased posterior cingulate and parahippocampal gyrus and a decreased medial prefrontal and dorsolateral prefrontal during an emotional "counting" Stroop paradigm with fMRI.

Studies have also used declarative memory tasks as specific probes of hippocampal function. We measured brain activation with a paragraph encoding task in conjunction with PET O-15 water measurement of brain blood flow. Women with abuse and PTSD showed a failure of hippocampal activation during the memory task, relative

to controls (Bremner et al. 2003a). Women with abuse and PTSD in this study also had smaller hippocampal volume measured with MRI, relative to both women with abuse without PTSD and non-abused non-PTSD women. The failure of hippocampal activation was significant after controlling differences in hippocampal volume as well as accuracy of encoding. Shin et al. 2004a also found a failure of hippocampal activation with a memory stem completion task in PTSD.

Although multiple studies have used symptom provocation with traumatic scripts or similar designs, little has been done in the area of fear conditioning in PTSD. To that end, we studied women with a history of severe childhood sexual abuse and the diagnosis of current PTSD ($N=8$), and women without childhood abuse or PTSD ($N=11$). All the subjects underwent positron emission tomographic (PET) measurement of cerebral blood flow and psychophysiology measurement of heart rate and skin conductance during habituation, acquisition and extinction conditions on a single day, with scanning during a control condition on another day separated by 1 week from the active condition. During habituation the subjects were repeatedly exposed to a blue square on a screen (conditioned stimulus (CS)); during active fear acquisition, exposure to the blue square (CS) was paired with an electric shock to the forearm (unconditioned stimulus (UCS)); and during extinction, subjects were again exposed to the blue squares (CS) without shock ("active" extinction). On the second day, the subjects went through the same procedure with electric shocks delivered randomly when the blue square was not present (unpaired CS–UCS). Acquisition of fear was associated with increased skin conductance (SC) responses to CS exposure during the active versus the control conditions in all the subjects. There was increased SC for PTSD during the first CS–UCS presentation. Extinction of fear was associated with increased skin conductance (SC) responses to CS exposure during the active versus the control conditions, in all the subjects. When PTSD and non-PTSD subjects were examined separately, the SC levels were significantly elevated in non-PTSD subjects undergoing extinction of fear following the active compared to the control condition during session one. PTSD subjects showed activation of the bilateral amygdala during fear acquisition compared to the control condition (Fig. 2). Non-PTSD subjects showed an area of activation in the region of the left amygdala. When PTSD subjects and control subjects were directly compared, PTSD subjects showed a greater activation of the left amygdala during the fear conditioning condition (pairing of US and CS) relative to the random shock control than healthy women. Other areas that showed increased activation with fear acquisition in PTSD included the bilateral superior temporal gyrus (Brodmann's Area (BA) 22), cerebellum, bilateral inferior frontal gyrus (BA 44, 45) and the posterior cingulate (BA) 24). Fear acquisition was associated with decreased function in the medial prefrontal cortex, the visual association cortex, and the medial temporal cortex, the inferior parietal lobule function, and other areas. Extinction of fear responses was associated with decreased function in the orbitofrontal and medial prefrontal cortex (including subcallosal gyrus, BA 25, and anterior cingulate BA 32), the visual association cortex, and other areas in the PTSD subjects, but not in the controls. Amygdala blood flow with fear acquisition was negatively correlated with medial prefrontal blood flow with fear

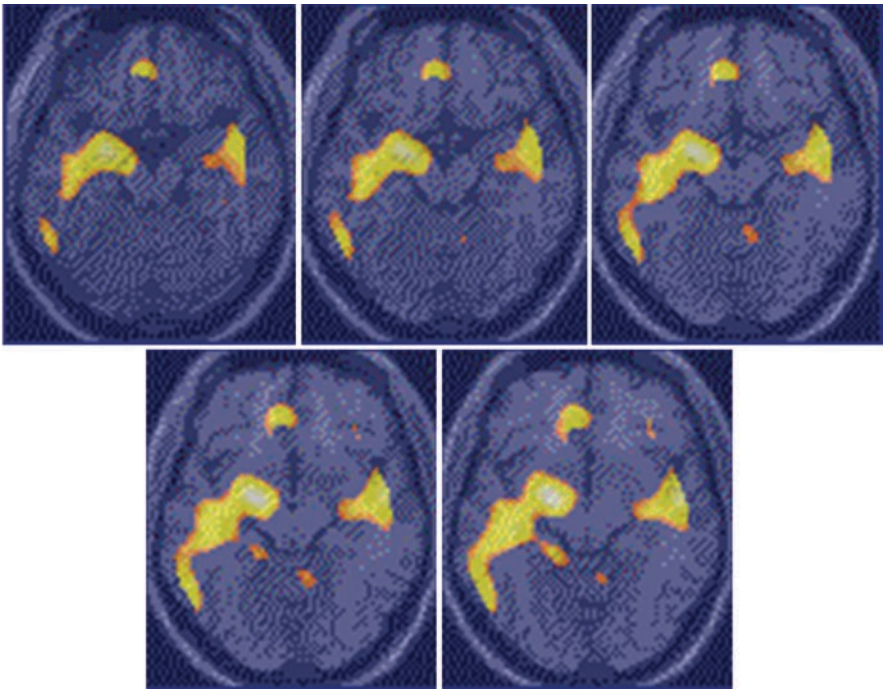

Fig. 2 Medial prefrontal dysfunction in PTSD. There was a failure of medial prefrontal activation in a group of combat veterans with PTSD compared to combat veterans without PTSD during exposure to traumatic combat related slides and sounds (*yellow* area in prefrontal cortex)

extinction (increased blood flow in the amygdala correlated with decreased blood flow in the medial prefrontal cortex) in all the subjects ($r = -0.48$; $p < 0.05$). Increased amygdala blood flow with fear acquisition was positively correlated with PTSD ($r = 0.45$), anxiety ($r = 0.44$) and dissociative ($r = 0.80$) symptom levels in PTSD (but not non-PTSD) subjects. There was a negative correlation between the medial prefrontal blood flow during extinction and anxiety as measured with the PASS during extinction in the PTSD group only which was significant after correction for multiple comparisons ($r = -0.90$; $p = 0.006$) (Bremner et al. 2005a). This study was consistent with increased amygdala function with fear acquisition, and decreased medial prefrontal (anterior cingulate) function during extinction in PTSD. This is consistent with the model of an over active amygdala and a failure of medial prefrontal cortex to extinguish, or shut off, the amygdala, when the acute threat is no longer present.

Few studies have involved imaging of receptors in the brain in PTSD. One study used single photon emission computed tomography (SPECT) to show a decrease in benzodiazepine receptor binding in the frontal cortex in Vietnam combat-related PTSD (Bremner et al. 2000). Another study of Gulf War-related PTSD showed a negative correlation between childhood trauma and right superior temporal gyrus benzodiazepine receptor binding (Fujita et al. 2004).

In summary, these studies are consistent with dysfunction of a circuit involving the medial prefrontal cortex, the dorsolateral prefrontal cortex, and the hippocampus and the amygdala, in PTSD patients that we hypothesize underlie symptoms of PTSD.

Effects of Pharmacotherapy on Brain Function and Structure in PTSD

We have begun to assess the effects of pharmacotherapy on brain structure and function in PTSD (Bremner and Vermetten 2004). We recently assessed the effects of phenytoin on brain structure and function. Studies in animals show that phenytoin, which is used in the treatment of epilepsy and is known to modulate glutamatergic function, blocks the effects of stress on the hippocampus (Watanabe et al. 1992a). We studied nine patients with PTSD in an open label function before and after treatment with phenytoin. Phenytoin resulted in a significant improvement in PTSD symptoms (Bremner et al. 2004c). Phenytoin also resulted in increases in both right hippocampal volume and right hemisphere volume (Bremner et al. 2005b). These findings indicate that phenytoin has an effect on PTSD symptoms as well as brain structure in PTSD patients. In a second study, patients with PTSD were shown to have an increase in hippocampal volume and memory function with paroxetine (Vermetten et al. 2003), and a decrease in cortisol responsiveness to a stressful cognitive challenge (Vermetten et al. 2006). One case report showed decreased inferior frontal, prefrontal, and insula blood flow measured with PET in response to war related sounds. These changes normalized with successful treatment with the SSRI fluoxetine (Fernandez et al. 2001). Another study assessed resting brain blood flow with SPECT Tc-99m HMPAO before and after 8 weeks of open label treatment with the SSRI citalopram in 11 adult patients with PTSD. The treatment resulted in a decrease in the left medial temporal cortex blood flow; decreased PTSD symptoms as measured with the CAPS were correlated with increased function in the medial prefrontal cortex (Seedat et al. 2003).

Summary and Conclusions

Brain imaging studies have shown that PTSD is associated with changes in brain function and structure. Brain areas implicated in the stress response include the amygdala, the hippocampus, and the prefrontal cortex. These brain areas also play a critical role in memory, highlighting the important interplay between memory and the traumatic stress response. Preclinical studies show that stress affects these brain areas. Furthermore, antidepressants have effects on the hippocampus that counteract the effects of stress. In fact, promotion of nerve growth (neurogenesis) in the hippocampus may be central to the efficacy of the antidepressants. Studies in patients with posttraumatic stress disorder (PTSD) show alterations in brain areas

implicated in animal studies, including the amygdala, the hippocampus, and the prefrontal cortex. Increased amygdala activation with acquisition of fear responses, and a failure of the medial prefrontal cortex to properly mediate extinction are hypothesized to underlie symptoms of PTSD. Treatments that are efficacious for PTSD show a promotion of neurogenesis in animal studies as well as a promotion of memory and increased hippocampal volume in PTSD. Future studies are needed to assess neural mechanisms in treatment response in PTSD. In addition, studies need to move beyond assessments of brain function and to examine areas such as neuroreceptor binding and changes in brain chemicals (e.g., with MRS).

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Part V
Integrative Processes

Integrative Processes: Neuroscience Clinical Imaging Biomarkers

Igor D. Grachev and Richard J. Hargreaves

Introduction

The pharmaceutical industry today is failing to convert early stage pipeline productivity to phase 3 clinical trials and the approval of drugs. The use of a portfolio wide biomarker strategy to facilitate early decision making is hoped to improve the probability of success of candidates moving into the later stages of development. By eliminating the failures early it is also expected that the overall cost-effectiveness of drug development can be improved.

This chapter will focus on clinical imaging biomarkers and their integration into drug development processes. Multimodality imaging technologies are playing an important role in defining biomarkers for the development of drugs in many therapeutic areas today. Their goal is to drive a paradigm shift in the development of drugs such that we identify molecule and mechanism – based failures for new therapeutic targets and lead optimization candidates earlier in the process and enable likely winners with a rapid go decision and information that can speed their developmental path. By improving decision making and decreasing cycle time through the early phases of the development of the drugs, imaging biomarkers and tools can help to stratify and prioritize a research portfolio to focus resources on the best probable drug candidates and therapeutic hypotheses.

Figure 1 shows a schematic of the multiple points at which molecular (PET) and functional (fMRI) imaging can impact upon decision making during different phases of the drug development process from target validation phase through to preclinical and clinical development (Phases 1–4). It is noteworthy that validation of target-specific biomarkers (e.g., PET) and platform-based approaches (e.g., fMRI) needs to be initiated at the pre-lead optimization phase within 18–24 months prior to nomination of new chemical candidates for clinical development.

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At this stage forward looking biomarker plans with clear pre-set decision metrics need to be formulated by project teams. These plans should cover the scientific rationale and justification for the generation of novel imaging reagents and selection of imaging technologies that are prioritized, "fit for purpose" and thereby most appropriate to the goals of the project, development status of the molecule or patient population being studied.

Clinical imaging biomarkers can provide early information about the drug time on target to help determine the optimal dosing regimen and support development of formulations with pharmacokinetic characteristics matched to intended applications. Imaging biomarkers can also be used to identify specific phenotypes and responder populations to stratify patient populations for inclusion in clinical trials and provide a basis for differential diagnosis and individualized clinical care. Imaging can be employed strategically to compare new drug candidates and older proven drugs or competitors to select active comparator/calibrating agents for clinical trials and better understand differentiating features that could impact product profiles. This chapter gives a strategic overview of potential clinical imaging approaches in drug development using some examples from Merck Imaging Research Laboratories. It is not intended to be a comprehensive technical review of imaging tools and technologies for central nervous system (CNS) drug development that have been covered by many authors in this book and in a number of recent articles and reviews (Frank and Hargreaves 2003; Rudin and Weissleder 2003; Massoud and Gambhir 2007; Sosnovik and Weissleder 2007; Gross and Piwnica-Worms 2005; Schuster 2007; Medarova et al. 2007; Bednar et al. 2007; Mamo et al. 2004; Bergström et al. 2004; Keller et al. 2006; Burns et al. 2007; Erondu et al. 2006; Hargreaves and Wagner 2006; Alzheimer's Disease Neuroimaging Initiative

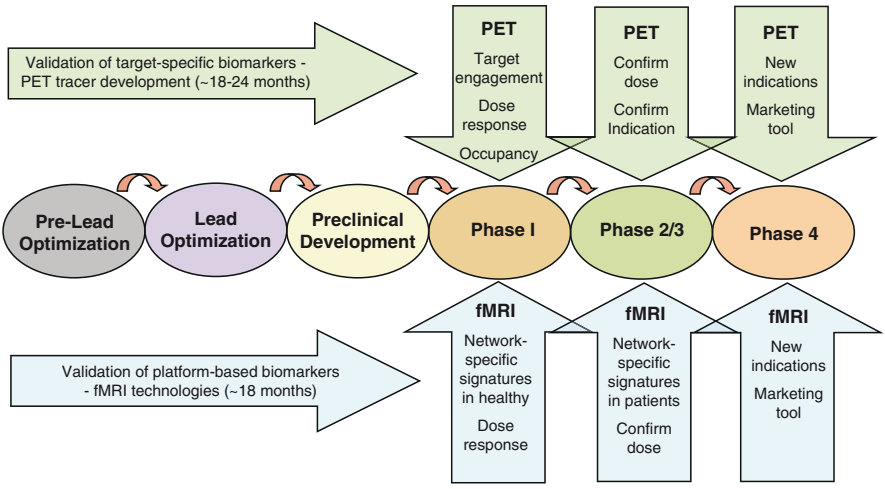

Fig. 1 Impact of PET and fMRI approaches on different phases of drug development process from the lead optimization phase through preclinical and clinical development (Phases 1–4) and associated decision-making applications

(ADNI) 2007; Borsook et al. 2006; Magistretti et al. 1999; Belliveau et al. 1991; Williams et al. 1992; Koroshetz and Gonzalez 1997; Grachev et al. 2000, 2001, 2002a, b, 2003; Salibi and Brown 1998; David et al. 1994; Breiter et al. 1996a; Breiter et al. 1996b; Whalen et al. 1998).

Imaging Platforms for Drug Discovery and Development

There is a wide range of non or minimally invasive imaging technologies and tools that can be leveraged into biomarker research and integrated with clinical development programs in a parallel or in a serial fashion. The most commonly used are positron emission tomography (PET), single photon emission computed tomography (SPECT), computer tomography (CT), morphometric (mMRI) and fMRI, ultrasound (US), and optical-based imaging. These multimodality molecular imaging technologies provide a choice of complementary information that can help to bridge studies in the laboratory to the clinic by visualizing, characterizing, and quantifying anatomical structures and physiological processes at the cellular, sub cellular, and intercellular levels with exquisite spatial, temporal, and biochemical (which is reflective of high sensitivity) resolution in living subjects. Imaging in vivo avoids many of the potential artifacts and pitfalls that can be associated with biopsy and tissue processing in ex vivo and in vitro model systems by making measurements in a physiologically relevant tissue context. Integrative imaging processes use a variety of different techniques to generate contrast and each has its strengths and weaknesses in terms of spatial and temporal resolution, sensitivity and imaging probe characteristics. All require close coordination of many disciplines including biology, chemistry, neuropharmacology, medical imaging physics, applied computer sciences, mathematics, modeling and bioinformatics. It is important to note that molecular imaging in the discovery of drugs and its development occupies a separate but complementary space to diagnostic imaging.

Nuclear PET Imaging

Nuclear imaging visualizes radiolabeled probes, or radiotracers interacting with protein targets within or on the surface of cells. The two key radionuclide imaging modalities are PET, which uses radiotracers typically labeled with the positron-emitting radioisotopes (e.g., ^{11}C and ^{18}F), and SPECT, which detects radiotracers labeled with gamma-emitting radioactive isotopes (e.g., ^{123}I). Radiotracers are versatile, specific and highly sensitive. They can be designed to track small molecular drugs or therapeutic proteins, image their targets or monitor key biochemical and physiological processes involved in the molecular pathology of health and disease. Novel molecular tracer probes are the only way to measure receptor populations and pharmacology (at picomolar to nanomolar densities) quantitatively in vivo in both

animals and humans. PET receptor binding studies can be viewed as a gold standard approach in pharmaceutical industry due to their superb sensitivity and reproducibility that require only small numbers of subjects per dose level or condition (e.g., time on-target vs. off-target) to guide internal decision-making. The simultaneous use of structural imaging modalities such as computer tomography and magnetic resonance imaging (MRI), particularly fully computerized and automated voxel-based morphometry (VBM), or functional imaging with fMRI or arterial spin labeling (ASL) enables relatively precise spatial, temporal and anatomical localization of molecular pharmacological activity in the brain. Advances in the development of small animal tomographic cameras (microPET or microSPECT with CT) has facilitated translational bridging between preclinical and clinical CNS research (Pomper and Lee 2005; Deroose et al. 2007) by linking responses (neurophysiological, behavioral) in animal assays to a degree of drug target engagement thereby setting targets for clinical testing of therapeutic hypotheses. In Merck Imaging Laboratories we have developed a "vertical integration" strategy which focuses on the use of CNS imaging biomarkers in animal experiments (mouse to monkey) linked to EEG biomarkers and behavioral tests that can be replicated in clinical experimental settings.

Radionuclide imaging, particularly PET, is a powerful tool for the discovery of drugs especially in the field of neuroscience due to the inaccessibility to the brain and the lack of CNS specific biomarkers to drive accurate drug dosing (Rueger et al. 2007; Hammoud et al. 2007).

The effective use of a PET tracer strategy in a drug development relies on parallel discovery efforts in medicinal chemistry and radiochemistry at lead optimization phase which is approximately 2 years before nomination of the drug candidate for development (Fig. 1). At this lead identification and optimization phase the tracer development program usually becomes a part of focused biomarker plan that assesses the drug candidates performance during development up to making go-no go decisions on clinical proof-of-concept and progression to Phase 2b testing. Medicinal chemistry programs are a rich source of novel imaging tracer candidate molecules as they are generated, yet often discarded as drug candidates due to limited half life for once a day dosing or lack of oral bioavailability. The best tracer molecules have distinct characteristics such as feasibility for ^{11}C- or ^{18}F-labeling and reproducibility of tracer synthesis, availability of analytical chemistry methods to study the disposition of the tracer and its metabolism, an adequate solubility for intravenous (iv) formulation, stability of iv formulation (at least up to 24 h at room temperature), short half-life ($T_{1/2}$ = 4–5 h or less), high on-target and low off-target affinity selectivity and specificity, good brain–blood barrier penetration (brain/plasma ratio > 1: not a PgP substrate and lipophilicity properties :LogP~1–3), lack of active metabolites in the brain, acceptable preclinical safety evaluation (e.g., single dose toxicology, cardiovascular safety, genotoxicity) and dosimetry.

PET imaging has been traditionally used to follow the target engagement of neutral orthosteric site antagonists where there is thought to be a relatively simple relationship between occupancy and efficacy. More recently PET has been used to study partial and inverse agonists where the relationship between occupancy and

efficacy is modified by receptor reserve that includes a factor related to the intrinsic pharmacological efficacy of the drug molecules. Here the value of PET comparisons between molecules is totally dependent on them having the same intrinsic efficacy profile. Recent studies with allosteric modulator drug classes highlight a new challenge for PET imaging in drug development that, in contrast to neutral antagonists, suggests structurally related drug-tracer pairs may be required for dose-occupancy studies. In Merck Research Laboratories, PET studies of different chemical series of negative allosteric modulators for mGluR receptors using different PET tracers confirmed the existence of binding differences between chemical structural classes and suggested that the antagonists and PET tracer pairs may each have a unique (but somewhat overlapping) binding site. It appears likely that unique tracers may be required to study each unique structural class of molecules generated during a lead optimization program and only one of these, together with the drug candidate will progress to early clinical trials to set dosing targets for clinical proof of concept testing. PET imaging tracers can therefore now be thought of as heterologous or homologous to the drug candidate they track. The use of PET imaging in neuroscience drug discovery programs of allosteric modulators will probably require increased pre-investment in PET tracer development compared to traditional antagonist programs. The availability of independent clinical efficacy biomarkers is clearly of value when making occupancy-efficacy comparisons between molecules that are not neutral antagonists.

Early discovery of a PET tracer helps the selection of lead molecules in the preclinical lead optimization phase by focusing research on those drug candidates that achieve the highest target engagement off the lowest exposure thereby maximizing the therapeutic window. As a general strategy in pharmaceutical industry, if tracer discovery and development does not start early at pre-lead optimization phase it is important to justify whether tracer development is worth doing at all. Synergizing radioligand and drug development programs reduces the time and cost of PET tracer discovery. PET tracers need to be clinically validated in advance of Phase I clinical studies so that they can be incorporated into early pharmacokinetics, safety and tolerability study paradigms in a parallel or staggered fashion (e.g., first dose in PET after completion of two higher doses in first-in-man safety and tolerability study). Tracers that come too late in a drug discovery and development program have limited value as early decisions affecting the fate of the molecule and clinical hypothesis testing will have been made using alternate traditional safety and exposure criteria. PET tracers in conjunction with clinical outcome data can be used to define therapeutic windows and support clinical trial designs.

PET can help determine the dose/plasma-occupancy relationships of novel targets to guide dose selection for clinical proof of concept testing and subsequent Phase 2b dose ranging trials. Proof of concept testing requires maximal target engagement to test a clinical hypothesis that the receptor or enzyme being targeted is relevant to the clinical outcome. This is critical for novel targets with no pre-existing proof-of-efficacy (e.g., NK1 for pain or depression (Bergström et al. 2004)) and for drugs that attempt to improve therapeutic windows to refine the tolerability of older therapeutic agents. It is important to remember that many receptor or enzyme occupancy studies are conducted in healthy subjects and it is a working assumption that the

data generated are applicable to patients with CNS disorders. This assumption may require subsequent validation in patient populations as there may be changes in receptor or enzyme densities due to the underlying pathophysiological processes (e.g., neurodegeneration, changes in neurotransmitter release) in the CNS disease being studied or as a result of chronic administration of therapeutic agents used in the patient's usual therapeutic care. It is also important to note that receptor or enzyme occupancies determined after single dose administration may not be representative of occupancy after multiple dose administration. This assumption can be tested by combining single and multiple dose experiments in one study protocol. PET studies comparing single and multiple dose occupancies can be used to assess target engagement at steady state trough plasma levels and may elucidate dissociations between time on target and plasma exposure levels that could be important for the selection of optimal dosing regimens. This is particularly important for decisions related to compounds with potential narrow therapeutic index in the early development.

For clinical proof of concept testing it is best to achieve the highest levels of occupancy, within the safety and tolerability of the molecule, in order to provide the best test of the clinical hypothesis. No occupancy and no efficacy is not surprising and a new molecule is needed but full occupancy and no efficacy means a project can be terminated saving resources. There has been a long debate over the "ideal" levels of receptor for CNS therapeutics. It has been proposed on the basis of registered classic neuroleptic dopamine D2 receptor antagonists, that clinically effective doses occupy over 75% of the target. However it is not known if better clinical efficacy could be achieved at higher occupancies due to the safety window available for this mechanism nor whether this level of engagement at Cmax plasma concentrations is required around the clock. For novel mixed pharmacology mechanisms, the occupancy-efficacy relationship for each drug molecule is determined by the drug interacting with more than one receptor site. Knowledge of target engagement levels from existing clinical agents targeted at a single site can be used to design studies to test whether the pharmacodynamics of novel mixed mechanism molecules are suitable for clinical proof of concept testing, accepting the risk that the occupancy required for the mixed mechanism may differ. An example of this is the atypical neuroleptics (Dopamine D2 and serotonin 5-HT2 receptor antagonists) where some molecules occupy only 60% dopamine D2 receptors at Cmax after oral dosing and decline to as low as 20–27% at trough for quetiapine or 33–55% at trough for clozapine. See Table 1 (Mamo et al. 2004; Tauscher et al. 2002a, b; Kapur et al. 1999, 2000; Jones et al. 2000; Gefvert et al. 1998).

Magnetic Resonance Imaging

fMRI is a fast developing technology in CNS drug research and development that can be used to detect brain activity within multiple associated neuronal pathways and networks. Over the last decade, fMRI has been used widely for the study of

Table 1 D2 receptor occupancies for atypical antipsychotics at peak and trough plasma concentrations or at 12–14 h post-dose (where applicable)

Atypical antipsychotic	Daily dose	Dosing frequency	D2 receptor occupancy at peak plasma concentration	D2 receptor occupancy at trough plasma concentration
Olanzapine	15–20 mg	QD	81–88% (Tauscher et al. 2002a)	78% (Tauscher et al. 2002a)72–76% (at 12–14 h) (Kapur et al. 1999)
Risperidone	3 mg	QD	81–88% (Tauscher et al. 2002a)	66% (Tauscher et al. 2002a) 72% (at 12–14 h) (Kapur et al. 1999)
Clozapine	300–600 mg 350 mg	BID	– 71% (Jones et al. 2000)	33% (Tauscher et al. 2002b) 55% (Jones et al. 2000)
Quetiapine	150–600 mg	BID or TID	60%(Kapur et al. 2000; Gefvert et al. 1998)	27%(Kapur et al. 2000; Gefvert et al. 1998) 20%(Tauscher et al. 2002b)
	300–700 mg		–	
Ziprasidone	40–160 mg	BID	–	56%(Mamo et al. 2004)
Aripiprazole	10–30 mg	QD	81–93%(Mamo et al. 2007)	–

cognitive processing, language, vision, movement, hearing, memory, emotion, psychiatric symptoms (symptom provocation designs), pain and psychiatric and neurological disorders (Borsook et al. 2006; David et al. 1994; Whalen et al. 2008; Shin et al. 2005; Baliki et al. 2008; Geha et al. 2007; Becerra et al. 2006; Wise et al. 2002, 2004; Schweinhardt et al. 2008). The widespread availability of MR scanners makes fMRI a rich area for collaboration in the neurosciences between academia and the pharmaceutical industry. It provides an imaging platform that can be used to study the pharmacodynamics of drug activity to support go/no-go decision making and dose selection for many CNS disorders and conditions (Borsook et al. 2006) especially when the development of target engagement biomarkers such as PET tracers is not feasible. The most commonly used fMRI technique is blood-oxygen-level dependent (BOLD)-fMRI. The BOLD-fMRI signal (small, voxel-associated signal changes usually within 1–5%) arises from deoxygenated hemoglobin and is altered by physiological factors that change blood oxygenation (blood blow, oxygen consumption, blood volume). The BOLD signal is a surrogate hemodynamic signal that represents mass neuronal activity within the spatial resolution of a neuroimaging voxel (Magistretti et al. 1999). It was recently estimated that an fMRI voxel of 55 μL in size contains 5.5 million neurons, $2.2–5.5 \times 10^6$ synapses, 22 km of dendrites and 220 km of axons and gives a population view of neuronal activity that reflects changes in the balance of brain excitatory and inhibitory neurotransmission (Magistretti et al. 1999; Logothetis 2008) The main advantage of BOLD-fMRI is that it is a totally non-invasive way to monitor networks of brain regions involved

in behavioral tasks and responses to drugs with high spatiotemporal resolution (~100 ms and 1–2 mm making it suitable to track rapid changes in CNS activation (Fig. 2). Unlike PET, fMRI does not involve an injection of radioactive materials (although intravenous contrast agents are sometimes used in some protocols to enhance signal detection) making it a suitable technology for imaging protocols that require multiple imaging sessions. BOLD-fMRI can be used to compare the responses of the brain in healthy subjects and patients to stimuli in the presence and absence of drugs, in different mental states, in psychiatric and neurological disorders and varying physiological conditions within an individual in a single session or over a period of time. Resting state BOLD can be also used to examine the pharmacodynamic effects of CNS active agents "off the base line" on neural networks that are active by default at rest.

fMRI studies use different designs and analyses. Block experimental designs compare brain activation (collected over 20–30 s periods) between investigator designated tasks and control states (normalized to the mean signal of all states). In block designs data is analyzed by subtraction methods to reveal signal differences, both increases and decreases, in the BOLD signal between conditions. The advent of faster methods of data acquisition has allowed the development of event related fMRI methodologies where, in contrast to the signal "averaging" that occurs over 20–30 s epochs in block designs, the temporal nature of signal changes detected perhaps more closely represents the time course of neuronal activity. It is

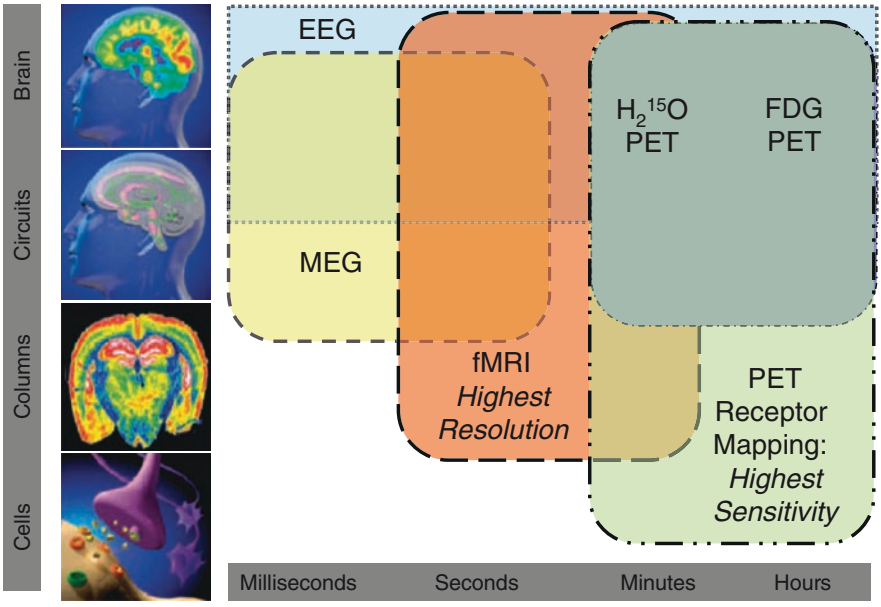

Fig. 2 Spatial and temporal resolution for the main bio-imaging modalities. This diagram shows the highest anatomical resolution for functional magnetic resonance imaging (fMRI), and the highest sensitivity for the PET receptor mapping

noteworthy that limitation of block designs is that neuronal networks that overlap between the test and control states, even if serving different functions, may be missed through the subtraction paradigm. Most fMRI studies used voxel based analysis of the time series of MRI BOLD signals within or across individuals and groups. More recently data has been examined by looking at patterns of brain activation across the brain to define state dependent "signatures" that can be used to compare and detect small differences between conditions in a concept similar to that behind expression profiling analyses.

Recent publications have highlighted the benefits and limitations of the use of fMRI to study brain function especially in the cognitive neurosciences (Logothetis 2008) and many of the practical aspects discussed apply to its use in drug discovery. fMRI can be used as a translational technology that links the neural circuits involved in responses in humans to behaviors in animals where more extensive electrophysiological, pharmacological and molecular testing are feasible. Figure 3 shows some of the gaps in the application of fMRI imaging to drug development. Understanding the reproducibility and translatability of fMRI signatures (personal fingerprints) and their responsiveness to different doses of drugs in healthy animals, healthy humans, animal models of CNS disease and patient populations is extremely important for integrative decision-making processes. When combined with PET proof of target engagement, fMRI has the potential to be a powerful tool that defines the pharmacodynamics of drug action in the brain even if the neural elements that drive change in the fMRI signal remain poorly understood. Some of the current limitations of a multicenter approach to fMRI relate to the wide differences in instrumentation, experimental approaches, analysis methods and modeling between expert groups. The lack of standards and consensus has the potential to limit the field to single center studies. Additionally, in the field today there is generally a paucity of good housekeeping studies that examine factors such as test/retest variability and reproducibility within and between subjects in single imaging sessions or where there are time intervals between them. These studies are essential to assess variability in the fMRI paradigms being used and enable investigations to be powered appropriately.

Whilst fMRI-BOLD is the main MR neuroimaging technology used today, there are other functional MRI technologies that measure perfusion and blood volume that can be used in drug development. We mention them here for completeness. It should be noted however that reproducibility and standardization across these techniques is yet to be reliably demonstrated and in this respect as tools for drug discovery and development they are probably behind fMRI-BOLD. (1) Perfusion fMRI, measures regional cerebral blood flow as a surrogate marker of neuronal activity. It relies on measurement of a T2*-weighted signal after the iv injection of gadolinium-containing contrast agents or super-paramagnetic iron oxide particles that pass through the cerebral vasculature within the brain tissue over a short period of time (Belliveau et al. 1991), (2) ASL or dynamic ASL (dASL), measures regional cerebral blood flow and arterial transit time using a non-invasive approach via internal magnetically labeled blood contrast, and it does not require any injection (Williams et al. 1992), (3) diffusion-weighted fMRI, measures random

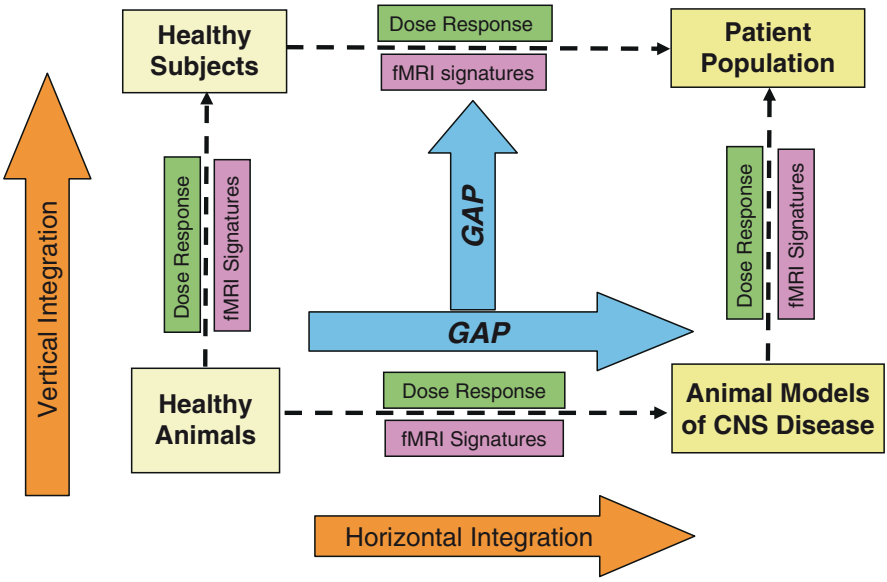

Fig. 3 Gaps in fMRI imaging in drug development. (a) Vertical integration: understanding the predictability and translatability from healthy animals to healthy humans and from animal models of CNS disease to patient population, (b) Horizontal integration: Understanding the predictability and translatability from healthy animals to animal models of CNS disease and from healthy humans to patient populations. The key information links specific fMRI signatures (fingerprints) to their dose responsivity

movement of water molecules (Koroshetz and Gonzalez 1997), and (4) MRI spectroscopy or spectroscopic imaging which can measure certain chemicals and metabolites in the brain and other tissues (e.g., ^{1}H-MRS (Grachev et al. 2000, 2001, 2002a, b, 2003), ^{31}P-MRS, ^{19}F-MRS, ^{13}C-MRS (Salibi and Brown 1998).

Examples of the Use of Clinical PET Imaging Biomarkers

Our clinical PET imaging studies in Merck Research Laboratories have had a high impact on our CNS drug development programs and have been used to enable go/no-go decisions or the acceleration of compound development timelines. There are several examples: (1) The ^{18}F-SPA-RQ, neurokinin-1 (NK1) specific tracer was evaluated before and after treatment with placebo or increasing doses of aprepitant (EMEND) (Bergström et al. 2004), a selective NK-1 receptor antagonist that prevents acute and delayed chemotherapy-induced nausea and vomiting. A clear dose and plasma concentration vs. receptor occupancy was observed, suggesting that high levels of central NK1 receptor occupancy (>90%) were required to achieve optimal anti-emetic effects. This finding helped justify the doses of EMEND®

selected for registration to have enough but not too much drug in the setting of complex chemotherapeutic dose regimens and also guided dose selection for clinical trials in other CNS indications such as, pain and depression where the NK-1 antagonists were found to be inactive. (2) The ^{18}F-MK-9470, a selective inverse agonist of the cannabinoid-1 (CB1) receptor also illustrated the value of PET tracers in CNS drug development (Burns et al. 2007; Addy et al. 2008). The ^{18}F-MK-9470 was used to characterize CB1 receptor distribution in the human brain and to measure the receptor occupancy of potential therapeutic doses of the CB1 inverse agonist taranabant. As the cannabinoid system is thought to be involved in modulation of a variety of CNS functions, ^{18}F-MK-9470 has the potential to be a valuable research tool to study CB1 biology and pharmacology in metabolic, neurological, and neuropsychiatric disorders. (3) ^{11}C-MK-0233, a selective NPY5 receptor antagonist was used to assess the ability of an anti-obesity drug candidate MK-0557 to engage NPY5 receptors in the brain, which were believed to be critical components of a pathway that stimulates food intake and decreases energy expenditure. However, despite full engagement of the therapeutic target (approximately 100% occupancy with the lowest tested dose at 24 h post-dose), the degree of weight loss induced using the drug candidate alone was relatively modest and therefore not clinically meaningful. The results suggested that further development of this drug candidate would not produce therapeutic value enabling reprioritization of the target and program (Erondu et al. 2006).

Pre-competitive Clinical Imaging Consortia – Shared Risk and Reward

Development of disease related clinical imaging biomarkers requires large efforts and investment from multiple stakeholders, including pharmaceutical and medical device companies, academic centers, government agencies and advocacy groups, which may help to define new imaging-based surrogate clinical biomarkers/endpoints. A good example is the Alzheimer's Disease Neuroimaging Initiative (ADNI), a public–private partnership between industry, academia and the National Institute of Health/Aging (NIH/NIA), that aims to validate imaging tools such as PET for identification of metabolic defects and amyloid burden, and MRI for identification of atrophy patterns and volumetric changes related to neurodegeneration, which will be combined with evaluation of fluid (CSF and plasma) biomarkers and cognitive tests. This large observational multi-center trial will be tracking a progression of Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD) after receiving a standard care with the best available therapies (Hargreaves and Wagner 2006; Alzheimer's Disease Neuroimaging Initiative (ADNI) 2007). Data collected from the ADNI initiative could lead to the development of standardized clinical fluid biomarkers and sensitive imaging technologies that can be used to stratify patients much earlier than neuropsychiatric diagnosis of AD. These tools could then be used to enrich future clinical trials with patients with amyloid pathology

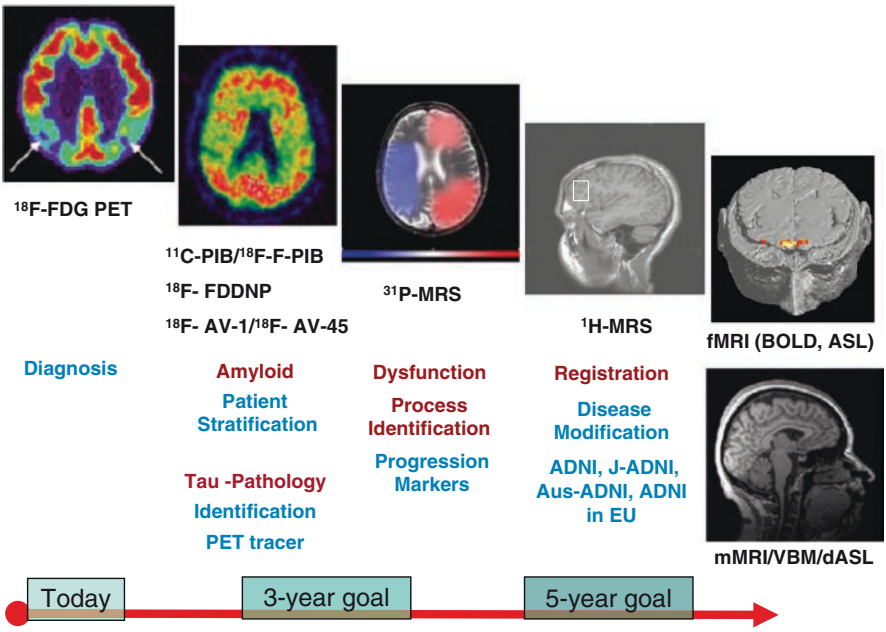

Fig. 4 Alzheimer's disease imaging roadmap. The figure represents future steps as we move from today's clinical imaging biomarkers to the development of new PET tracers that label amyloid deposits in the brain to imaging biomarkers of Tau pathology and identification of disease process and progression imaging biomarkers in coordinated Alzheimer's disease neuroimaging initiatives. Thanks to Drs WE Klunk, CA Mathis, AV Apkarian and M Weiner for use of the images

and the highest risk of progression to give an early assessment of the effects of novel disease-modifying drugs and perhaps support registration. Figure 4 represents steps of increasing value and probability of success as we move from today's clinical imaging biomarkers for AD staging and progression with ^{18}F-FDG PET, to the development of new PET tracers that label amyloid deposits in the brain (^{11}C-PIB/^{18}F-F-PIB, ^{18}F-FDDNP, ^{18}F-AV-1/^{18}F-AV-45), for patient identification and clinical trial stratification to imaging biomarkers of Tau pathology and identification of functional and disease progression imaging biomarkers (^{31}P/^{1}H-MRS, BOLD & ASL fMRI, mMRI/VBM/dASL). There is now a coordinated network of Alzheimer's disease Neuroimaging initiatives across the globe, in the USA (ADNI), Japan (J-ADNI), Australia (Aus-ADNI) and EU (ADNI initiatives in EU) that are using common platforms to characterize Alzheimer's disease, its pathology, fluid biomarkers and PET and MRI-based imaging biomarkers. These consortia efforts will help to characterize today's patient populations and apparent progression rates by imaging modality and diagnostic tests thereby setting the baseline metrics for future clinical trials evaluating disease-modifying agents in the context of today's standards of clinical care (you can visit http://www.adni-info.org for more information).

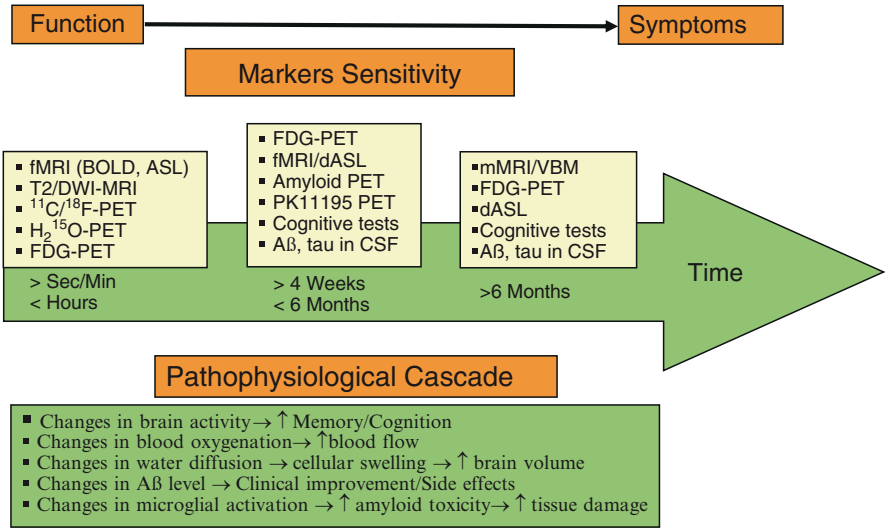

Fig. 5 Imaging biomarker strategy for Alzheimer's disease (AD): Understanding the time – pathophysiology – biomarker sensitivity relationship

The development of clinical imaging biomarkers requires an integrated understanding of the relationships between several processes. These are the time course of changes being studied, (e.g., neuronal degeneration and brain atrophy as a result of AD progression), the molecular pathophysiology of the disease and sensitivity of the imaging biomarker modalities being used to track these changes. Figure 5 shows a schematic view of the complex relationships between changes in brain activity, blood oxygenation, water diffusion, and Aβ level and microglial activation as a part of pathophysiological cascade of reactions in AD. The selection of an appropriate clinical imaging biomarker is based on an understanding of their relative sensitivity and reproducibility in the context of clinical studies and trials of differing durations. For example, for changes within >seconds/minutes and <hours, which might be applicable for single dose studies, fMRI (BOLD, ASL), T2/DWI-MRI, $^{11}C/^{18}F$-PET, $H_2{}^{15}O$-PET, and FDG-PET technologies can be considered. For changes within >4 weeks and <6 months timeframe FDG-PET, fMRI, dynamic ASL (dASL), Amyloid PET, PK11195 PET, cognitive tests, Aβ, and Tau CSF levels can be considered, with mMRI/VBM as potential endpoints in longer term (>6 months) studies. The schematic in Figure 5 is theoretical yet these clinical imaging biomarker tools and technologies with their associated hypotheses can be tested and validated today in clinical trials of potential disease-modifying agents for Alzheimer's disease. (e.g., vaccines, monoclonal antibodies, and small molecular therapeutics).

Another good example of the development of disease related clinical imaging biomarkers using shared resources is the Imaging Consortium for Drug Development (ICD), that currently involves Merck, Lilly and McLean Hospital (Harvard Medical School), and is open for other participants. The ICD objectives are to conduct basic

and applied research activities, both preclinical and clinical to assess the utility of fMRI in the development of pharmaceutical products to treat pain disorders. The ICD efforts have established an fMRI database for human and animal imaging containing the "signatures" of several well-known CNS active compounds and their activity in functional imaging experimental pain models. These efforts are currently examining fMRI in pain perception and the dose response of analgesic (e.g., buprenorphine) and non-analgesic (e.g., fosaprepitant) CNS active compounds. The long-term goal of ICD is to define industry standards for the use of fMRI as a translational technology in drug development (you can visit https://meitner.mclean.harvard.edu/index.jsp for more information).

Conclusions

Imaging biomarkers hold immense promise to accelerate the discovery and development of CNS drugs by accelerating early decision making around molecules and clinical hypotheses. However they increase early investments in drug discovery, often at the expense of drug discovery efforts, and in order to be successful will have to be used rigorously on a portfolio wide basis for decision making. Clearly this approach has yet to have a clear impact on phase 3 clinical trial starts or drug registration although it is perhaps too early to judge. There are a number of important questions to be considered by the imaging experts and drug developers in the future (1) which imaging technologies will prove operationally feasible in multicenter versus single site settings? (2) Which imaging biomarker technologies should we invest in the most– universal functional imaging or proprietary target engagement markers? (3) Should we develop clinical disease markers in parallel with clinical trials or focus on pharmacodynamic, proof of biology and target engagement markers only? (4) What level of confidence is needed to consider novel imaging biomarkers scientifically validated and clinically qualified? (5) Which imaging biomarkers translate best from animals to man (6) what gaps still need to be filled by new imaging biomarkers? Many of these questions are being and will be answered as the strategy for imaging in the discovery of drugs and its development. All of these imaging approaches will provide scientific knowledge of great value to the neurosciences but not all will find a place in the drug development tool box. It is our challenge to work out which imaging agents and technologies will have the greatest value and to focus on their integration into our research processes in order to help deliver safe and effective medicines to people who need them.

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Reasons to Believe: The Potential of Imaging in CNS Drug Development

Richard Hargreaves, Edward Bullmore, Lino R. Becerra, and David Borsook

Introduction

There has never been a better time to explore imaging as a legitimate tool to enhance decision making in drug discovery and development. Imaging technologies are now being used in preclinical and clinical discovery by many pharmaceutical companies and academic groups and have become an integral part of the research process particularly in the neurosciences (Borsook et al. 2006; Rudin et al. 2003; Beckmann et al. 2004; Wise and Tracey 2006; Matthews et al. 2006; Pohlmann et al. 2007; Borsook et al. 2008). The use of CNS imaging as a biomarker in drug development has also been supported by the Food and Drug Administration (FDA) Critical Path initiative and the advent of the Exploratory Investigational New Drug (IND) guidelines (http://www.fda.gov/CDER/guidance/7086fnl.htm) that facilitate the development of imaging agents. The current volume has reviewed the remarkable progress that has been made in the development of diverse imaging technologies and methodologies that have revolutionized our ability to monitor functional (evoked and resting state networks – RSNs), anatomical (diffusion-tensor imaging – DTI – and magnetic resonance – MR – morphometry), and chemical (e.g., magnetic resonance spectroscopy – MRS) changes in the living brain systems of animals and humans to study the effects of drugs and disease (Prichard et al. 1999; Gur 2002; Logothetis 2002; Logothetis 2008; Hyder et al. 2001; Beckmann et al. 2005; Mori and Zhang 2006; Jissendi Tchofo and Baleriaux 2009).

Positron emission tomography (PET) has been the dominant imaging technology used in CNS drug development over the last decade and it has featured in most of the early development of New Drug Applications (NDAs) approved from 1995 through 2004 in the Division of Neuropharmacological Drug Products at the FDA (Uppoor et al. 2008). PET imaging has very specific place in decision making for

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CNS drugs as it provides a way to verify that there is true CNS target engagement by drugs at well tolerated exposures before clinical hypothesis testing in patient populations. Receptor occupancy is not however proof of efficacy and PET has now been progressively augmented by other neuroimaging approaches that provide clear evidence of the pharmacological, pharmacodynamic, and therapeutic consequences of drug receptor interactions in healthy volunteers and patient populations particularly in the early stages of CNS drug development.

Imaging in Preclinical Drug Discovery

Minimally invasive imaging techniques have a unique place in preclinical CNS drug development as they can be used to link target engagement and drug-induced functional biological changes that can be cross validated using detailed invasive methodologies utilizing neuronal markers or electrophysiology. Once a CNS target has been identified, imaging can (1) help to choose the best molecules to study by selecting those that reach the highest levels of target engagement from the least exposure, thereby opening therapeutic safety windows, (2) monitor the functional effects of drugs on specific brain regions and neural networks, (3) repurpose failed drug candidates to potential new indications, and (4) assess strategies to enhance brain penetration and activity.

Minimally invasive imaging also gives a unique opportunity to improve the interpretation and predictability of preclinical animal models by studying them with techniques that can be used in early clinical trials. The read-out of behavioral animal assays often reflects the output of multiple processes taking place across CNS networks. Functional imaging can be used to reveal these pathways in the brain of living animals, thereby providing unique information on the consequences of drug–target interactions. It is hoped that by using CNS networks as a "language of translation" (Borsook et al. 2006; Wong et al. 2009), in either current or new models adapted for imaging, the levels of failure from preclinical to clinical trials may be decreased.

Imaging in Clinical Development

The potential uses of imaging in clinical development are summarized in Fig. 1.

The value of CNS imaging in clinical development comes from its use in (1) early phase clinical studies and (2) evaluations of disease state. It is hypothesized that smaller populations of individuals or patients will need to be studied to reach go/no go decisions with imaging relative to traditional trial designs. Examples of early phase data that could be obtained by imaging small cohorts of volunteers or patients early on, following the initial "first in man" tolerability trials, are (1) CNS

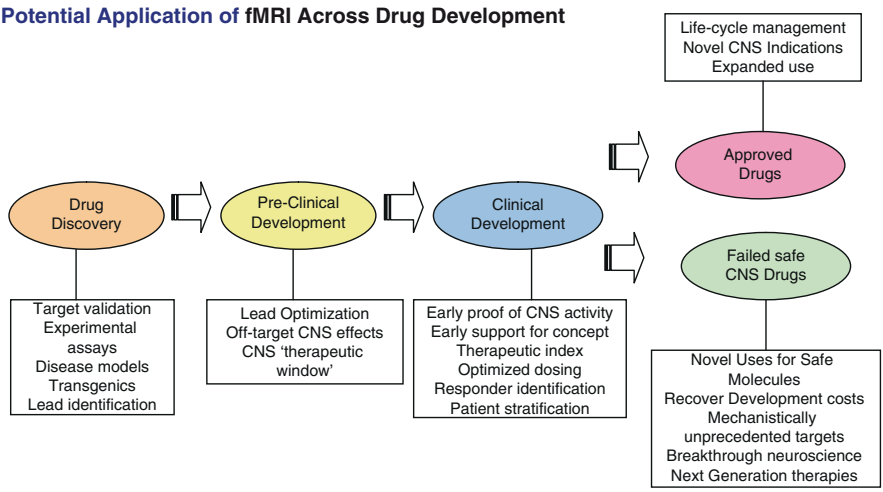

Fig. 1 Potential application of fMRI across drug development (From Borsook et al., 2006, Nature Review Drug Discovery, with permission)

target engagement to optimize dosing, (2) proof of CNS activity, (3) support for therapeutic concept, (4) a CNS phenotype for a disease state that allows for greater specificity of selection of patients for early and late clinical trials, and (5) more speculatively, a potential to warn of potential side effects including nausea and vomiting, potential addiction, psychosis, or somnolence based on specific neural circuit activation. Current strategies often involve the parallel assessment of novel imaging paradigms in small cohorts of patients as investigational endpoints alongside traditional clinical measures. An important benefit of being able to study smaller number of volunteers or patients than would be required for more traditional study designs is a reduction in human risk during the early phase assessment of novel chemical entities that may ultimately have no therapeutic value. It should however be cautioned that, in general, proof-of-principle studies that define the reproducibility and specificity of many clinical imaging paradigms (other than PET) are lacking or still in progress (http://www.imagingdrugs.org) today. Only when these studies are complete will we be able to design trials with sufficient power to have confidence in their outcome. The big question is whether the level of reproducibility is such that the use of imaging actually does save time and money, and help decision making.

Many pharmaceutical companies are currently in the scientific validation and clinical qualification stages with proof of concept imaging trials using diverse imaging paradigms with drugs of proven or failed clinical benefits. Such efforts are essential to validate robust imaging platforms that can be used routinely in drug development independently of proprietary drug targets. Clinical applications clearly also need to establish protocols for the evaluation of novel drugs in the imaging environment that have appropriate operating procedures that meet the guidelines for good clinical practice (GPC) for industry (http://www.fda.gov/cder/guidance/959fnl.pdf).

The role of CNS imaging in late phase clinical development (phase II or III or IV – post marketing) studies, especially registration, is less well defined as here there is more of a regulatory rather than development risk associated with decision making. Imaging has been used in the registration of drugs for the treatment of multiple sclerosis (MS) and yet today its value has been reassessed as it is now known to be a more sensitive endpoint than the symptoms of disease. As a consequence, imaging today provides a go/no go decision point in the evaluation of novel drugs to treat MS because if the CNS lesions do not resolve treatment is not working and the trial can be terminated but if they do resolve it is still necessary to continue dosing to evaluate the impact on disease symptomatology.

Recently it has been proposed that imaging is a key technology for the conduct of clinical trials in Alzheimer's disease. In suspected Alzheimer's disease, PET imaging of amyloid can be used to identify patients for clinical trials that test the amyloid hypothesis and then anatomical volumetric CNS imaging used to follow disease progression and the neuroprotective effects of the potential disease modifying agents. Longitudinal studies have suggested that cortical, entorhinal, and hippocampal volumes decline at monitorable annual rates and therefore may provide early indices of clinical response to treatments (Jack et al. 2003). These observations formed the basis for the formation of a consortium, the Alzheimer's Disease Neuroimaging Initiative (ADNI), a publicly funded initiative with private company members, which is being run by the Foundation for the National Institute of Health (FNIH). The ADNI is studying the progression of mild cognitive impairment and Alzheimer's disease using magnetic resonance imaging (MRI), molecular imaging with PET, and cerebrospinal fluid (CSF) biomarker candidates. The ADNI program will characterize today's Alzheimer's disease patient population and define standards and baselines that can be used to develop improved methods for clinical trials of drugs that could alter disease progression (Jack et al. 2008). Brain volumetric/anatomical imaging in this context will potentially "enrich" neuropsychological cognitive registration endpoints (Cummings 2008) and provide a biological basis for any observed improvements in the core symptoms of Alzheimer's disease.

Another example of academic/industry consortium is that formed by Merck, Eli Lilly, Sepracor, and the McLean Hospital/Harvard Medical School, namely, The Imaging Consortium for Drug Development (ICD). This industry initiative is investigating whether functional magnetic resonance imaging (fMRI) can be used to enhance our ability to find new drugs for neuropathic pain. This combination of academic/industrial expertise pools knowledge, and funds an initiative that is beyond any of the group's resources alone. Once validated, these fMRI signatures can be used by each member company confidentially in early development to evaluate their proprietary pain drug candidates (Borsook et al. 2008). However, as a reality check, it should be noted that functional imaging is in its infancy and has so far made little contribution to regulatory drug approval processes in contrast to CNS anatomical and PET imaging.

Reasons for Optimism

The verdict is not out, but there are good reasons to be optimistic that imaging will change the CNS drug development model. The application of current imaging paradigms and future technological advances promise to lead to more efficient methods of CNS drug development that will decrease the risk of development and reduce cycle times. The relationship between drug target engagement (occupancy) and drug efficacy (function) will be progressively re-evaluated in the light of novel insights from molecular imaging and functional imaging. Imaging technologies are therefore "process transforming tools that have the potential to fundamentally alter the way we see CNS disease and our approaches to drug development" (Paul Shea, MPI). Functional as well as molecular imaging can provide potential markers of disease as well as drug effects and moreover could provide a means for endophenotyping (defined as "of a complex behavioral phenotype that can facilitate the identification of relevant genes and elucidate their function" http://www.nature.com/nrg/journal/v6/n4/glossary/nrg1575_glossary.html) diverse CNS diseases. Indeed it has been suggested that CNS imaging can be viewed as a form of *high content screening* (Bickle 2008), as it can provide information on CNS drug effects and disease processes that can be used as biomarkers in the development process.

Reasons to Believe

It is not expected that all the ideas discussed in this book will be successful and there are always risk and cost to exploring new tools and technologies that may ultimately be fascinating and highly successful scientifically but have limited or no value in the drug discovery and development environment. However, given the current risk and associated high costs of CNS drug development together with low success rates in this therapeutic area, it seems prudent to explore new technologies that could enhance our drug discovery processes.

Imaging has the potential to impact CNS drug discovery at many stages in the development process. Imaging is expected to compliment rather than replace current drug development methodologies. We expect it to provide early readouts that can help advance or stop discovery and development programs at key stage gates and decrease risk by generating novel information that can be used to improve traditional decision making processes. Imaging can enhance the probability that we will successfully choose the best molecules and clinical hypotheses to progress from the lab to the clinic, from healthy volunteers to patients and on to more extensive and time consuming clinical outcome trials. Imaging therefore has the potential to decrease cycle times and thereby help us to bring effective treatments to patients sooner.

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