

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).

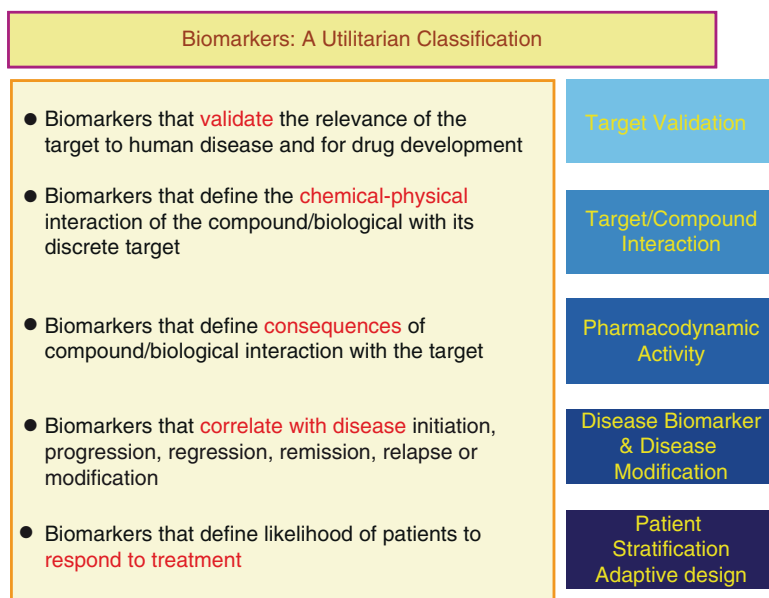
One factor contributing to this inverse relationship between escalating drug development costs and successful new drug approvals can be attributed to the continued decreasing probability of successful transition through critical proof of concept studies in early clinical development (Feuerstein, 2007). 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.



Feuerstein et al, *American Drug Discovery*, 2007

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

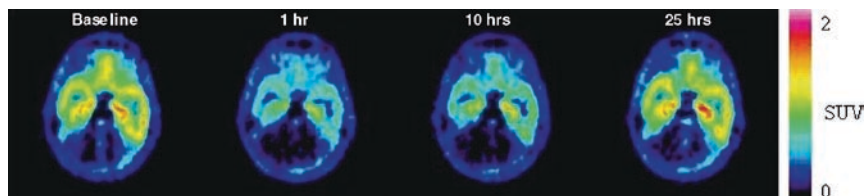


Fig. 2 [^{11}C]-WAY-100635 uptake in human temporal cortex. The scans were taken before and at different times (hours) after administration of 5 mg of lecozotan IR formulation to a young healthy subject. $5\text{HT}_{1\text{A}}$ receptor occupancy of lecozotan, calculated based on uptake, serves as a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

allowed a clear understanding of the relationship between lecozotan $5\text{-HT}_{1\text{A}}$ RO and drug plasma concentrations and enabled the development of a PK/PD model which predicted peak $5\text{-HT}_{1\text{A}}$ 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 $5\text{-HT}_{1\text{A}}$ 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 Alzheimer'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

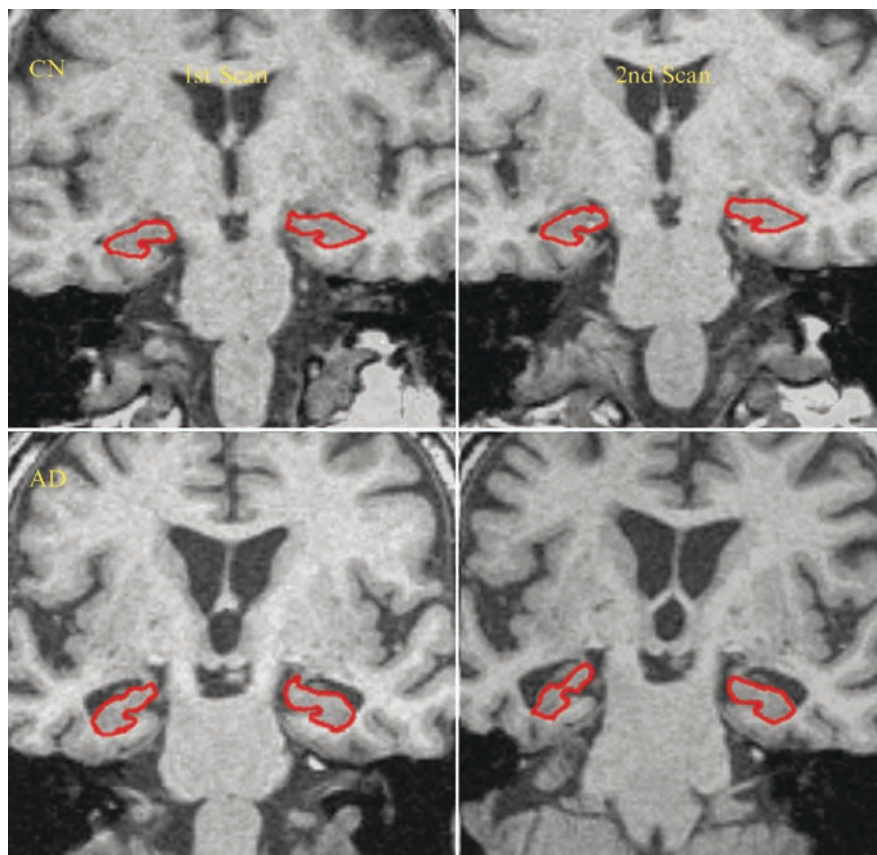


Fig. 3 Hippocampal atrophy detected in AD patients. Two MRI scans 1 year apart in the same individuals are shown. Hippocampi are indicated by the red trace on coronal sections. Marked atrophy (reduction of hippocampus area) is observed in an AD patient compared to an age-matched control subject. The 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.

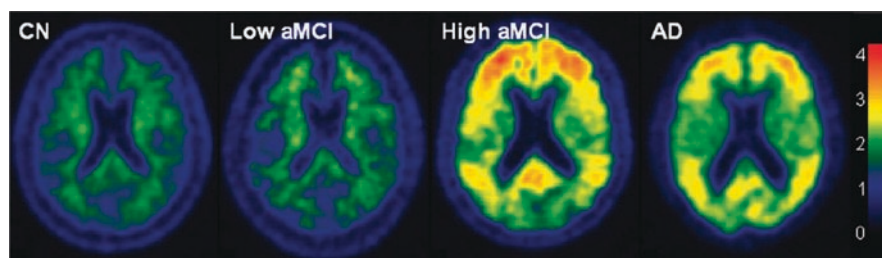


Fig. 4 Amyloid deposits detected with ^{11}C -PIB PET. Increased retention of PIB signal in cortical and temporal areas is observed in AD patients and subset of amnesic MCI patient compared to cognitive normal controls. The global PIB retention ratio can be used as a biomarker of amyloid plaques and 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 A β 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 -H₂O, ^{18}F 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

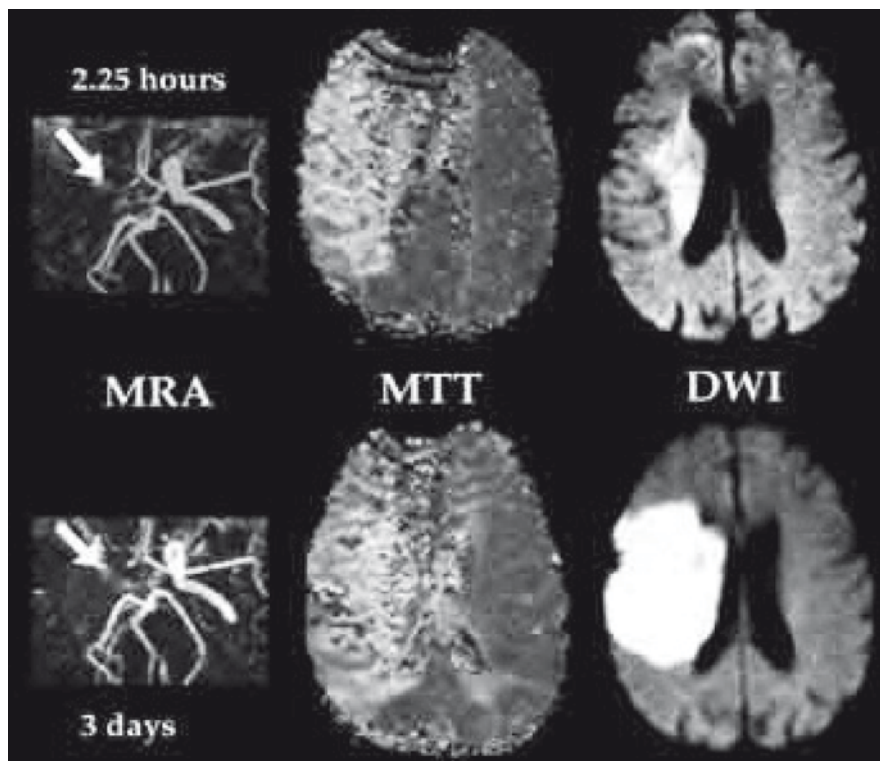


Fig. 5 PWI/DWI mismatch can be used to identify the presence of penumbral tissue. Initially (2 h post ictus) a large perfusion deficit (MTT) and small DWI lesion suggest the presence of large penumbral region. In the absence of reperfusion or other affective therapy, ischemic lesions mature into large 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 mismatch 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., $\geq 72\%$ for hyperprolactinemia, and $\geq 78\%$ 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.

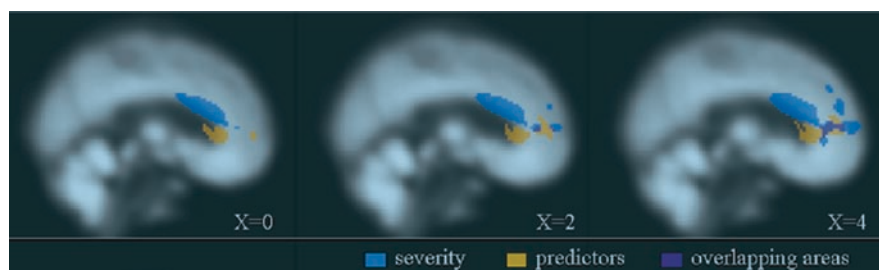


Fig. 6 Activation in pregenual cingulate cortex predicts antidepressant response independent of depression severity. This figure shows substantial dissociation between brain areas predicting antidepressant response, and those associated with severity of depressive symptoms. From Chen et al. (2007)

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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