Pharmacological MRI Approaches to Understanding Mechanisms of Drug Action

Michael J. Minzenberg

Abstract Functional neuroimaging is a novel technique for the study of drug action in the brain. The emerging role of this method is intimately tied to the unique challenges to advancing drug development for neuropsychiatric disorders. This chapter first presents a brief overview of the important treatment needs that remain to be met, which serve as clinical targets for drug development. Important factors that hinder progress in drug development, which arise from clinical, scientific and economic issues, are acknowledged. This sets the stage for the unique advantages of functional neuroimaging modalities such as functional MRI (fMRI) as a biomarker and drug development tool, in both clinical and preclinical phases. The physiological basis of the fMRI signal is briefly outlined, and aspects of neural signaling related to this signal change, with emphasis on implications for pharmacology studies. The utility of fMRI for evaluating the full anatomic extent of central neurotransmitter systems in a dynamic manner is then described. This is a critical advantage, and particularly important for studies of how systems such as the monoamines modulate distributed neural networks during cognitive processes in both health and illness, and how these actions are modified with pharmacological intervention. Central catecholamine systems are seen as paradigmatic targets amenable to pharmacologic fMRI. fMRI is observed to occupy a unique position in the armamentarium of methods available to the pharmacologist and the drug development process, and poised to play an expanding role in basic and clinical neuroscience.

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1 The Status and Challenges of Drug Development in Neuropsychiatric Illness

Any discussion of new methodologies available to advance neuropharmacology must first acknowledge the tremendous personal and public health impact of neuropsychiatric illness, and the substantial challenges to furthering our knowledge of how drugs work in the human brain. Psychiatric disorders are among the most debilitating illnesses that humans face. Contemporary global epidemiological surveys such as those conducted by the World Health Organization (Murray et al. 1994) have established a number of high-prevalence psychiatric conditions, including schizophrenia, mood disorders and substance abuse, as among the highest impact in medicine overall, reflected in measures of long-term disability. Not surprisingly, these conditions are associated with inordinate suffering on the part of the afflicted, their families and loved ones, and considerable economic cost incurred as direct costs of care and enormous levels of lost productivity.

A number of important unmet treatment needs persist among the more serious and chronic conditions encountered in psychiatry. These include symptom domains such as negative symptoms and cognitive dysfunction in schizophrenia, which confer the greatest impact on clinical outcome in this illness yet have no established treatment; treatment-refractory states and relapsing–remitting courses of illness, even for symptoms which serve as primary treatment targets, in mood, anxiety, psychotic and substance-use disorders; “secondary” symptoms that arise from these disorders and strongly modify outcome, such as impulsivity and

suicide; the deleterious effects of persistent symptoms on health-related behaviors and medical co-morbidity; and uncertain and variable effects of existing treatments on subjective well-being and long-term functional outcome, even for many patients who achieve full remission from primary target symptoms such as psychosis. The single-minded emphasis on monotherapy for disorders that are heterogeneous (at least as currently defined) may be one factor that tends to preclude attention to neglected clinical targets (Hyman and Fenton 2003). On the other hand, inappropriately narrow clinical indications are likely to be disapproved by the FDA as "pseudospecific" (Laughren and Levin 2006), leading to the need to "walk the tightrope" in adequately addressing these targets. More generally, there is little evidence that the morbidity or mortality associated with psychiatric illness has changed in the era of modern psychopharmacology (Insel and Scolnick 2006). Furthermore, the notion of either primary or secondary prevention remains a neglected goal in psychiatry (Insel and Scolnick 2006). Therefore, genuine advances in psychiatric treatment hold the promise to alleviate considerable suffering and global health burdens.

Unfortunately, the remote and recent history of drug development in psychiatry poses numerous challenges to achieving this aim. As is well known, the early history of drug discovery in mental health is characterized largely by serendipity (Ban 2006), with major successes initially arising from keen clinical observations made during use of certain compounds for unrelated medical conditions (such as surgical procedures, respiratory infections, hypertension and epilepsy). More recently, the industry has virtually uniformly pursued the strategy of mining existing targets (such as D2 receptors for antipsychotics, or monoamine transporters for depression) with a proliferation of so-called "me-too" products. One important consequence is that the newer agents available for major mental illness generally lack enhanced efficacy for existing treatment targets, nor clinically significant efficacy against new targets, but rather extend the range of adverse physiological effects that patients are exposed to, including some (e.g. metabolic syndrome) that appear likely to adversely affect long-term clinical outcomes such as life expectancy. Unfortunately for patients, families and clinicians, there may be a vicious cycle where the patients in most need of treatment advances (e.g., schizophrenia patients with prominent negative symptoms or cognitive dysfunction) are the least likely to benefit from new medications.

While some pharmaceuticals for central nervous system (CNS) indications have been among the most successful throughout medicine in recent years, generating staggering revenues for large pharmaceutical companies, with massive, expanding markets for these drugs, there has been a recent withdrawal overall of the pharmaceutical industry from CNS. The proximal causes appear to be the inordinate and expanding cost of bringing new drugs to market, particularly costly for CNS drugs, which show a relatively high failure rate compared to other classes of agents (Kola and Landis 2004). This scenario is accounted for in part by the increasing cost of obtaining regulatory approval. This trend away from CNS was set in motion even prior to the recent economic downturn, and appears likely to worsen, given the trend in costs (Breier 2005).

Many reasons for this inordinate cost dovetail with the scientific challenges to advancing drug development. There is now a considerable dialogue in the clinical/scientific and industry literatures addressing the myriad challenges facing the scientific, medical, regulatory and industry communities to advance the state of drug development for neuropsychiatric disorders (Agid et al. 2007; Conn and Roth 2008). These challenges, in the aggregate, provide a context for the emerging role of noninvasive functional neuroimaging in pharmacology, and are discussed in turn.

The inevitable starting place for any discussion of these challenges is the system for classifying illness in psychiatry. The current standard, instantiated in the Diagnostic and Statistical Manual (DSM, now undergoing a revision to a fifth full edition), has a long and complex history, originating in committees of experts in clinical psychiatry. This system of diagnosis and classification remains based on clinical phenomenology, that is, reportable or clinically observable signs and symptoms, without reference to underlying theoretical models of mental illness, nor empirical knowledge about etiology or pathophysiology available from clinical or basic neuroscience. While more recent editions of the DSM have relied increasingly on empirical field trials to evaluate the measurement properties and performance of these criteria sets in discriminating among discrete illness types, there remains no rooting of categories in distinct etiologies or pathophysiological processes. In addition, it remains unclear whether individual syndromes (including schizophrenia-spectrum disorders, depression and personality disorders) are better represented as dimensional conditions. As a result, heterogeneity within categories, and co-morbidity across categories, are the rule for patients. The lack of correspondence between diagnoses and distinct pathophysiologies is a particular obstacle for drug development, as the resulting heterogeneity confounds efforts to establish molecular targets and useful biomarkers of drug action (see below).

The underlying pathophysiology also remains obscure for virtually all major mental illness. The biological events or fundamental cellular/molecular processes that form the basis for illness is essentially unknown for schizophrenia, autism, bipolar disorder, etc., despite a wealth of epidemiological clues about antecedents and risk states for many disorders, and a range of consistent biological and cognitive abnormalities observed in these disorders. This makes it hard to characterize and capitalize on treatment targets, as the field is to some extent "shooting in the dark." Beyond the nosological constraints, both the polygenic nature of mental illness, and the inherent complexity of the brain, may be root causes of this obscurity. While this is increasingly recognized in psychiatric genetics and systems neuroscience, there is no visible way yet around the roadblock.

The unique challenge of modeling the complex clinical phenomenology of psychiatric illness in animals remains an unresolved problem (Nestler and Hyman 2010). Many of the core features of mental illness, such as hallucinations, delusions, depressed mood and guilt, cannot be induced or detected in animals, including non-human primates. While cellular and molecular processes implicated in psychiatric disorders (such as monoamine transport, signaling via D2 receptors,

brain-derived neurotrophic factor, etc.) can be reliably and specifically manipulated with diverse methods, animal models of clinical phenomenology tend to express at best very partial (and typically very nonspecific) features of illness. For instance, animals with targeted gene knockouts or brain lesions that lead to social withdrawal are often suggested to variously model schizophrenia, autism and depression, which are distinct clinical conditions with widely divergent patterns of gene association, biological abnormalities, treatment response and clinical course. In addition, many cognitive processes that are important to psychiatric illness (e.g. language, abstract thought) are not found in any other species. Among treatment considerations, the precise drug treatment regimen (e.g. dose, duration) in animals that properly models treatment in clinical settings is typically uncertain. Furthermore, variation across species in both pharmacokinetics and pharmacodynamics confers hard limits on the utility of animal models for predicting the efficacy of drugs in humans. Not surprisingly then, there is poor predictive utility of animal models for effective treatments for cognition, for instance (Hagan and Jones 2005). Animal models remain an important pillar of both basic and clinical neuroscientific knowledge, given the inaccessibility of brain tissue in patients, the range of manipulations possible in animals, and the close correspondence of physiology across mammalian species. Nonetheless, significant refinement in how these models are developed and utilized will be necessary to improve their contribution (Nestler and Hyman 2010).

Interestingly, even for those drugs with clinical efficacy established beyond doubt, such as D2 antagonists for psychosis, or monoamine transport inhibitors for depression, the critical mechanism of action remains unclear. The consequences for neurotransmission of administering these drugs are well established; however, clinical effects tend to exhibit a considerable temporal delay beyond the onset of effects on neurotransmitter receptor activity. Delayed changes in neuronal signaling rates or patterns, intracellular signaling cascades, gene transcription and structural changes in synapses have each been addressed in recent work as likely mediators of clinical efficacy. Nonetheless, how an individual's brain can get from any of these changes relief from auditory hallucinations or suicidal thoughts remains essentially unknown. In addition, for many drugs (e.g. aripiprazole, clozapine, lithium, valproic acid, mirtazepine) there is evidence for a multiplicity of actions, each of which may be responsible for efficacy. This may be why investigators remain divided on whether more, versus less, specificity in the profile of drug action matters for clinical efficacy (Hopkins 2008; Roth et al. 2004). Indeed, the history of failed versus successful agents for major mental illness suggests that "magic shotguns" should be preferred to "magic bullets" (Roth et al. 2004). The trouble with this observation follows from the complexity of effects associated with a single drug that has multiple actions on numerous highly interacting neurotransmitter systems. This may necessitate both non-conventional small-molecule screening approaches to identify promising agents, and significant advances in direct testing in humans by a variety of experimental methods, including functional neuroimaging.

2 Functional Magnetic Resonance Imaging as a Biomarker

The question of which molecular process is most essential for clinical efficacy, and therefore most worthy of investigation and development, is often framed as a problem of "target validation." This notion is intimately tied to the construct of "biomarkers" in drug development. The NIH Biomarker Definitions Working Group has defined a biomarker as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention" (Lesko and Atkinson 2001). The FDA has proposed a similar definition of a "marker" or surrogate endpoint for testing medical treatments (US Senate Bill 830), and the FDA Critical Path Initiative (http://www.fda.gov/oc/initiatives/criticalpath/) has identified Imaging as a critical technology to surmount the drug development roadblock. A typical role for a biomarker is to properly "measure the delivery of drugs to their intended targets, and to understand and predict pathophysiology, and how it is altered by therapy, through monitoring variables known to have clinical relevance" (Frank and Hargreaves 2003). The NIH Working Group defines three levels of biomarkers: those that track the natural course of illness (type 0); those that examine intervention effects, where the drug's mechanism of action is known but where a strict relationship to clinical outcome has not been established (type 1); and those where changes in the biomarker are truly predictive of clinical outcome (type 2). Wong and colleagues suggest that at present, most neuroimaging measures can be considered to have the status of either type 0 or type 1 biomarker (Wong et al. 2009). Useful biomarkers can uniquely enable proof-of-concept studies for novel agents early in the drug discovery process, and thus reduce risk in safety and efficacy determinations in the course of drug development. They can be used to directly compare competing interventions, to evaluate pharmacokinetics, to stratify clinical populations, to ascertain treatment considerations such as dose and duration. They are also often sensitive predictors of adverse events associated with a given agent, including the detection of central effects of agents with primarily peripheral actions. As a surrogate for a clinical endpoint, they ideally provide earlier (and less expensive) measures of clinical efficacy and side effects. Furthermore, useful biomarkers may support the extension of a known compound into new therapeutic areas as its effects in the human brain are elaborated, and importantly, may also permit the "rescue" of drugs that have failed a primary CNS indication (O'Connor and Roth 2005; Shorter 2002). Biomarkers are now ubiquitous in drug development for indications throughout medicine, with variable levels of success which depends in a large part on the degrees of validation of the measure as a biomarker.

Given the foregoing review, it should be clear why the development of biomarkers in psychiatry has lagged considerably behind that of the rest of medicine. Nonetheless, CNS may be the vanguard in the use of imaging biomarkers in medicine, with other specialties following this lead, and it is reasonable to assume that each of the goals of a good biomarker outlined above will be attained with

functional neuroimaging measures in the foreseeable future. Wong et al. (2009) emphasize two critical phases in the course of drug development where noninvasive neuroimaging has emerging potential. First, clinical experiments that are conducted relatively early in development that aim to demonstrate "proof of biology" by testing a novel hypothesis by associating target engagement with a biological change that is proposed to lead to a therapeutic response. Second, subsequent clinical studies that demonstrate "proof of concept", defined as proof that this engagement of the target is linked to a clinically significant change in a clinical endpoint, or in other words that this target engagement by the drug has a true clinical impact that is likely to change how patients with the given condition are treated. This is typically established in phase II clinical trials.

A number of other practical advantages are observed with pharmaco-fMRI in drug development. The method does not involve exposure to ionizing radiation, rendering it more useful for repeated imaging studies in individual subjects. The capacity for easy, reliable repeat scanning is a significant advantage, as this is necessary to permit full washout of drugs with a long elimination half-life (which is a desirable feature of agents for clinical use), and to evaluate effects that may be region- and time-sensitive, such as those underlying a latency to clinical efficacy, as well as tolerance, sensitization and withdrawal effects (Stein 2001). Time-sensitive effects may be important to identify limits to effective drug action. Repeat studies also allow within-subject study designs, to confer greater statistical power for the evaluation of dose–response relationships (and dose–response interactions with brain region) and for the comparative efficacy of different agents, by varying doses or agents within subjects across sessions (Honey and Bullmore 2004).

3 Functional Magnetic Resonance Imaging in Preclinical Drug Development

The utility of fMRI is in fact observed even prior to clinical phases of drug development. One distinct advantage of this methodology is that it can be used noninvasively and in a repeated manner across diverse species, yet it is also feasible to combine with more invasive measures of neural activity in animal models. Rodents and primates alike are now readily investigated with fMRI, including with MRI scanners with small bores to accommodate rodents and particularly high magnetic field strengths (e.g. 12 Tesla) that are not yet approved for use in humans. The use of high field strength and combination with invasive measures such as intracranial electrical recordings or microdialysis has afforded insights into the relationship between BOLD signal change and cellular processes, to inform the use of fMRI in clinical populations. fMRI in animal models provides "functional signatures" by identifying distributed neural circuits engaged by a certain drug. It may be more sensitive to changes in a circuit underlying behavioral or cognitive effects than the overt behaviors themselves that are measured in traditional behavioral assays. Active circuits modulated by drugs may also remain

undetermined by overt behaviors in behavioral assays, yet may nonetheless have clinically relevant effects. It is less dependent on a limited set of receptor ligands, which significantly restricts the range of pharmacological assessments available in positron emission tomography (PET). fMRI may also have a particular advantage for specific classes of agents. For instance, full receptor agonists, partial agonists and inverse agonists are now entering the pharmacopoeia as novel agents for a variety of neuropsychiatric conditions (with aripiprazole as a successful example). Agents with these pharmacological actions, or potent agonists acting at low-density CNS receptors, are difficult to evaluate with other modalities such as PET. fMRI is uniquely capable of evaluating the neural effects of agents such as these, as it primarily indexes post-synaptic membrane polarization states, without relying on competition between a given agent and a (radiolabelled) competitor for ligand binding (see below). fMRI may in fact be the only existing method which can provide proof of target (at the circuit level), and target engagement, for these classes of agents (Borsook et al. 2006).

4 The Biophysical and Physiological Basis of Signal in fMRI

Because a fundamental concern of neuropharmacology is the effects of chemical compounds on signaling processes in the brain, it is essential to consider the types of signaling processes that can be measured by fMRI. As with all MR-based imaging procedures used in humans, the fMRI signal from which neural activity is inferred is derived from complex biophysical phenomena, and dependent on a particular set of physiological features of neural and hemodynamic properties of the brain and cerebrovasculature. While a full treatment of this is precluded by length considerations, a proper basic understanding is necessary in order to consider how fMRI measures brain activity, and the challenges of elucidating the action of drugs which act on both the neurons and the vasculature of the brain. Many excellent, detailed overviews of these phenomena are available elsewhere (Logothetis and Wandell 2004; Norris 2006); these are briefly outlined here as an introduction, along with some basic procedural aspects of MRI. First, a large, doughnut-shaped magnet with a circular electric current (maintained at very low temperatures to perpetuate the current) establishes a static magnetic field with relatively high strength, on the order of 40,000 times that of the earth's magnetic field. Subjects are placed inside, with humans typically reclining awake and relaxed in a supine position on a table inserted into the bore of the magnet; they typically maintain both visual contact with a monitor to present visual stimuli during cognitive tasks, and auditory contact with the investigator at the MRI console (though other tasks and monitoring are feasible, such as auditory, gustatory or somatosensory stimuli, physiological monitoring, eye tracking/pupillometry, etc.). The magnetic field is experienced in the subjects' soft tissues, with the most important effect being the alignment of protons with the direction of the static field. These protons are primarily available as hydrogen ions in water;

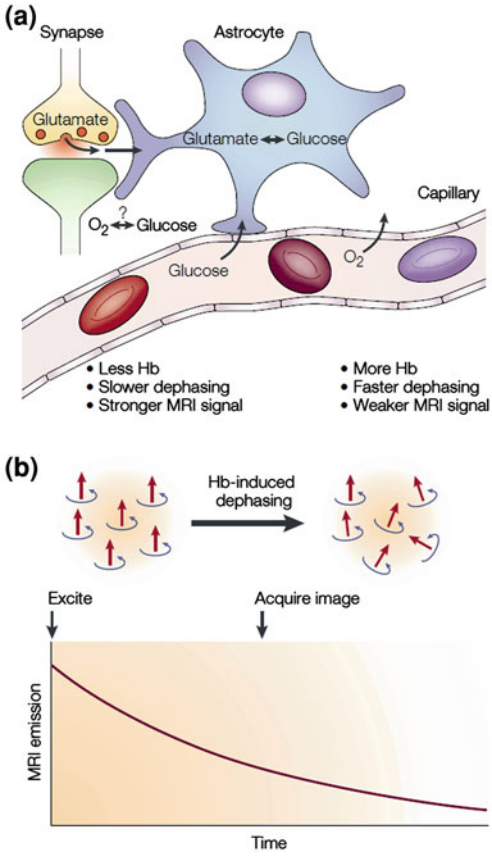

Fig. 1 BOLD functional magnetic resonance imaging (fMRI)

at relatively low field strength this source is primarily intravascular, but as the field strength increases, an increased proportion of protons from water are contained within the parenchyma of the brain. A radiofrequency pulse is then triggered in a repetitive manner during the course of image acquisition. This pulse alters the angle of the protons' spin in a manner that is controlled by the pulse sequence (the "flip angle") set by the investigator. With the brief cessation of the pulse, the original angle of the protons' spin is assumed again. This is referred to as "relaxation", and during this process energy is released which is detected by the scanner. A gradient coil distributes the field in a graded manner across space (i.e., the subject's head); this creates signal variation in two spatial dimensions that permits a two-dimensional image "slice" of the brain to be acquired, and these slices are in turn acquired in a progressive, repetitive manner across the third spatial dimension so that a three-dimensional image can be constructed. The signal acquired is maximized with the proportion of protons that resonate, that is, spin in phase with each other. Protons that spin out of phase tend to cancel each other's contribution to the signal recovered by the scanner. This "dephasing" is strongly

affected by inhomogeneity in the local tissue environment; in the vasculature, this is determined most critically by the oxidation state of hemoglobin contained in erythrocytes that course through the tissue.

Here, the physiology of the cerebrovasculature comes into play. The vasculature of the brain has complex autoregulatory processes to maintain blood flow and oxygen delivery to the brain (Heeger and Ress 2002) (see Fig. 1).

Among these, as relative increases in local neural activity occur (e.g. in response to a sensory stimulus or another information processing demand), there is a transient increase in flow to the local area. While the increased neural activity elicited in this manner may rely primarily on "aerobic" glycolysis, the vasculature tends to deliver more oxygen than needed locally. Therefore, the oxygen extraction fraction tends to decrease with increased local neural activity, and the fraction of oxygenated hemoglobin coursing through this vascular bed increases. As the paramagnetism of hemoglobin decreases with increasing oxygen content, the local field inhomogeneity is transiently decreased, permitting a relatively higher rate of signal recovery by the scanner, due to relatively less dephasing of the signal among the affected protons. Thus, the signal measured in fMRI is referred to as the Blood Oxygen Level-Dependent contrast, or BOLD signal (Bandettini et al. 1992; Kwong et al. 1992; Ogawa et al. 1992) [reviewed in (Logothetis and Wandell 2004)]. This signal is measured as a relative signal, as it does not strictly quantify blood flow, oxygen content, or energetics in the neurons or local tissues (though other emerging MR-based methods are now available to quantify flow, such as arterial spin-labeling). Therefore, the degree of neural activity determined with this method and attributed to the experimental condition(s) must be measured relative to a "baseline" condition. This baseline can be either an implicit baseline, typically determined as the residual (i.e. the error term) in a statistical model of experimental effects, or alternatively, the signal associated with another explicit (baseline) experimental condition.

A critical feature of this complex physiological process, with particular implications for the use of fMRI for pharmacology, is the link between neural activity and hemodynamics. This functional link, between neurons/glia and the adjacent vasculature, is referred to as *neurovascular coupling*. The precise cellular and molecular mechanisms that form the basis of this coupling remain incompletely elucidated (Raichle and Mintun 2006). However, a number of distinct yet interrelated biochemical processes and mediators have been implicated. These include vasoactive ions, nitric oxide, glutamate, adenosine, lactate, prostaglandins and other endogenous compounds, with a special role for astrocytes (Girouard and Iadecola 2006). Most of the energy need of neurons, especially in primates, appears budgeted for postsynaptic activity (Attwell and Iadecola 2002). However, other investigators have found evidence for presynaptic activity as a major energy consumer as well, and alternatively, there is evidence that neurotransmission may directly drive changes in hemodynamic activity, not dependent per se on energy utilization [see (Logothetis and Pfeuffer 2004) for review]. What is common to each of these models of neurovascular coupling is an emphasis on signaling activity by neurons as the basis for changes in local hemodynamics. Importantly, for the

present discussion, a considerable range of pharmacological agents, including numerous therapeutic CNS drugs, may have either direct or indirect effects on one or more of these biochemical processes. There is evidence for cerebrovascular regulation by a range of neurotransmitter systems, including glutamate [via NMDA receptors: (Faraci and Breese 1993; Lovick et al. 1999)], dopamine (Krimer et al. 1998), norepinephrine (Palmer 1986), serotonin (Palmer 1986) and acetylcholine (Sato et al. 2002). How the precise sites of action of these neurotransmitters in the vasculature may affect neurovascular coupling, or other hemodynamic responses that affect BOLD signal change, remain to be elucidated. This is an active area of research, not merely because of the implications for interpreting BOLD signal change in pharmaco-fMRI studies, but also because the neurochemical regulation of the cerebrovasculature is important to numerous disorders of this system, including migraine, stroke and Alzheimer's disease (Girouard and Iadecola 2006). In the meantime, particular experimental methods have been recommended to support disambiguation of neural versus vascular sources of drug effects on the BOLD signal. These include fMRI experimental design that includes proper control conditions that are not expected to show neurally mediated drug effects; direct measures of blood flow and vascular reactivity, other physiological monitoring such as oxygen consumption, and complementary measures of neural activity that are not dependent on blood flow, such as electroencephalography (Iannetti and Wise 2007). It should be appreciated that the use of these measures is not necessarily straightforward, as drug-induced changes in flow for instance may arise from changes in local central neural activity rather than directly on the local vasculature. Nonetheless, convergent evidence for a neural mechanism of action of drugs evaluated by fMRI remains an important goal.

5 Features of Neural Signaling that are Measured by fMRI

5.1 Local Field Potentials Versus Action Potential Generation

Recent studies have begun to elucidate which of the diverse types of signaling processes manifest by neurons may be measured in BOLD signal change. A very influential study conducted by Logothetis and colleagues (Logothetis et al. 2001) investigated electrical activity in awake monkeys concurrently with measurement of BOLD contrast by fMRI. Microelectrode recordings were made in the V1 visual cortex to derive measures of both local field potentials (LFP) and multi-unit activity (MUA). The LFP is a measure of slow electrical signals and subthreshold activity in neuronal membranes (not action potentials), reflecting primarily a weighted average of synchronous dendritic and somatic components of synaptic signals of a local neuronal population, with LFP amplitude a function of the extent and geometry of the recorded dendrites. MUA, in contrast, is a measure of overt spiking (action potential) activity in a recorded neuronal population, with cell size a critical determinant of amplitude, and the MUA is variably heterogeneous as a

result, especially across brain regions. Both the LFP and MUA are measured in a local population of neurons adjacent to the recording electrode, though the LFP probably is detected from a larger expanse of space than the MUA. Logothetis evaluated how each of these measures compared in their relationship to BOLD signal change in the monkeys, first in the absence of visual stimulation, to determine how to model the relationship of each to the BOLD signal. Then, they compared visually evoked BOLD responses with those predicted by the models derived from LFP and MUA. The authors found that both the LFP and the MUA predicted BOLD signal change very well in visual cortex, with a slight but significant advantage for the LFP. The difference appeared due to the transient nature of the MUA, which returned to baseline prior to stimulus offset, in contrast to both the LFP and BOLD signal, which were sustained for up to 20 s. The authors concluded that BOLD signal primarily reflects input and processing within a local area rather than spiking output. In contrast, a different study addressing this problem found that LFP and MUA were comparable in the strength of their relationship with BOLD signal change, measured with intracranial recording in awake humans (Mukamel et al. 2005). However, other studies may provide definitive evidence in favor of the LFP account of BOLD signal change (Mathiesen et al. 1998; Thomsen et al. 2004). Using a rat cerebellar preparation that permits the uncoupling of LFP with MUA, these investigators employed electrical stimulation of parallel fibers that leads to both a monosynaptic excitatory postsynaptic potential, and a disynaptic inhibitory postsynaptic potential in the Purkinje output neurons of the cerebellar cortex. With strong synaptic excitation but minimal change in the net spiking activity of these neurons, they found that blood flow and LFP nevertheless increased. This critically suggests that the mechanism underlying BOLD contrast is more directly determined by the LFP than spiking activity (though it is important to acknowledge that under most conditions, LFP and MUA are themselves highly correlated). These and other studies have also suggested that there may not be a simple linear relationship between LFP and the BOLD signal, and that the association of BOLD with both LFP and spiking activity may vary with the design of experiments, including stronger associations among all measures seen with more transient stimulus presentations that are typical of rapid event-related designs in fMRI (see Heeger and Ress 2002). More generally, with these studies BOLD signal change is presently thought to primarily reflect the postsynaptic integrative processes that occur in a local brain region, which are more diverse and arguably more informative of neural integration than spiking activity (Logothetis and Wandell 2004).

5.2 Oscillatory Brain Activity

One of the more interesting features of this synaptic integration which relates to BOLD signal change is the oscillations observed among populations of neurons. A wide range of oscillatory phenomena is seen across a wide frequency band and

throughout the brain of mammals (Buzsaki and Draguhn 2004). A number of oscillatory frequency bands have been implicated in information processing, and more generally, they may serve as signatures of dynamic functional assemblies of neurons that are responsible for cognition. Oscillatory activity can be detected by subjecting the LFP to filters to restrict the electrical potentials to certain oscillatory ranges. It can also be detected in the EEG recorded non-invasively from the scalp, when the electrical signal recorded is subjected to signal processing methods such as wavelet deconvolution. While scalp-recorded oscillatory activity maintains a lower frequency limit due to attenuation and filtering of the signal by tissues intervening between brain and scalp, brain oscillations detected at the scalp nonetheless correspond well to that measured intracranially, in their relationship with other biological and cognitive processes. Like the LFP more generally, brain oscillations are not strictly associated with spiking activity. Single neurons can oscillate, typically as a function of a set of coordinated cell membrane ion conductances; however, large-scale oscillations tend to emerge from complex dynamics between multiple neuronal types. A critical element appears to be the activity of inhibitory interneurons, which in the cortex use gamma amino-butyric acid (GABA) as a neurotransmitter. Oscillation-based synchrony is the most energy-efficient mechanism for temporal coordination (Buzsaki and Draguhn 2004). Oscillation may be an important mechanism of gain control in the nervous system (Salinas and Sejnowski 2001), as well as supporting resonance (Hutcheon and Yarom 2000), which can be seen as a form of tuning of neurons. Gain control and resonance are two important features of neuronal activity that confer sensitivity and specificity to neuronal signals and codes. One high-frequency oscillation that appears particularly related to BOLD signal change is in the gamma range (typically defined as 30–80 Hz, though higher gamma frequencies can be detected in intracranial recordings). A number of research groups have now reported strong and specific associations between BOLD signal change and gamma-range oscillations in the LFP, with diverse experiments such as the comparison of intracranial responses of neurosurgical patients to BOLD signal change in healthy adults in response to the identical visual/auditory stimulus (Mukamel et al. 2005; Nir et al. 2007); combined fMRI/EEG recording in epileptic patients performing a semantic decision task (Lachaux et al. 2007); and simple visual stimulation of V1 in monkeys (Goense and Logothetis 2008). It thus appears that BOLD signal change may reasonably index changes in power in the gamma range. This is fortuitous given the diverse cognitive processes that are also associated with gamma oscillations. These include perceptual integrative processes such as binding of sensory representations into a coherent percept (Fries 2009); episodic memory (Herrmann et al. 2004); working memory (Tallon-Baudry et al. 1998); and cognitive control processes (Cho et al. 2006; Minzenberg et al. 2010). These cognitive processes are generally highly dependent on prefrontal cortical function and ascending modulation by catecholamines and other neurotransmitter systems. Gamma oscillations (measured primarily by scalp EEG) are disrupted in a number of neuropsychiatric conditions (Herrmann and Demiralp 2005; Uhlhaas and Singer 2006) which are also characterized by cognitive deficits in these processes and their neural

substrates, measured by functional and structural imaging and post-mortem histopathology. This critically suggests that altered brain oscillations may represent a physiological signature of neural circuit dysfunction that is expressed in impaired cognition and perhaps even clinical symptomatology. While brain oscillations are fundamentally dependent on the complex temporal-spatial dynamic interactions between GABAergic interneurons and glutamatergic principal cells in regions such as neocortex and hippocampus, nonetheless there are a number of other neurotransmitter systems that modulate the power and frequency of these oscillations, and perhaps the neuronal membership in an active assembly that participates in a given oscillation (Whittington et al. 2000). These observations suggest the potential for fMRI to characterize the sites and mechanisms of action of pharmacological agents (including monoaminergic and peptidergic agents, anesthetics, and other drugs) which affect brain oscillations, and the relationship of these oscillatory effects with clinical efficacy.

5.3 Negative BOLD Response

In typical traditional fMRI studies, investigators process the acquired images and derive the signal used to infer brain activity using a linear statistical model of BOLD signal change. Statistical inference regarding the predicted experimental effects is made by contrasting the targeted experimental manipulation against the (implicit or explicit) baseline, with increased activity (typically beta values derived from the regression model of BOLD signal change) above the baseline leading to rejection of a null hypothesis. However, approximately 10 years ago, various research teams began to appreciate certain experimental conditions associated with changes from baseline in a *negative* direction, i.e. phenomena where local neural activity appeared higher in the baseline, or "resting" state and subsequently decreased during certain cognitive demands. Directed interrogation of brain regions that exhibited this pattern of task-related deactivations from baseline revealed a previously unidentified functional network in humans, centered on midline cortical regions in the medial prefrontal and parietal cortices, and lateral cortical areas including the inferior parietal lobule and lateral temporal lobe. Remarkably, this same network has been consistently identified in functional connectivity analyses of so-called "resting state" BOLD fluctuation, where subjects are awake and alert during fMRI but unengaged in any externally directed cognitive task. This network is now commonly referred to as the "default mode network", which implies a significant level of activity in the absence of an overt or externally directed task, and it is observed in both humans and non-human primates (Buckner et al. 2008; Raichle and Mintun 2006). More recently, there is evidence that the default mode network is negatively correlated with the fronto-parietal "task-positive" network, implying a reciprocal relationship that may be controlled in accordance with the behavioral state or current goals of the organism (Fox et al. 2005). In the context of the present discussion, one important implication of this discovery is that BOLD signals may be used to meaningfully

evaluate neuronal inhibition with certain cognitive processes, especially those with significant demands on attention or control processes.

An important consideration for fMRI studies of pharmacology is how the "negative BOLD response" (NBR) may inform the nature of neuronal membrane polarization states or signaling activity in the brain, and how these may be sensitive to pharmacological intervention. The metabolic budget associated with inhibitory neuronal processes is incompletely known at present, and it has been argued that quantification of the energy cost of inhibition cannot be meaningfully isolated from that of excitation in the brain (Buzsaki et al. 2007). Nonetheless, recent fMRI studies in animals have offered some intriguing hints that the NBR may be associated with increased neuronal inhibition in a local brain region. These studies have found that NBR is associated with decreased blood flow and blood volume and increased deoxyhemoglobin locally, and decreases in both the LFP and MUA (Boorman et al. 2010); and that cortical hyperpolarization occurs with local decreases in oxygenation, as would be expected with the NBR (Devor et al. 2007). fMRI studies in humans are consistent with these observations (Shmuel et al. 2002; Wade and Rowland 2010) and suggest that the NBR is not merely due to vascular steal or altered neurovascular coupling (Lin et al. 2011). Furthermore, the NBR in humans has been associated with cortical GABA concentration measured by magnetic resonance spectroscopy (Northoff et al. 2007). These various observations suggest that fMRI has utility for detecting inhibitory neuronal processes, and therefore potential for the evaluation of pharmacological effects that are manifest in neuronal hyperpolarization or decreased neuronal activity. Neuronal inhibitory processes are not only essential to brain oscillations (as indicated above), but form a fundamental feature of the operation of neural networks in general, both at the local circuit level and in long-range intracortical and cortical-subcortical network operations as well. Indeed, the "small-world" nature of cerebral circuitry that is increasingly apparent in the mammalian brain is critically dependent on these inhibitory processes, which are probably mediated primarily by GABA (Buzsaki et al. 2004). Furthermore, the actions of modulatory neurochemical systems (including those at virtually all monoamine receptor subtypes) are predominantly inhibitory. These observations suggest that for a neuroimaging method to comprehensively interrogate the effects of drugs on signaling in the brain, a valid and sensitive measure of inhibitory processes is essential. fMRI is promising in this regard, and cellular model-based tests of neuronal inhibitory effects of pharmacological agents have recently appeared (Minzenberg et al. 2008, 2011).

6 fMRI as a Tool to Evaluate the Functional Role of Central Neurotransmitter Systems

fMRI has at least three key methodological features which confer important advantages over other neuroimaging and non-imaging methods used in human pharmacology. First, the spatial resolution with the currently standard fMRI

methods for image acquisition and processing allows the discrimination of activity in local brain regions at approximately millimeter spatial resolution. While this is not yet a degree of spatial resolution that can discriminate cortical columns, or subregions within small subcortical nuclei, for instance (at least not with standard methods), it is increasingly adequate for the resolution of adjacent subcortical regions in tightly packed subcortical regions such as the thalamus or midbrain dopaminergic areas. Second, the temporal resolution of fMRI is critical to monitoring the time course of information processing as it unfolds in typical cognitive task paradigms. Virtually all experimental tasks that are in use to evaluate cognitive processes comprise few to many subcomponent processes. These include investigations of perception, attention, various forms of memory, control processes, incentive and reward processes, and motor output, not to mention more complex phenomena such as social and emotion processes. While fMRI is constrained by the hemodynamic response from the millisecond sampling rate of EEG, for instance, it still can be easily used to isolate neural activity associated with discrete events ("event-related" fMRI) or processing stages within single trials. This stands in contrast to either PET or the older approach in fMRI, which both rely on blocked sequences of trials to compare brain activity under different experimental conditions. This older approach does not permit the isolation of the varied subcomponent cognitive processes that might be demanded in a single trial: for instance, a typical working memory task paradigm requires a subject to encode a stimulus, maintain a representation of the stimulus or its associated information across a delay, perceive the subsequent target stimulus and reactivate the stored information and use it to make a decision and an associated overt motor response. This entire sequence of information processing stages typically occurs over a span of 3–20 s. Because it is entirely possible, and of great theoretical and practical interest, that a given drug modulates the maintenance of information and not the motor response that follows from the use of that information, event-related fMRI represents a clear advance over other noninvasive methods in achieving specificity in drug modulatory effects on cognition and its neural basis. The third major advantage of fMRI is the lack of dependence on the development of brain-penetrating, potent and selective ligands for neurotransmitter receptors of sufficient density in the brain, to evaluate effects on signaling. Many important neurotransmitter systems are not yet amenable to interrogation with ligand-PET in humans (e.g. noradrenergic system), yet these systems remain fully amenable to the study of drug effects on signaling processes using brain-penetrating compounds such as beta adrenergic receptor antagonists and norepinephrine transporter inhibitors. In principle, any chemical agent that enters the brain and has psychological effects can be evaluated by fMRI, both in the presence or absence of information-processing demands.

As a consequence of these particular methodological features, fMRI represents an advance in the evaluation of drug action on neurotransmitter systems in modulating distributed neural networks and the discrete cognitive processes that they support. The capacity to interrogate small structures located deep in the brain is a major advantage over EEG for instance, which only measures electrical

activity arising from large cortical regions. In order to fully understand how neurochemical systems operate, and how they are affected by drug interventions, it is critical to have a method that can evaluate drug effects on activity at the cell bodies of neurons that give rise to these systems. This is because of a single-minded focus on terminal fields (e.g. in the cortex) of projections arising from these subcortical areas, while representing the "business end" of the system as the local influence on signal throughput, nonetheless cannot account for how a drug may affect neurotransmission by modulating its major determinant, which is activity arising from the cell body. In addition, these subcortical structures have numerous reciprocal projections with their projection targets, and therefore drug effects on cognition may result from changes in descending influence on subcortical nuclei. For these reasons, it is important to interrogate the full anatomic extent of these systems, that is, the activity from the origin of cells in subcortical regions to their full terminal fields throughout the brain. A recent and exciting development in functional neuroimaging therefore is the emerging capability of fMRI to measure activity in subcortical regions such as the ventral tegmental area, substantia nigra and locus coeruleus, which give rise to monoamine neurotransmitter systems which are of great importance to both the etiology and treatment of much neuropsychiatric illness.

6.1 fMRI Studies of Subcortical Catecholamine Systems

The neurochemical systems that arise from the human brain stem and use dopamine (DA) or norepinephrine (NE) as a neurotransmitter subserve critical roles in a number of cognitive and behavioral processes. For DA, these include incentive and reward-based learning (Haber and Knutson 2010), behavioral and cognitive control (Montague et al. 2004), attention, integration of related affective information such as pleasurable and aversive features of environmental stimuli to modulate cognition and guide behavior, and integration of brain activity as a function of general overt activity levels and the sleep–wake cycle. Cognitive and behavioral processes subject to modulation by NE overlap with those of DA to a larger degree than has been traditionally appreciated. Reward-based learning may be the only one of these processes that has been largely unaddressed with respect to the central NE system. NE additionally has long been recognized for its role in arousal, elementary aspects of perception and autonomic functions. In general, these systems, arising from the ventral tegmental area (VTA) and the substantia nigra (SN) of the ventral midbrain for DA, and the pontine locus coeruleus (LC) for NE, serve as critical links between the environment and the organism, for the valuation and use of information to guide information storage and goal-directed action (Aston-Jones and Cohen 2005; Bouret and Sara 2005; Montague et al. 2004; Sara 2009) [see Figure from Sara (2009) below for projection patterns arising from VTA and LC]. In keeping with this general role, the circuitry that these areas participate in is extensive, involving a large share of the major functional areas in

cortical and subcortical regions. For the VTA, this primarily includes limbic regions (e.g. hippocampus, hypothalamus, amygdala, ventral striatum, basal forebrain cholinergic nuclei), association neocortices, and other monoamine systems. For the SN, this includes dorsal striatum, thalamus, and more distant targets in the motor cortices, other cortical areas and cerebellum. For the LC-NE system, virtually the entire brain serves as a target, with the dorsal striatum as the only region in the mammalian brain that contains a paucity of NE or adrenergic receptors. These networks are organized as discrete functional loops which give rise to much of what the brain can achieve in cognition and behavior. As might be expected, these networks are also disrupted in a wide range of neuropsychiatric illnesses, including Parkinson's disease, schizophrenia, obesity and drug addiction, psychopathy, dementias, mood and anxiety disorders, attention-deficit disorder and many others. Accordingly, these catecholamine systems are important targets for the action of existing and candidate drugs for the treatment of these disorders, currently including l-DOPA, DA and NE transporter inhibitors, adrenergic antagonists, and DA receptor agonists and antagonists.

It is therefore of great interest to interrogate the functional dynamics of catecholamine systems during cognitive and behavioral processes, as model systems in which to evaluate the potential of new and existing drugs to modulate these processes and remediate the clinical syndromes arising from disturbances in these processes.

Recent work from several research groups has shown that these subcortical areas can be reliably evaluated during experimental cognitive tasks in humans, and that the functional role of these areas in humans is consistent with those determined in animal models (Duzel et al. 2009). While the LC-NE system has been relatively less well studied in humans, there is evidence that drug effects on this system can be tested in a manner that is informed by physiological and cognitive model-based predictions (Minzenberg et al. 2008). In addition, concurrent pupillometry can be acquired in the MRI environment, both to aid in topographic localization of the LC in BOLD images, as well as to provide a novel concurrent behavioral measure of LC-NE system functioning that can be related to the fMRI measure of LC activity during cognitive processes (Sterpenich et al. 2006). Among the midbrain DA regions, there are a number of distinct and important species differences between rodents and humans or non-human primates (Duzel et al. 2009). These species differences highlight the need for a valid and sensitive method to study these systems directly in humans. In addition, in humans, the functional and anatomic distinctions between the VTA and SN in particular are also much less well drawn than is commonly appreciated. For example, the midbrain region most representative of the rat VTA resides in the dorsal part of the primate SN; these two nuclei are more continuous with each other in humans than in the rat, leading some anatomists to distinguish dorsal/ventral tiers of the midbrain DA complex rather than VTA versus SN (and the retrorubral field); the projection patterns of VTA versus SN are not easily distinguished in humans; and the response properties of VTA and SN neurons show no significant differences (reviewed in Duzel et al. 2009). Due to these observations, plus the limits on

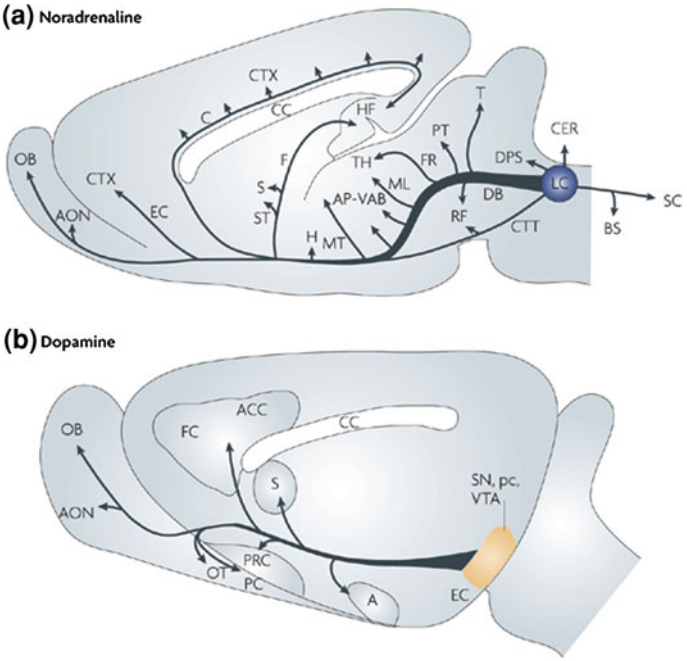

Fig. 2 Comparative anatomy: **a** Noradrenaline. **b** Dopamine

spatial discrimination between adjacent, small subcortical structures, many investigators who study these regions in humans with functional neuroimaging refer generally to the "dopaminergic midbrain." In these studies, the VTA/SN complex is typically localized in BOLD images by reference to structural scans acquired as either magnetization transfer scans, proton density scans or T1-weighted scans sensitive to neuromelanin, which in the brain stem is restricted to catecholamine neurons.

It is important to consider the various possible underlying physiological sources of BOLD signal change in the neurons found in this area [see (Duzel et al. 2009) Fig. 2]. These could include LFPs arising from either glutamatergic or GABAergic inputs to tonically active DA neurons, glutamatergic inputs to silent DA neurons, or inhibition of GABAergic input to DA neurons. BOLD signal change could also arise from burst firing of DA neurons, or local DA release from DA neurons in either burst or tonic firing modes. While the relationship of LFPs in these DA-rich areas to discharge of these neurons remains to be characterized in animals, the most parsimonious account, based on single-unit studies in monkeys, suggests that DA neuron discharge is primarily a function of direct excitatory glutamatergic input from the neocortex or elsewhere in the midbrain. In addition, fMRI studies in animals have supported a relationship between BOLD signal change and DA neurotransmission. These studies find a strong association of DA release (induced

with stimulants) and BOLD signal change in limbic terminal fields of midbrain DA projections e.g. nucleus accumbens; (Chen et al. 1997), and that this relationship persists with DA lesions and fetal DA neuron grafts (Chen et al. 1999). Stimulant-induced changes in blood volume (which itself is highly-related to BOLD signal change) show similar associations (reviewed in (Knutson and Gibbs 2007), and these stimulant effects show predictable interactions with co-administered D2 antagonists (Chen et al. 2005; Schwarz et al. 2004). An elegant study of healthy human subjects, using fMRI during a reward-based learning paradigm and ligand-PET, found BOLD signal change in the VTA/SN to correlate with [^{11}C] raclopride displacement as a measure of increased DA release in the nucleus accumbens (Schott et al. 2008). These various studies indicate that BOLD signal change closely follows DA neurotransmission, at least in some brain areas that receive strong input from the VTA/SN. Nonetheless, a number of physiological features of these systems may have important implications for interpreting studies of cognition and pharmacology in humans, including the role of local inhibition in the VTA/SN, the role of silent DA neurons, and the temporal relationship of BOLD signal change to discharge of these neurons. Studies combining functional neuroimaging with genetics or pharmacology in humans, or invasive measures in animals, will further elucidate the underlying basis of BOLD signal change in these brain areas to optimize the utility of neuroimaging in studies of human pharmacology.

7 Summary

The emergence of noninvasive functional neuroimaging methods such as fMRI for neuropharmacology is intimately tied to the status of drug development for CNS disorders, and the importance of currently unmet treatment needs among patients with these disorders. These imaging methods are seen to have a number of unique advantages to meet these goals, as well as serving as a tool for basic neuroscience. The physiological basis for signal change in fMRI is complex, and the readout in BOLD signal change is a function of a number of signaling processes in the brain, each of which are of interest for pharmacology. Widely distributed neurotransmitter systems such as the monoamines are readily amenable to study using fMRI, and the dynamics of these systems' operations over space and time can be interrogated in a uniquely advantageous manner. fMRI and related methods therefore stand poised to occupy a unique position in the armamentarium of experimental methods available to the basic and clinical scientist, to achieve progress in our understanding of the pharmacology of the brain and how we may attain greater success in relieving the massive burden of neuropsychiatric illness.

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