# Functional Magnetic Resonance Imaging in Drug Development

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## Introduction

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

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## Measuring the BOLD Response with fMRI

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

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

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

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

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

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

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

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

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

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

## Previous and Common Uses of fMRI

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

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

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

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

## Pharmacological fMRI: Applications in Drug Development

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## Standardization and Reproducibility

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

### *Nonphysiological*

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

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

### *Physiologic or Cognitive*

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

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

## Conclusion

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

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