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Pharmacologic Magnetic Resonance Imaging (phMRI): Imaging Drug Action in the Brain

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Abstract

The technique of functional magnetic resonance (fMRI), using various cognitive, motor and sensory stimuli has led to a revolution in the ability to map brain function. Drugs can also be used as stimuli to elicit an hemodynamic change. Stimulation with a pharmaceutical has a number of very different consequences compared to user controllable stimuli, most importantly in the time course of stimulus and response that is not, in general, controllable by the experimenter. Therefore, this type of experiment has been termed pharmacologic MRI (phMRI). The use of a drug stimulus leads to a number of interesting possibilities compared to conventional fMRI. Using receptor specific ligands one can characterize brain circuitry specific to neurotransmitter systems. The possibility exists to measure parameters reflecting neurotransmitter release and binding associated with the pharmacokinetics and/or the pharmacodynamics of drugs. There is also the ability to measure up- and down-regulation of receptors in specific disease states. PhMRI can be characterized as a molecular imaging technique using the natural hemodynamic transduction related to neuro-receptor stimulus. This provides a coupling mechanism with very high sensitivity that can rival positron emission tomography (PET) in some circumstances. The large numbers of molecules available, that do not require a radio-label, means that phMRI becomes a very useful tool for performing drug discovery. Data and arguments will be presented to show that phMRI can provide information on neuro-receptor signaling and function that complements the static picture generated by PET studies of receptor numbers and occupancies.

Keywords

Drugs; fMRI; hemodynamics; neurotransmitters; phMRI; receptor

Introduction

The technique of functional magnetic resonance imaging (fMRI) using either relative cerebral blood volume (rCBV) (Belliveau et al., 1991), blood oxygenation level dependent (BOLD) (Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1992) or T1-based

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cerebral blood flow (CBF) techniques (Kwong et al., 1992) has led to a revolution in brain mapping. This is largely due to the fact that the advent of a non-invasive tool with reasonable contrast to noise, no requirement of radiotracers and relatively high spatial and temporal resolution, allows for studies to be conducted more easily than the prior positron emission tomography (PET) studies of brain activation using CBF, oxygen and glucose metabolism (Kuhl et al., 1975; Kuhl et al., 1977; Phelps et al., 1977; Raichle et al., 1976). Both fMRI and PET studies of brain activation are based upon the coupling between neuronal activity, metabolism and hemodynamics. The possibility that fMRI may help understand the organization and flow of information in the brain has led to an explosion in the number of centers dedicated to performing fMRI.

Most fMRI studies have used task activation such as photic stimulation or finger movements or a cognitive challenge to elicit neuronal activity. However it is also possible to elicit neuronal activity using various pharmacological agents as a stimulus or as a means of modifying the response to some other stimulus (such as a cognitive task). The latter two applications are what can be termed pharmacologic MRI (phMRI) and, similar to conventional fMRI, have their antecedents in prior PET or autoradiographic studies of metabolic changes elicited by drugs.

In the case of a drug challenge, maps are generated of the metabolic/neurotransmitter signaling consequences of receptor stimulation of relevance to a large number of cerebral disorders. Autoradiographic and PET studies have previously examined metabolic changes (both blood flow and glucose utilization) after drug stimulation using, for example, the dopamine releaser amphetamine (Carlsson et al., 1975; Dewey et al., 1993; Dolan et al., 1992; Friston et al., 1992). While PET is the gold standard tool for measurement of regional changes in glucose utilization in vivo, the same cannot be said for PET studies of metabolic activation using CBF. A number of studies have appeared imaging the change in CBF after drug stimulation using PET. These studies suffer from the fact that the CBF is usually only measured once after administration of the drug whereas with MRI one can obtain the entire hemodynamic time course with temporal resolution on the order of 1s. The technique of fMRI is thus well suited to study these hemodynamic changes follwing a drug challenge (Chen et al., 1997). Some early reports using MRI to study the acute effects of amphetamine (Chen et al., 1997; Silva, 1995; Zhang et al., 2001), cocaine or cocaine analogs (Chen et al., 1999; Chen et al., 1997; Marota et al., 2000), apomorphine or L-dopa (Chen et al., 1996; Nguyen et al., 2000; Zhang et al., 2000), nicotine (Stein et al., 1998), heroin (Xu et al., 2000) and serotonin ligands (Houston et al., 2001; Scanley et al., 2001) appeared more than ten years ago. More recently, the number of receptor targets has increased to a number of other neurotransmitter systems or drugs including neuropeptides (Gozzi et al., 2005); cholinergics (Choi et al., 2006b; Goekoop et al., 2006; Gozzi et al., 2006; Hoff et al., 2010); serotonergics (5-HT) (Anand et al., 2005; Anderson et al., 2008; Canese et al., 2011; Klomp et al., 2012; Martin and Sibson, 2008; Rauch et al., 2008; Sekar et al., 2011); glutamatergics (Gozzi et al., 2008a; Gozzi et al., 2008b; Jones et al., 2008; Jones et al., 2005; Littlewood et al., 2006a; Littlewood et al., 2006b); cannabinoids (Bossong et al., 2011; Chin et al., 2008; Dodd et al., 2009; Rabinak et al., 2011; van Hell et al., 2011; Winton-Brown et al., 2011) and opioids (Liu et al., 2007; Sell et al., 1997; Upadhyay et al., 2011; Xi et al., 2002, 2004; Xu et al., 2000). Nonetheless, there are a number of issues that render interpretation of the

signal changes induced more difficult than in conventional task-related fMRI. Because drugs are used as the stimuli of interest, and because drugs can have effects very different from functional activation tasks, we coined the term pharmacologic MRI (*phMRI*) to describe these experiments (Chen et al., 1997).

Unlike in conventional task-related fMRI studies where the time courses of the stimuli can be controlled at will, in phMRI the time course is determined by the pharmacokinetic and pharmacodynamic profile of the drug administered to induce the signal changes. Since most drugs can be anticipated to have long time courses compared to task-related stimuli (minutes or more compared to seconds for conventional fMRI), data collection and analysis schemes become important for accurate determination of metabolically induced signal changes after pharmacologic stimulus. The inability to control, or even know ahead of time, the timing and amplitude of the stimulus renders this considerably more challenging that conventional resting state or task-related fMRI studies.

There are generally two “flavors” of what might be considered pharmacologic MRI. The first is what has been discussed in most of the papers referenced above. This flavor is most often run in the form of a ***drug challenge*** study in which MR signal changes are monitored after the acute administration of the drug of interest. Clearly, there are many permutations on this basic model, such as antagonism of the effects of one drug with another, or examining perhaps the acute effects of one drug upon the chronic effects of another (useful perhaps for studying cocaine addiction). The second flavor of what might be considered phMRI is the observation of the ***pharmaco-modulatory*** effects of a pharmaceutical upon a conventional task-related fMRI study, such as the effects of dopaminergic drugs upon cognitive tasks (Dodds et al., 2009; Ersche et al., 2010; Kimberg et al., 2001). The latter type of study much closer to what is performed in conventional fMRI and can be analyzed using the same types of approaches as in conventional fMRI. The effects of such a drug administration, however, must be kept in mind when interpreting the hemodynamic changes – as the drug itself may have modulatory effects upon basal and/or stimulated hemodynamics. Common neurotransmitter systems forming targets for phMRI studies, as well as their effects upon hemodynamics, is shown in Table 1.

This brief survey is not intended to dwell too much on the past, and in that sense is not a review of the field. We wrote a fairly thorough survey of the issues and techniques involved where much more detail can be found (Jenkins et al., 2002; Jenkins et al., 2006). Rather, after some looking back, it will focus on some key questions about the nature and interpretation of phMRI that will hopefully spur some in depth considerations of the potentialities of the technique for examining drug effects on the brain - particularly those targeting neurotransmitter receptors - and suggest some future studies. There is some critical opinion mixed in with objective review, however this is primarily intended to evoke some discussion. Much of the discussion, due to our own interests, is heavily focused upon the dopaminergic system, and many of the examples discussed come from our own studies of the dopamine system. However, when we use these studies as exemplars, it is clear that similar types of approaches and thinking would generalize across many different receptor systems, and indeed much work has proceeded into other neurotransmitter systems since the first studies using dopaminergic drugs. Pharmacologic MRI has the true potential to be

a ”molecular imaging” technique, with many properties complementary to, for instance, PET that can be used to probe receptor circuitry and function in the brain. That, in a nutshell, is the take home message of this manuscript.

What’s in a name?

In science it is necessary to have precise language, and to have unambiguous concepts with mutually agreed upon meaning. This is most important when describing physical quantities such as entropy or magnetic field strength or molarity where common units are agreed upon by all. Precision in language is also required for fields like taxonomy where names of species and their cladistics must be agreed upon by all in the field otherwise things quickly devolve into a chaotic soup of pet names. In the case of fields such as paleontology, the terminology is an intrinsic part of the system of description that needs to be used consistently by practitioners in the field. Other terms become part of the common vernacular even though they may not have a very precise definition but, because of widespread usage, they are rarely misunderstood. Into the latter category falls the term fMRI. What is commonly understood as fMRI can be a measurement of CBV or CBF or, most commonly, BOLD signal changes. The term really signifies measurement of an hemodynamic response function in the brain to some sort of stimulus. The reality is, though, that the term is used simply as a shorthand – as are many terms and acronyms that have developed in the MRI community. When we first coined phMRI it was meant somewhat tongue in cheek such that it could still be pronounced *ef* MRI. As explained above, we used it because it seemed a convenient terminology to refer to it while still keeping it in the family of fMRI techniques. Indeed there is considerable promiscuity between what might be termed an phMRI study with what is referred to as fMRI, and many practitioners still just use the latter term. Unfortunately, some people take such shorthand terminology much more seriously than do others. When Iris Chen, then a student in our laboratory, gave a young investigator finalist award talk at the International Society of Magnetic Resonance Meeting (Vancouver, BC; Canada) on this topic in 1997, the first question asked of her, by one rather distinguished and well known member of the audience, was not about the science but about the term phMRI. To paraphrase he said “If I use a hammer when I image do I now call it hammer-MRI?” Aside from the obvious safety issues of bringing a hammer into an MRI scanner, I believe he over-stated the import of the term. As long as people understand what you mean I would argue that it really isn’t that important. If a term gains currency, usually due to its utility, it will develop a life of its own, otherwise it will be ignored. Like all forms of slang – the market decides.

Somewhat later, a review article was written by Leslie and James in Trends in Pharmalogical Science with the title using the appellation phMRI (Pharmacological magnetic resonance imaging: a new application for functional MRI) (Leslie and James, 2000). As happened then, and has happened subsequently, the term incited some vociferous opposition including a letter criticising the term, as well as other concepts laid out in the article with the title “Pharmacological MRI: a nebulous concept?” That critique missed the boat on a number of items, including the intrinsic sensitivity of phMRI to which we shall return later, however they did raise some valid points about potential confusions with other studies of pharmacological effects using various MRI techniques. A number of times our laboratory

has submitted manuscripts only to get some rather heated reviews where the primary objection seemed to be the term phMRI rather than the science. To be fair, there are some arguments to be made about using neologisms that may not convey the full extent of phenomena that may fall into the category of pharmacological MRI such as studies of contrast agent clearance in the kidney, or use of dynamic contrast enhanced (dce) MRI to examine brain tumor shrinkage. These are legitimate concerns, but would also hold for other commonly used terms such as fMRI, for example, couldn't one examine kidney function using dce-MRI and call it fMRI? In short, it often comes down to a matter of opinion. It *is* important, for instance, whether a new hominid fossil is called *Homo florensis* versus *Homo sapiens* since the name connotes whether it is a different species. However, when it comes to fMRI or dce-MRI or phMRI I say lighten up – are you really confused or just cranky? Of course there should be some constraint on proliferation of short hand terms, but it is best to let the market decide which survive.

Pharmacologic MRI: How well do hemodynamic changes reflect receptor distributions and/or receptor function and signalling?

Both PET and autoradiography have the potential to measure specific binding of a drug to its target. Although the quantitative ability to measure specific receptor parameters is quite complicated, and involves multiparametric models with extensive fitting and estimates of non-specific binding; binding parameters such as Bmax/Kd (Bmax = number of receptors; kd dissociation constant – determination of each indepdendently requires at least two separate studies) or receptor occupancy can be extracted from such measurements. In addition, radiolabel studies can also determine metabolic coupling, most sensitively using measurements of either glucose utilization (CMRglc) or cerebral blood flow (CBF). PET does have limitations in terms of temporal sampling for both CMRglc and CBF. The possibility of using indirect coupling, as in phMRI, for measuring specific receptor parameters based upon simple hemodynamic changes seems rather remote on the face of it. At best, one might hope to verify the assumption that the hemodynamic changes observed after administration of a particular drug correlate with the activation of the receptor systems targeted by the drug. In this manner the pharmacologically induced "metabolic" coupling is analogous to the metabolic coupling usually assumed in standard fMRI studies. There is one important difference, however, in that many drugs of interest specifically target a given receptor or neurotransmitter system and therefore can be expected to produce a map that reflects the regional distribution of the receptors or neurotransmitters. In conventional task-related fMRI much of the discussion of the neurovascular coupling problem tacitly uses the context of glutamatergic neuronal stimulation to relate to the signal changes. The use of drugs that target alternative systems such as the dopaminergic, cholinergic or serotonergic opens up the possibility of learning much about neurovascular coupling.

Autoradiographic techniques have the obvious "terminal" limitations as an invasive method. However, autoradiography can measure not only many of the same parameters measurable by PET, but can also examine mRNA expression levels, receptor protein levels as well as the direct ligand binding to the receptors. Histologic/autoradiographic studies have exquisite spatial resolution, however the quantitative measurements of CBF using iodoantipyrene, and

CMRglc have the problem of trying to catch a metabolic snapshot by allowing enough time for accumulation of labeled compound (such as glucose, where it can be 45 minutes) in a system where the metabolic parameters may be changing dynamically. This factor may account for the differences that may be observed between phMRI and autoradiographic and PET measurements of CMRglc when using pharmacologic challenges.

One question that needs to be addressed is how well do the hemodynamic changes observed after administration of a particular drug correlate with the activation of the receptor systems targeted by the drug? Unfortunately, for the purposes of validation, there is often not a direct relationship between the regional mRNA expression levels, ligand binding, or protein levels (Palacios et al., 1991; Pompeiano et al., 1992; Schalling et al., 1990). This means that selection of a “gold standard” for comparison of an phMRI map can be problematic. Should we compare phMRI maps to receptor mRNA expression levels; drug binding patterns; the immunohistochemical distribution of the receptor proteins; or to the pattern of CMRglc? There are a number of reasons that one or the other of these markers can be expected to correlate with the phMRI data. Since phMRI clearly falls into the category of *functional* MRI techniques one has to ask the question of whether the static picture (i.e. receptor number, or ligand binding or protein density distribution) is the appropriate comparison or does the phMRI better reflect signaling processes that can be determined using such techniques as mRNA expression levels, or G-coupled protein activities?

As a simple example of such issues we examined the phMRI response to various dopamine receptor ligands targeting a specific dopamine receptor sub-type, the D3 receptor. This receptor has a circumscribed distribution in the brain primarily targeting the limbic circuitry. We found that dopamine D3 receptor agonists produce negative changes in CBV whereas the antagonists produce positive changes in CBV. Further, although the pattern of regional CBV changes matched well with the distribution of D3 receptor ligand binding – it matched better with the pattern of mRNA expression of the D3 receptors (Choi et al., 2010). In another experiment we found that the CBV after different doses of amphetamine correlated quite well with cyclicAMP levels which reflect the dopamine receptor signaling (Ren et al., 2009a). Thus, there is no *a priori* reason to expect that the pattern of static receptor binding or protein levels will best reflect the phMRI data. There are many experiments necessary to determine the relationship between receptor distributions and function. Most manuscripts dealing with phMRI of drugs (including many of our own) simply do not perform enough ancillary experiments to unequivocally assign receptor circuitry to the observed signal changes. The coupling between pharmacologic stimulation, neural activity, and a hemodynamic change is a very complicated issue. Therefore, the first time one performs an phMRI experiment with a given drug or neurotransmitter system, one should take great pains to determine that the hemodynamic changes observed are due to the neurotransmitter or receptor system in question. This should not be assumed as a foregone conclusion without proving it, otherwise, to paraphrase Lord Kelvin’s quote about measurement and numbers, one’s ability to interpret the signal changes is of a meager and unsatisfactory kind. This is especially true in systems where the neurotransmitter may also be vasoactive. As discussed below, many neurotransmitters are indeed vasoactive. It should be stated that this stimulation may activate not only specific neuronal subtypes targeted by the pharmacologic ligand, but may include as well the downstream circuitry that will likely include other neurotransmitter

systems. With this in mind, we outline below some of the criteria that should be fulfilled. Many of these experiments by necessity have to be performed in animals, possibly in support of human studies.

Criteria for Demonstration that Hemodynamic Changes induced by a given ligand are specific to neurotransmitter in question.

1. phMRI signal changes should demonstrate adequate decoupling to changes in systemic physiologic parameters (e.g. pCO_2, blood pressure).
2. The regional pattern of phMRI signal changes should be correlated with the known distribution of receptors of the neurotransmitter in question and/or associated circuitry measured using other techniques such as autoradiography or PET.
3. Selective lesioning of receptor system in question (or depletion of the neurotransmitter levels) should modulate phMRI signal changes in predictable ways (e.g. in Parkinson's disease the known loss of dopamine transporters should lead to diminished response to dopamine transporter ligands).
4. Administration of agonists and antagonists of the receptor system should modulate the signal in a manner consistent with the proposed mechanisms of action of the agonists and antagonists – including post-synaptic signaling.
5. phMRI signal changes should correlate with behavioral and or neurotransmitter dynamics (the latter measured using, for instance, microdialysis, or perhaps PET) or with markers of post-synaptic signaling (e.g. cyclic-AMP).

In order to make these criteria more concrete we will review the steps we took in animal studies of the dopamine system to establish that the hemodynamic changes observed after stimulation with dopaminergic drugs were truly due to stimulation of dopamine receptors. Luckily, in the case of the dopamine system, there was a large body of prior literature with which to compare to cross validate the phMRI studies.

In these studies we injected the drugs in an acute challenge design, and also examined the effects of agonists and antagonists. In our first paper in 1997 (Chen et al., 1997), we showed that challenges with the dopamine releaser amphetamine or the dopamine transporter blocker β-CFT induced BOLD signal increases in the striatum and cortex. The time course of the BOLD changes correlated temporally with the time course of extracellular dopamine release measured using microdialysis and did not correlate with changes in pCO_2 or blood pressure. We further lesioned the animals unilaterally with 6-hydroxydopamine a chemical that leads to destruction of dopamine fibers in the striatum, but leaves serotoninergic, cholinergic norepinephrine and noradrenaline neuron intact (Perese et al., 1989). If one compares the BOLD or CBV changes induced by amphetamine or the dopamine transporter blocker CFT one can see that the signal changes are largely restricted to the intact side, and further, the intact side looks similar to a control (Chen et al., 1999; Chen et al., 1997; Nguyen et al., 2000). Interestingly, the cortical response is also lost showing that this response must also depend upon dopaminergic function. The behavioral data in the animals showed that their unilateral rotation after amphetamine stimulation had a time course that

correlated with both the BOLD and dopamine release (Chen et al., 1997). About the same time the group in Kentucky showed that the response to levo-DOPA, a drug that can increase dopamine levels in animals or people with loss of dopamine neurons, could be detected using BOLD in a primate model of Parkinson's disease (Chen et al., 1996). These results suggest that dopamine itself may well drive the hemodynamic response.

One more experiment provides additional evidence that the phMRI signal changes are due to stimulation of dopamine neurons. Transplantation of fetal dopamine cells into the unilaterally lesioned striatum of a rat not only restores the behavioral profile of these animals (stopping them from rotating after injection with amphetamine) but it also restores binding of CFT as measured by PET scans as well as the phMRI response at the very same location where the graft is. This is readily verified using post mortem histology on the same animal. This may prove useful for study of fetal and stem cell grafting in Parkinson's disease (Bjorklund et al., 2002; Chen et al., 1999).

The neurovascular coupling problem - opening the black box

The neurovascular coupling problem lies at the heart of the interpretation of both fMRI and phMRI signals. While one can derive correlations between local field potentials and spike activity with BOLD signal intensity during task or drug stimulations, until the discovery (if ever) of any voltage-gated vascular receptors such correlations will always be problematic to interpret. One unifying feature that has been proposed is that it is increases in intracellular calcium in neurons and astrocytes that leads to the release of vasoactive substances that subsequently couple to CBF changes (Jakovcevic and Harder, 2007; Lauritzen, 2005). Such a coupling mechanism could potentially lead to very different outcomes for different neurotransmitter systems. For instance, stimulation of glutamatergic neurons leads to reuptake of glutamate through the astrocytes and this uptake is coupled to calcium flux. Uptake of glutamate through astrocytes has been shown, in vitro, to be coupled to release of a number of vasoactive molecules such as arachidonic acid or vasoactive intestinal peptide (Sorg and Magistretti, 1991; Stella et al., 1994), thus providing possible coupling mechanisms via numerous different astrocytic pathways (Attwell et al., 2010). For dopamine neurons things are different in that the uptake of dopamine occurs pre-synaptically through the dopamine neurons and is not calcium dependent, although release of dopamine is. Therefore, for instance, dopaminergic stimuli will have contributions from both pre- and post-synaptic mechanisms. There is no reason to believe that all neurotransmitter systems will couple to a change in CBF in the same manner or with the same neurovascular coupling agents (vasoactive molecules). While good evidence has accrued that fMRI stimuli of, for instance, the glutamatergic sensory system are blocked by inhibitors of nitric oxide (NO) synthesis (Burke and Buhrle, 2006; Gsell et al., 2006), we found that blocking NO slightly potentiated dopaminergically related hemodynamic changes (Choi et al., 2006a). In addition to well known coupling agents such as NO, many neurotransmitters are directly vasoactive such as dopamine, serotonin and acetylcholine. The neurons associated with these receptors can directly synapse upon both microvessels and capillaries. Thus, stimulation of such a neuronal population, leading to neurotransmitter release, can have a direct vasoactive effect in addition to any other hemodynamic effects seen via stimulation of post-synaptic, in some cases, pre-synaptic neurons.

Several different brain neurotransmitters have axonal projections to the microcirculature in many brain regions. Many of the amino acid neurotransmitters, in particular glutamate, appear to couple to changes in blood flow via nitric oxide (NO) (Fillenz et al., 1999; Iadecola, 1993). As discussed above, this coupling is thought to arise at the level of the astrocytes. Serotonin took its name from the fact that it was a potent vasoconstrictor – this was discovered before its role as a neurotransmitter was known (see review in (Cohen et al., 1996)). Acetylcholine also has vasoactive properties (Sato and Sato, 1995). A coupling of cholinergic M5 receptors on the vasculature apposed to NO neurons (which are vasodilatory) in the basal forebrain has been worked out to provide control of cerebral microcirculation (Elhusseiny and Hamel, 2000; Vaucher et al., 1997). Strong histological evidence accrued over the past twenty years indicates that dopamine neurons are intimately associated with microvasculature in brain parenchyma (Head et al., 1980; Jones, 1982). It has even been suggested on the basis of such data that much of the hemodynamic change observed in neuronal activation may be dopaminergic in origin (Krimer et al., 1998). This latter study by Krimer et al. showed direct immunocytochemical evidence for termination of central dopaminergic neurons on penetrating arterioles and the pericytes of capillaries. The pericytes are the contractile motors regulating capillary flow. The highest density of dopaminergic innervation of these microvessels was in areas of cortex known to be high in dopaminergic innervation in the parenchyma such as frontal cortex. They found that iontophoretic application of DA to isolated microvessesl from ferret cortex led to constriction. Thus, control of microvascular flow via dopamine release is certainly one important factor in regulating changes induced by dopaminergic drugs. One component missing from the latter study was the identification of which dopamine receptor sub-types were present on the microvessels. Our group, in collaboration with Edith Hamel identified different dopamine receptor sub-types on both arterioles and capillaries as well as astrocytes. Interestingly, the arterioles and capillaries had only D1 and D5 receptors, both of which lead to vasodilatation, whereas the capillaries had D3 receptors stimulation of which leads to vasoconstriction (Choi et al., 2006a). It is well known that dopamine is a vasoactive substance in the peripheral vascular system and is an important regulator of systemic blood pressure (Amenta et al., 2000; Tayebati et al., 2011). Adenosine is another neurotransmitter whose vasoactive properties are well known. Adenosine antagonists such as caffeine and theophylline have the interesting property of increasing energy metabolism and at the same time decreasing CBF (Nehlig et al., 1992). The list of vasoactive molecules in the brain also includes many of the important neuropeptides (Attwell et al., 2010; Gulbenkian et al., 2001).

Dissecting out the various components involved in the neurovascular coupling is obviously a difficult problem and it is clear that obtaining a complete mechanistic interpretation of such effects for fMRI is far from being a solved problem. The metabolic effects of stimulation of a given set of neurons leads both to local changes as well as changes in the attendant circuitry. This question also needs to be reframed in light of the specific distribution of vascular receptors. In general, the distribution of vascular receptors is much less well known than the distribution of parenchymal receptors. Also the spatial correlation between the vascular and parenchymal receptors is not well known. What can be stated with some certainty is that if, in general, the pattern of activation induced by a given drug is *not*

consistent with any of the known receptor distributions of the drug administered, then the interpretation of the phMRI data is much less interesting than it would otherwise be.

Drug challenges allow one the opportunity to start to open up the black box that represents the coupling between neural activity and release of vasoactive substances (see Fig. 1). For instance, we showed in rats that there was a tight temporal relation between dopamine release and either BOLD or CBV coupling after stimulation with the dopamine releaser amphetamine (Chen et al., 2005a; Chen et al., 1997; Chen et al., 1999; Chen et al., 2005b; Choi et al., 2006a). Further, at high dopamine concentrations the coupling was linear. At low dopamine concentrations there was a negative coupling (Ren et al., 2009b). Using pharmacologic challenges affords the opportunity to determine the role a given neurotransmitter system plays in regulation of hemodynamics. This has the potential to allow for a window onto receptor modulation in a manner that may be complementary to that of PET.

Changes in blood pressure can also have an effect upon hemodynamics, however numerous studies of autoregulation suggest that maintenance of CBF in the face of decreased arterial pressure occurs over a fairly wide range of pressures. Measurements of simultaneous CBV and CBF using blood withdrawal and MRI suggested that between 65 and 140 mm Hg pressure CBF and CBV are constant (Zaharchuk et al., 1999). Another study found that use of a drug that increases blood pressure, but doesn't cross the BBB (norepinephrine) did not alter brain CBV between 60–120 mmHg (Gozzi et al., 2007). Many drugs of interest can cause changes in mean arterial blood pressure. Although one can deconvolve the systemic physiologic changes from the drug changes using modeling, complete discrimination may not be reliable unless there are differences in the temporal or spatial profiles of the drug and physiologic parameters.

The confounds of anesthesia are even more challenging than those of changes in systemic physiologic variables. This is because the latter effects can be readily measured and potentially corrected for, whereas those of anesthesia can often selectively affect different neurotransmitter systems. Many studies have investigated the effects of differing anesthetics on the coupling of cerebral metabolism and anesthetic dose (Stullken et al., 1977) and such studies will not be reviewed here except to say that for somatosensory stimuli there are often very large differences in the CBF increases observed for the same stimulus with different anesthetics (Lindauer et al., 1993). These results have not generally examined the neuro-vascular coupling problem with respect to the differing neurotransmitter systems selectively affected by the various anesthetics. It can safely be said that for animal imaging the choice of anesthetic (or whether to anesthetize the animal at all) is one of the most important choices the investigator has to make before embarking upon a study.

The Yin and Yang of receptor stimulation: Positive and negative hemodynamic changes – more common than not

A few years ago there was great excitement in the fMRI community about negative BOLD effects observed in the visual cortex (Shmuel et al., 2002) that might be attributable to neuronal inhibition (Shmuel et al., 2006). It is true that for most cognitive and sensory

stimuli positive BOLD changes were observed notwithstanding notable exceptions such as the ephemeral “pre-dip” (Menon et al., 1995) or decreases in the precuneus (Cavanna and Trimble, 2006) and default network (Buckner and Vincent, 2007) that may be attributable to GABAergic inhibtion (Northoff et al., 2007). However, it appears it will be difficult to attribute positive hemodynamic changes to excitatory activity and negative changes to inhibitory neuronal activity (Lauritzen et al., 2012). When using a drug as a stimulus, however, it is more common than not to observe both positive and negative hemodynamic changes simultaneously in various brain regions using drugs targeting many different neurotransmitter systems such as the opioid (Liu et al., 2007) or dopaminergic (Dixon et al., 2005; Jenkins et al., 2004). For example, for the neurotransmitter dopamine there are two families of receptors (D1 and D2) that couple to adenylate cyclase activities in opposite manners. We have found as a general rule that D1 receptor agonism induces positive hemodynamic changes whereas D2 family receptors induce negative hemodynamic changes, and this is also supported by others (Chen et al., 2005a; Chen et al., 2010; Choi et al., 2006a; Choi et al., 2010; Dixon et al., 2005; Shih et al., 2009). Thus, in brain regions where both sub-types are represented one expects to see a balance between vasodilatation and vasoconstriction; in other regions an excess of one sub-type over the other may lead to either an increased or decreased hemodynamic response. As an example we show data from a cocaine challenge in a rodent and an amphetamine challenge in a primate. In both cases the predominant changes are positive in dopaminergic regions, but there are also considerable negative changes. In the case of cocaine, we observed an “initial dip” followed later in time by positive CBV (Chen et al., 2011), a finding also observed by Luo et al. (Luo et al., 2009). This is readily interpreted due to the fact that D2 and D3 receptors have higher affinity for dopamine than do post-synaptic D1 receptors. If one makes a map of the two signals (initial negative – later positive) the negative changes resemble maps of D2 receptor agonism as would be expected based upon the differing affinities of the receptors (Chen et al., 2011) (Fig. 2). As another example we show data taken from an amphetamine stimulus in a monkey and from Joe Mandeville’s group using a remifentanil (a mu-opioid agonist) challenge that produced both negative and positive changes in CBV (Liu et al., 2007). The negative change was due to opioid stimulation similar to morphine and the positive change was attributable to inhibition of gabaergic neurons. These differing signs were brain region dependent as shown in Fig. 3.

Monitoring hemodynamic changes after administration of a drug will lead to a number of general outcomes. First, one may obtain no response or correlation whatsoever with other measured parameters. In such an instance the only conclusion to be drawn is that a drug targeted towards a specific receptor has no hemodynamic effect. This does not mean that it has no behavioral effect or even that it has no metabolic effect - for instance - the vasodilatory and vasoconstrictive properties of the drug may cancel one another. The most copacetic possibility is that a very good correlation exists between the hemodynamic changes induced by the drug and a given receptor distribution. This situation may occur when the administered drug causes release of a vasoactive molecule reflective of the targeted receptor system or when the pattern of vascular targets is reflective of the receptor targets in the brain tissue. However, even in such a case the possibility that a drug will have a functional (in the mathematical sense) mapping with a given receptor is a rather remote

possibility. This is because very few drugs can be presumed to act upon only one receptor. Even in cases where this may be so, the receptor may couple to hemodynamics in more than one manner (i.e. vascular and post-synaptic receptors) and the circuitry may be subject to both inhibitory and excitatory influences that may be reciprocal. Thus, extensive work is required, using multiple antagonists and agonists as a function of dose to parse out a consistent interpretation of the data from any single drug. Failure to do so can lead to erroneous reasoning when trying to interpret the signal changes. For instance, I have reviewed multiple manuscripts where investigators administer drug X that causes a positive hemodynamic change. Then, they administer drug Y (that may lead to a negative hemodynamic change) and since the positive changes are blocked, they therefore conclude that drug Y blocks the effects of drug X. All this has really proved is that drugs X and Y have opposite hemodynamic changes that may be operate completely independently. As an example, we showed that administration of L-NAME and 7-NI (inhibitors of NO synthetase and hence NO) led to negative changes in CBV in multiple brain regions. Treatment with amphetamine (either before or after NO inhibition) led to increases in CBV in the striatum that were not blocked by NO inhibition. In the cerebellum, however, amphetamine induces negative changes in CBV that are additive to those of NO inhibition (Choi et al., 2006a). It is also important to tests agonists and antagonists in the same study using pre- and post-treatments. Although such experiments can be tedious - especially when one considers that multiple doses may be necessary to really figure out what is going on - they are necessary. Of course such experiments are more difficult still in humans, which demonstrates the benefits of performing translational studies.

Detailed study of stimulation of neurotransmission quickly leads one to the conclusion that no single neurotransmitter is an island. That is, there is a coupling between the many neurotransmitters in a given brain region such that increasing the level of one leads to reciprocal changes in coupled systems to maintain homeostasis. For example, a large body of literature also exists on interactions between dopamine and glutamate, dopamine and serotonin and dopamine and gaba, dopamine and adenosine and dopamine and acetylcholine! Thus, the administration of a given pharmacologic agent may often need to be considered in light of interactions with multiple neurotransmitter systems. Indeed, many of the most behaviorally efficacious drugs - whether a drug of abuse such as cocaine, or an anti-psychotic such as clozapine - target multiple neurotransmitter systems. Cocaine clearly blocks the serotonin as well as the dopamine transporter, whereas clozapine may be one of the dirtiest drugs ever studied having activity at multiple serotonin receptors, dopamine D4 receptors, and adrenergic receptors. In light of this fact, one must interpret potential phMRI data with an eye towards parsimonious interpretation of the data. This problem is especially acute in human studies where separation of all the competing hemodynamic interactions is not likely possible. In animal studies there should be little excuse for such a lack of thoroughness.

Different Flavors of phMRI “Maps”

With conventional fMRI, many different methods for analyzing the spatio-temporal dynamics of the brain have been developed. These include both parametric and statistical parametric maps of changes in BOLD signal, CBF or CBV, modeling of the hemodynamic

response function, maps of time to peak or full width half max, or even fitting CBV curves to a logistic function as a function of drug dose similar ot analyses performed in dose dependencies using other pharmacologic parameters (Chen et al., 2005a). In addition, a myriad of means to examine the "functional connectivity" under both tasks as well as during the resting state. All of these methods can be adapted to examine both pharmacologic challenges or alterations in brain circuitry as a function of chronic drug use whether for therapeutic purposes or as a consequence of drug abuse. A number of investigators have published detailed analyses of use of the functional connectivity in phMRI studies (Honey et al., 2003; Schwarz et al., 2009; Schwarz et al., 2007a; Schwarz et al., 2007b). These types of approaches have been used to examine drug abuse (Hong et al., 2009; Jacobsen et al., 2004; Khalili-Mahani et al., 2011; Kobiella et al., 2011; Li et al., 2000; Meunier et al., 2012; Tomasi et al., 2010) or in aging and AD (Li et al., 2012; Wink et al., 2006). Clearly, there is alternative information available for investigating drugs from more traditional analyses. We found, for instance that there were much more marked changes in wide-spread limbic and somatosensory networks in rats sensitized to cocaine using functional connectiviy than there were in the standard parametric maps looking at changes in CBV amplitude or full width half maximum of the CBV data (Chen et al., 2011).

This isn't the place to review such studies, however, most of the analyses cited in the preceding papers can be directly adapted to and from more conventional non-pharmacologic fMRI data analytic techniques. In the following section we detail data analytic approaches that may be unique to phMRI (as opposed to fMRI) studies and may have the potential to bring phMRI closer to a "molecular imaging" technique as well as a tool for pharmacologic investigation.

Pharmacokinetics and Pharmacodynamics Measured using phMRI

Another arena that has been little exploited in phMRI studies is the ability of the technique to provide exquisite temporal information with high temporal resolution. We probed this idea with the dopamine release studies discussed above (relating the dopamine release to the BOLD or CBV changes). A more formal development of this idea first came out of the laboratory of Eliot Stein and Alan Bloom using a nicotine challenge (Bloom et al., 1999). In that study, they measured the nicotine plasma profile and came out with a pharmacokinetic model where they used the ph/fMRI temporal profile to determine parameters releated to half-life of the drug in humans. Although the modeling is rather difficult (as are any situations where a time course ends up being fit by a double exponential model) this study was the first to really try to use the temporal phMRI data, combined with plasma pharmacokinetic data, to produce quantitative results about pharmacodynamics. The nicotine time course in the brain is consistent with the plasma and brain temporal nictoine profile, as well as the pharmacodynamic profile representing the "rush" and "high" (Stein et al., 1998). Thus this raises the very interesting possibility that the phMRI data can be used to determine pharmaco-parameters. A number of subsequent studies in both humans and animals have supported this idea by showing consistency between phMRI time courses and the time courses of nicotine and dopamine release as well as behavior (Choi et al., 2006b; Gozzi et al., 2006). The question then must be asked as to how general is the principle that phMRI time courses can be used to model brain pharmacokinetics? It may not be, and therefore

needs to be addressed with each drug examined. As a counter-example to the situation with nicotine we show data from the dopamine transporter blocker CFT. In this case we compared the brain time course of the drug using PET with the time course of the phMRI studies and found it was discordant. PET data indicates a very long half-life for the drug in the brain (with little corrected decay over the course of 70 min). The phMRI data show a time course that returns to baseline in about 60 min. As we previously published, this time course reflects the dopamine release rather than the drug's brain lifetime. Given the good temporal correlation between the dopamine release and the CBV values, one could use the CBV values to extract parameters related to dopamine release from the time courses (Jenkins et al., 2002, 2006; Jenkins et al., 2007). We began to address this issue in our first paper (Chen et al., 1997a) when we compared the time course of the BOLD signal changes induced by two different dopaminergic drugs – the dopamine releaser amphetamine and the dopamine transporter blocker CFT with invasive measurements of dopamine release using microdialysis. We showed that there was a strong coupling. This idea was tested in the magnet using microdialysis and a cocaine challenge by Schwarz et al. (Schwarz et al., 2004). They concluded that there was a complicated relation between CBV and dopamine release using 0.5mg/kg cocaine challenge. Although the microdialysis data in that study were sub-optimal due to the large dead volume of the sampling line out of the magnet leading, essentially, to a convolution of the true temporal curve with a low pass filter, it nonetheless showed that the linear coupling idea was incomplete. We subsequently followed this up numerous times using both BOLD and CBV measurements and various dopamine drugs (Chen et al., 2005a; Chen et al., 1999; Chen et al., 2005b; Chen et al., 2010; Choi et al., 2006a; Ren et al., 2009b). These studies showed that there was a linear coupling between dopamine and CBV (or BOLD) at high concentrations of released dopamine (> ~450% over baseline) and a negative relation at lower levels of released dopamine. In fact it was possible, in cases where there was a linear coupling, to calculate a coupling constant that relates a change in CBV to a change in DA concentration. (Jenkins et al., 2007).

PET imaging studies can provide excellent information on the time course for specific drug binding in the brain. Shown in Fig. 4 below are data from PET studies on ^{11}C-CFT binding in rat brain along with microdialysis measurements of dopamine release as well as changes in rCBV. It is seen that there is a temporal correlation between CBV (or BOLD) and dopamine release (Fig. 4a). The FWHM of the curve is much larger for CFT than for cocaine (Fig. 4b). The PET time course for CFT binding in the caudate/putamen is much longer than that of the CBV or DA release. Lastly, we see a good correlation between DA and CBV at high levels of released DA with coupling constants (%CBV/%DA) that are close for both CFT and amphetamine (approximately 0.014% CBV change for each percent of DA).

In this latter case of DAT blocker versus nicotine one can postulate that the phMRI is reflecting the pharmacodynamics rather than the pharmacokinetics. With nicotine, the latter two may fortuitously coincide. Unlike the case for the dopamine transporter blocker CFT, the brain lifetime for cocaine (Fowler et al., 1992; Fowler et al., 1989; Telang et al., 1999) parallels that of the CBV and dopamine release (Chen et al., 2010; Chen et al., 2011; Luo et al., 2009; Mandeville et al., 2011; Marota et al., 2000). Thus, no general rules can likely be drawn as to the relation between brain kinetics and the hemodynamic time courses for even

classes of drugs. It is patently clear, however, that far more effort needs to be expended in this direction and the surface has barely been scratched in utilizing the potential of phMRI in this domain.

Pharmacologic MRI as a brain molecular imaging technique with very high sensitivity using hemodynamic transduction of neurotransmitter signaling

The term “molecular imaging” has been a buzz word in the MRI community for close to a score of years. While many fascinating probes have been developed, the impregnable forttress of the blood brain barrier (BBB) has limited the ability of charged molecules with large paramagnetic metals, such as gadolinium or iron oxide nano-particles, to reach their targets. Even if it were possible, it is still an open question whether or not there would be adequate sensitivity generated by binding to targets in the sub-nanomolar range. It is perhaps something of an indictment of the field that Magnevist (Gd-DTPA), a contrast agent that came of age at the same time as MRI in the early 1980s, still has a dominant market position for probing brain physiology. CEST agents have promise by using a long chain polymer that can attain adequate sensitivity via large numbers of exchangeable protons (McMahon et al., 2006; Snoussi et al., 2003; Wu et al., 2010) – however these agents still face delivery problems that have yet to be solved. Optogenetic approaches also show great promise, but at the present time are invasive.

In contrast to this, phMRI can use drugs that have already been developed to penetrate the CNS and effect behavioral, metabolic or functional changes. Although the indirect nature of hemodynamic coupling is rightly construed as a limitation – it has one particularly strong attribute. When a drug, such as amphetamine (a dopamine releaser; used to treat attention deficit hyperactivity disorder or depression) enters the brain it causes release of a neurotransmitter - dopamine. Since dopamine is vasoactive it produces a change in CBF. *Thus, the strong attribute of phMRI is that it can use an exquisitely sensitive coupling mechanism provided by nature.* The coupling, of course, is the same traditionally considered in conventional task, or resting state releated fMRI studies, but with a twist. With phMRI we can target specific receptor systems and use an extremely large array of molecular probes that have already been developed. Two questions naturally ensue from this fact. Can we directly probe receptor modulation? The power of phMRI is that one can postulate that we can probe both increases and decreases in receptor function and its effects on the attendant circuitry. A second question is that if one believes that receptor modulation can be measured then can one get some kind of quantitative metric that would allow for modeling of receptor or neurotransmitter levels. As we showed above, since there is about a 0.014% change in CBV per 1% change in DA, and since the basal extracellular [DA] ≈ 10–100 nM, this implies that we have a sensitivity to changes on the order of a nanomolar of DA. As an example, we show unpublished data we collected using transplantation of fetal dopamine cells in a macaque (Fig. 5). After MPTP lesioning there was little response to amphetamine and little binding of ^{11}C-CFT in the putamen (Jenkins et al., 2004). After transplantaton, there was a tiny graft, that showed up with a few tyrosine hydroxylase cells in histology and a very small increase in ^{11}C-CFT binding. The PET images, though were quite noisy due to the very low number of cells. Nonetheless, we readily pick up the graft with little noise

using phMRI suggesting that nature's coupling mechanism may be more sensitive than one might anticipate. This is merely illustrative of the possibilities. A number of studies have shown upregulation of the phMRI response in, for instance, Parkinson's disease models in response to alterations in receptor numbers – going either up or down (Delfino et al., 2007; Nguyen et al., 2000).

Before we conclude this section one has to ask: would you rather examine receptor modulation using libraries of literally hundreds or thousands of compounds that are known to cross the BBB, many of them in current clinical use, or would you rather spend years trying to determine if your targeted chelate crosses the BBB, has little toxicity, attains adequate target to background delivery and then hope that the change in relaxivity from a nanomolar or picomolar target concentration will produce enough T2* or T1 relaxivity to detect with adequate CNR using MRI? Obviously, this is a biased and leading question, but the truth is solving this problem has stymied the field for a very long time.

In the spirit of being fair, one must also raise an issue that multiple colleagues have raised with me. The gist of the objection goes something like this: well sure, you get a response when you challenge an animal with a whopping dose of drug that may lead to a very non-physiologic concentration of dopamine in the brain or some other neurotransmitter. What kind of sensitivity does one have with normal changes seen with relevant stimulation? The answer to this question is that this is the same coupling seen in ordinary fMRI – just subject to the black box of unknown vascular coupling agents. Further, many of the drugs that we and others have administered are administered at concentrations that are quite relevant clinically. Nonetheless, there are numerous studies that need to be done to measure the coupling of say dopamine or serotonin release under "normal" conditions (for instance while playing video games or dreaming about choclate eclairs). Using simultaneous PET of dopamine release and phMRI one can start piecing apart such puzzles.

Drug Discovery using phMRI

One application that is still relatively lightly populated is the use of phMRI techniques to test novel drugs targeted towards receptors (or other targets) in the brain to see if they indeed hit their targets. Ideally, one would like to radio-label every ligand developed in a laboratory and run a PET study to ascertain brain distribution. This is practically unfeasible. With phMRI one obviates the need for a radio-label and, in addition, can determine information on not only whether the drug is hitting its primary targets, but whether it is also activating (or deactivating) the associated functional circuitry. As an example, our colleague Amy Newman's medicinal chemistry lab has synthesized a number of compounds targeting the dopaine D3 receptor sub-type. While there are a number of in vitro assays that can demonstrate that the compounds are selective for D3 receptors (over D2 receptors) it is another thing entirely to demonstrate that this selectivity inheres in vivo. Given the rather circumscribed distribution of dopamine D3 receptors in the brain, we were able to compare some novel D3 ligands with the receptor distribution and show that there was very good evidence for the D3 selectivity of the selected compounds (Choi et al., 2010; Grundt et al., 2007). Similar studies were performed for D3 compounds by Schwarz, Gozzi, Bifone et al. (Schwarz et al., 2007b). This approach can be attempted with any number of molecules

assuming they have reasonable brain penetration. In cases such as neuropeptides, where BBB penetration may be incomplete, one can resort to intracerebroventricular injections (Gozzi et al., 2005). These however, often present with variable distributions for different agents that may not reflect uniform penetrance to all brain regions (Liu et al., 2004).

Another twist on this scenario is to examine known circuitry, say for nociception, and determine whether a given drug has the ability to modulate the phMRI response in the known circuitry (Borsook et al., 2011; Schweinhardt et al., 2006; Tracey, 2011; Upadhyay et al., 2010). Similarly, use of complementary PET and ph/fMRI studies can be combined to differentiate between different opiod receptor antagonists targeting reward circuitry as a tool for drug discovery (Rabiner et al., 2011). In this case the strengths of the two different techniques are revealed. Clearly this arena should provide fruitful interactions between both pre-clinical and clinical worlds as well as between the academy and the apothecary.

Conclusion

We have attempted to demonstrate that although phMRI studies fall into the general category of fMRI there are additional attributes, related to the ability to stimulate selective receptors and neurotransmitter pathways, that allow for unique information to be obtained. We believe it is reasonable to construe this technique as a molecular imaging technique using exquisitely sensitive neurovascular hemodynamic signal transduction mechanisms. In addition, the high temporal and spatial resolution of MRI may well allow for more fine discrimination of subtle pharmacodynamic effects than is possible with other techniques and make it very complementary to PET studies of brain pharmacology and receptor mapping.

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Abbreviations

7-NI	7-nitroindazole
BBB	blood brain barrier
BOLD	blood oxygenation level dependent
CBF	cerebral blood flow
CBV	cerebral blood volume
CFT	((2β-carbomethoxy-3β-(4-fluorophenyl) tropane)
CMRglc	cerebral metabolic rate glucose
DA	dopamine

fMRI	functional MRI
mRNA	messenger ribonucleic acid
MRI	magnetic resonance imaging
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
L-NAME	Nω-nitro-l-arginine methyl ester
NO	nitric oxide
phMRI	pharmacologic MRI

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Highlights

- Drug-induced hemodynamic changes reflect receptor distributions, function and signaling.
- Selective pharmacologic challenges can open the black box of neurovascular coupling.
- Most drug stimuli produce positive and negative hemodynamic responses.
- Pharmacodynamics and pharmacokinetics can potentially be measured with phMRI.
- PhMRI is a molecular imaging tool with very high sensitivity rivaling PET.

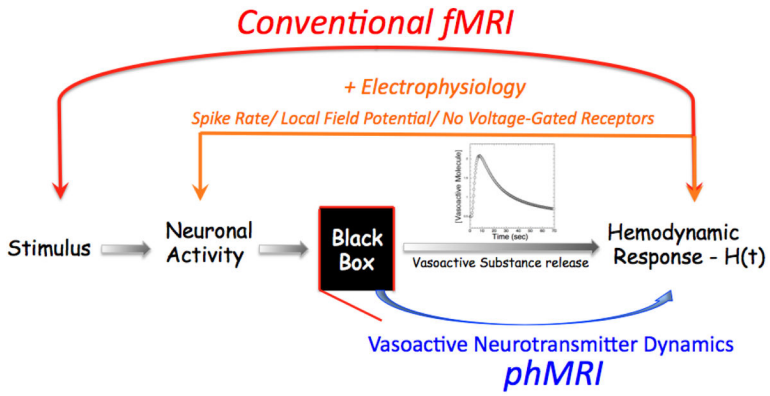

Figure 1. Schematic of the relation between fMRI or phMRI data and the couplings of electrophysiology, metabolism and vasoactive molecule release

In the top is shown the case for the bulk of fMRI studies where the coupling between the neural activity and an hemodynamic change is treated as a black box. In this case spatial and temporal information is obtained that is difficult to relate to the underlying neurophysiology. A number of studies have performed simultaneous electrophysiology (+electrophysiology) and derived correlations between spike rate or summed local field potential and an fMRI response (BOLD, CBF, CBV). However, in the absence of discovery of any voltage-gated vascular receptors, there is still an unknown coupling relating the neurotransmitter release and uptake leading to signaling and release of vasoactive molecules. It is likely that this coupling is non-linear since the release of the vasoactive molecules will lead to diffusion to blood vessels and pericytes. Shown is a small inset figure with simulations using the known diffusion coefficient of dopamine in tissue with its concentration as a function of time for 3D diffusion in an MRI-sized voxel (0.33mm) for an phasic release at time zero. Also shown is the use of phMRI to open the black-box by using drugs, such as amphetamine – a dopamine releaser, that can directly lead to release of vasoactive molecules.

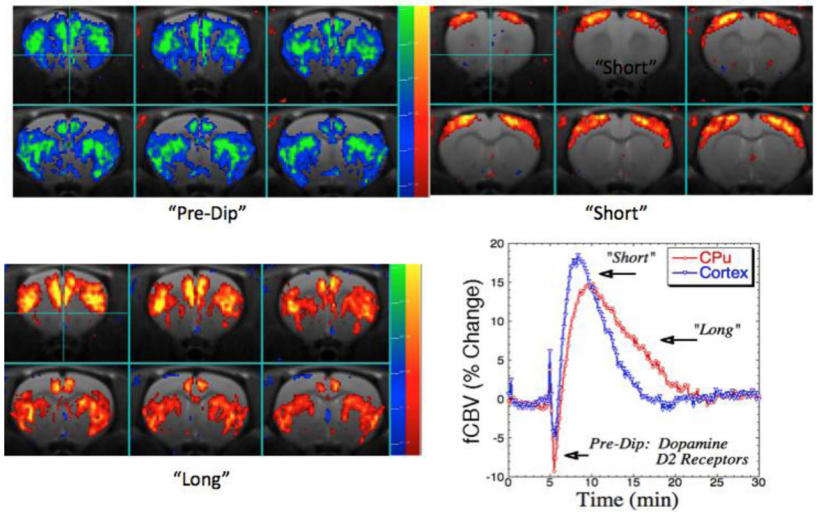

Figure 2. Positive and negative hemodynamic changes follwing cocaine injection

This figure shows the averaged response to 0.5 mg/kg cocaine challenge (similar to data we reported in Chen et al., 2011) in rats. There is an initial decrease in CBV followed by an increase with differing time courses in different brain regions. The initial decrease is due to stimulation of D2/D3 receptors which have higher affinity than dopamine D1 receptors and yield negative hemodynamic responses. The D1 receptors give positive hemodynamic responses. A map of the pre-dip response shows a response in the basal ganglia and medial prefrontal cortex similar to what is seen with agonism of D2 receptors using D2 agonists. A map of the early and late positive components shows both cortical and sub-cortical responses similar to what is seen using a D1 agonist (see Figs. 2 and 3 of (Choi et al., 2006) and Fig. 3 of (Chen et al., 2010) for a comparison of the D1 and D2 agonist maps).

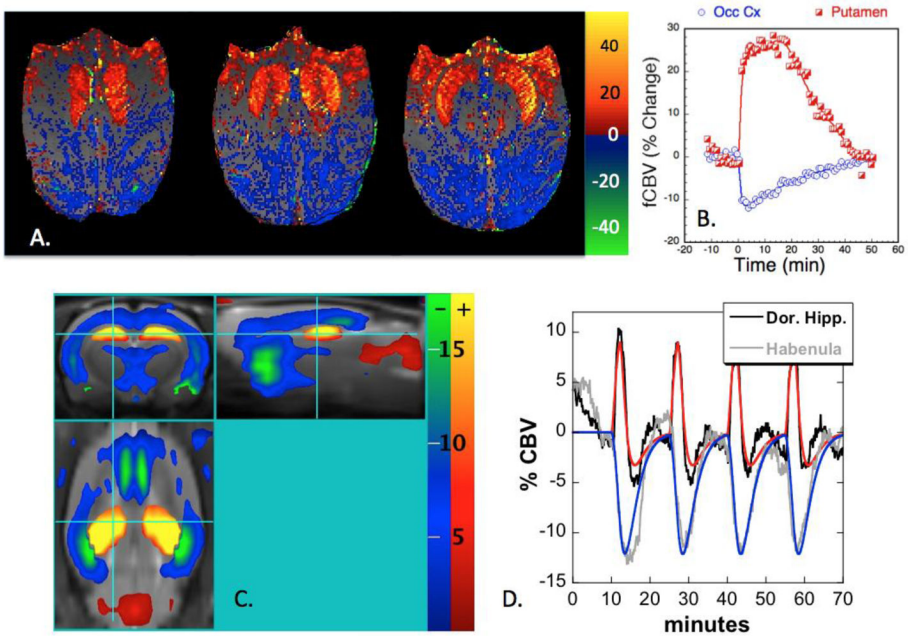

Figure 3. Positive and negative hemodynamic changes follwing amphetamine in a primate and remifentanyl in a rat

A) Map of significant changes in CBV induced by 2.5 mg/kg of amphetamine in a macaque. There are positive CBV changes in striatum and negative CBV changes in occipital cortex (B). Data replotted from (Jenkins et al., 2004).

C) Effects of serial injections of 10ug/kg of remifentanil in a rat. Maps of the percent change in CBV (+ or −) windowed by a high-threshold F test for either contrast alone (fast or slow regressor), with the color scale set to the magnitude of the dominant regressor. The cross-bar is at the dorsal hippocampus. D) Fits to CBV data following sequential injections of remifentanil. The fit was comprised of a rapid and a slow regressor. A negative, slow, change comes from the frontal area in green on transverse slice, but this response also is seen in habenula, cortex, striatum and accumbens similar to morphine. A positive, rapid response is shown in dorsal hippocampus due to inhibition of gabaergic receptors. Data come from the laboratory of Joe Mandeville and are reanalyzed from (Liu et al., 2007).

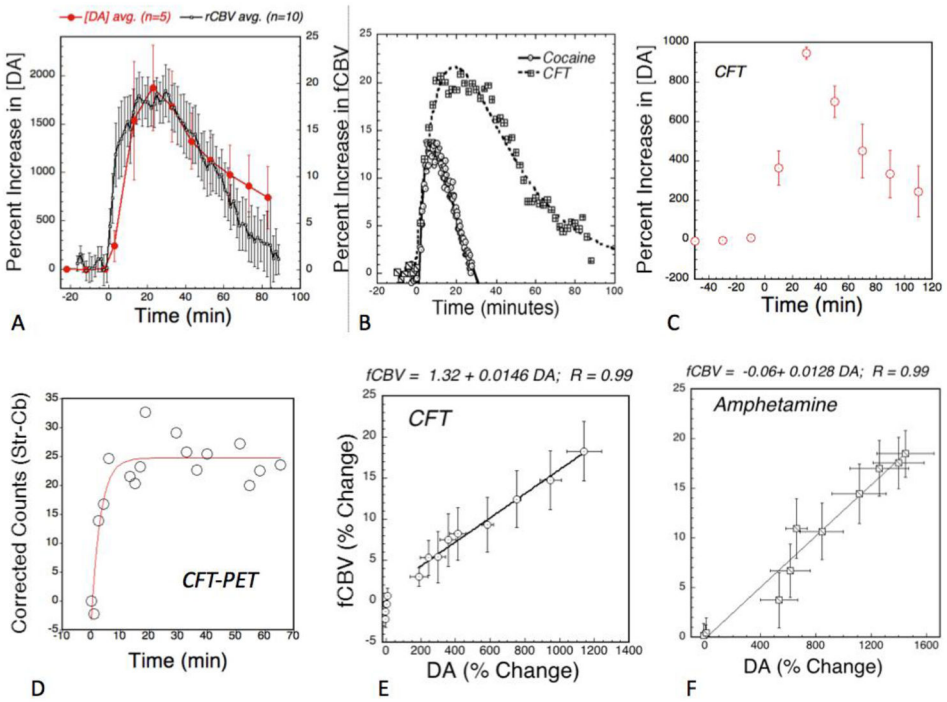

Figure 4. Coupling of dopamine release and hemodynamic changes in rats

A) Data from (Choi et al., 2006a) showing the change in the percent extracellular dopamine release and CBV induced by thethe dopamine releaser amphetamine. The microdialysis data has 10 min time bins. B) CBV changes induced by the dopamine transporter blockers cocaine or CFT. The CFT has a long brain lifetime compared to cocaine, and has a much longer FWHM compared to cocaine. C) Plot of extracellular dopamine release measured using microdialysis for CFT. In this case there were 20 min time bins. D) Specific binding of ^{11}C-CFT in the striatum of the rat (data collected with Dr. Anna-Liisa Brownell, MGH). Note how, unlike for the CBV and microdialysis data, there is little decay over 70 mins. This suggests the lifetime of the drug is NOT what determines the hemodynamic time course, but rather it is the dopamine release.

E) Correlation between CBV and extracellular DA release for the transporter blocker CFT. At high DA concentrations the correlation is linear (n=5). F) Correlation between amphetamine and DA release showing, again, a linear correlation at high levels of released DA. The slopes are similar for the two drugs.

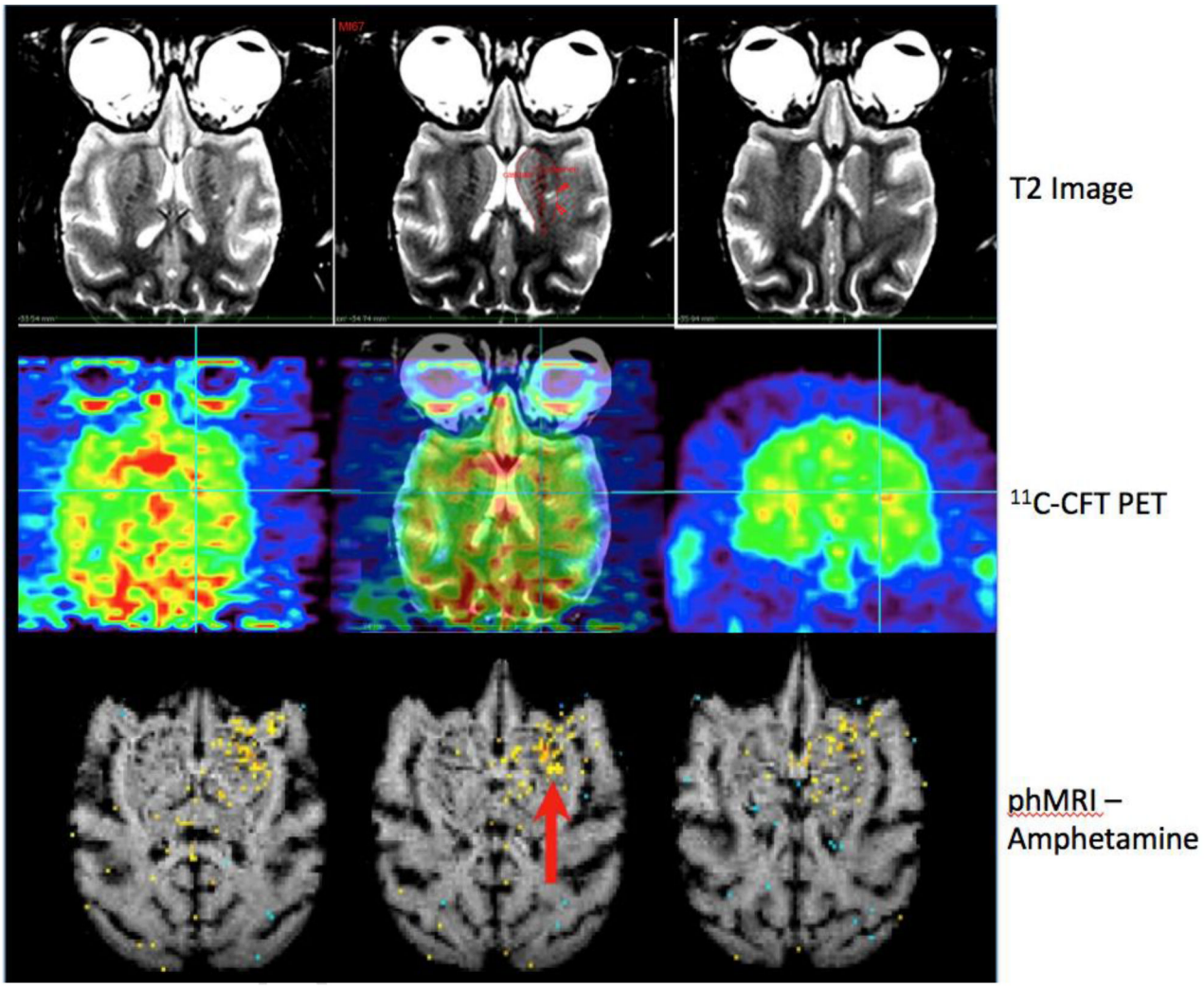

Figure 5. High sensitivity of phMRI for detecting very small fetal cell grafts

Data are shown for a monkey with severe parkinsonism following long term chronic treatment with MPTP. The monkey was then scanned following transplantation with fetal dopamine cell grafts. The data are shown following 4 months growth showing the T2 weighted image (top) and the PET image of ^{11}C-CFT showing loss of DA transporters. There is a (possibly) small recovery of CFT binding at the site of the blue cross-bar (middle). PhMRI map following stimulation with 2mg/kg amphetamine. Data show a recovery of signal with relatively high sensitivity compared to the PET image. Data taken in collaboration with Dr. Ole Isacson and Rosario Sanchez-Pernaute (McLean Hospital) and Drs. Anna-Liisa Brownell, Iris Chen and Ji-Kyung Choi at MGH. Grafts were confirmed post-mortem using TH staining and showed two very small grafts at the sites where the CBV increase following amphetamine.

Table 1

Important Neurotransmitter Targets for phMRI and their Hemodynamic Effects

Neurotransmitter System	Primary Hemodynamic Effect (CBF/CBV)	Clinical Targets	Examples
Acetylcholine	Increase	Alzheimer's Disease, Tobacco Addiction	Nicotine, Scopalamine, Rivastigmine
Adenosine	Increase	Parkinson's Disease, Stimulants	Caffeine, Theophylline (antagonists)
Dopamine	Increase (D1/D5) Decrease (D2/D3/D4)	Drug Abuse, Schizophrenia, Parkinson's Disease, ADHD	Cocaine, Amphetamine, L-DOPA
GABA	Increase ($GABA_A$) Decrease ($GABA_B$)	Epilepsy, Anxiety	Vigabatrin, flumazenil, diazepam
Glutamate	Increase	Drug Abuse, Schizophrenia	Ketamine, phencyclidine (PCP), LY2140023
Histamine	Increase (H1, H2)	Allergies, Multiple Sclerosis	Benadryl, Ranitidine (Zantac)
Opioid	Decrease	Drug Abuse Pain	Fentanyl, morphine
Serotonin	Decrease	Drug Abuse, Depression, Sleep Disorders	LSD, MDMA, Fluoxitine (Prozac)

The primary hemodynamic effects are described for agnoism of the receptors with consensus from the literature. In most cases antagonists will have the opposite hemodynamic effect. These effects may be brain region dependent, as well as dose dependent.