fMRI: A New Tool for the In Vivo Localization of Drug Actions in the Brain

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Abstract

Functional magnetic resonance imaging (fMRI), a still-emerging, non-invasive neuroimaging tool, has been applied to a wide range of questions in sensory, motor control, and cognitive psychology. Only more recently has it been applied to understand the sites and mechanisms of action of pharmacological agents within the human CNS. However, in so doing, a new set of problems and concerns surrounding the technique must be addressed because of the unique transduction mechanisms (both physiological and biophysical) that exist to produce the fMRI signal from the underlying neuronal activity. Experimental design and control issues become paramount in performing fMRI pharmacological protocols and in signal interpretation. With these caveats, the use of pharmacological agents with fMRI is likely to greatly increase in the near term as new questions about both brain physiology and neuropharmacological mechanisms become addressable for the first time. Examples are given using nicotine and cocaine as a prototypical agents.

Introduction

Functional magnetic resonance imaging (fMRI) is an application of MRI developed in the early 1990s that uses radio frequency pulses in a strong static magnetic field to non-invasively detect changes in brain activity. It possesses several key advantages over such older human imaging modalities as positron emission tomography (PET) and single photon emission tomography (SPECT). Perhaps foremost of these is that fMRI uses no ionizing radiation to detect brain activity changes. Thus, as dosimetry limits are not a concern, repeated measurements within a given individual are permissible, enabling within-subject design experiments that can follow a given effect or treatment over long periods of time with multiple manipulations. Furthermore, with temporal resolution generally in the seconds range and spatial resolution as small as a millimeter, fMRI can follow brain activity changes in the awake, behaving individual with a time resolution comparable to that of sensory, motor, and cognitive functioning and processing. In contrast, perhaps the biggest limitation of the technique versus PET and SPECT is the inability to measure static or dynamic alterations in neurotransmitter or receptor concentrations, a distinct strength of these radioisotope-based modalities.

fMRI indirectly measures electrical brain activity by taking advantage of the coupling between changes in local neuronal activity and changes in regional cerebral blood flow. Neurons are the principal brain cell types responsible for information signaling, although other cell types, notably the glia, are also necessary for normal brain functions, both in a supportive role and possibly also in information processing. However, only neurons signal information by rapidly changing their electrical potential and transmitting these potential differences rapidly down their output elements called axons. At the axon terminus, neurons release a chemical substance, a neurotransmitter, that diffuses across the synaptic cleft to adjacent cells where it bind to specific receptors to ultimately change the electrical potential in the postsynaptic cell. These postsynaptic potentials can either be excitatory or inhibitory and are linearly summed in the postsynaptic cell. When a certain potential is reached in the postsynaptic cell, a new action potential is generated, which is then transmitted down its axon where the process is repeated. Although action potentials carry most of the information processing in the brain, it is the postsynaptic potentials (both excitatory and inhibitory) that are the largest consumers of energy in the brain. Thus, changes in local energy consumption, either in the form of glucose or oxygen utilization or substrate delivery (i.e., blood flow), are considered good surrogate markers for brain information processing.

The coupling process between neuronal activity and blood flow, originally described more than 100 years ago (1), provides for rapid increases in energy substrates needed by metabolically active brain tissue. Because the brain does not store these substrates, it is dependent upon a constant, high blood flow rate that can be rapidly phasically shunted to local regions of
higher than normal activity (2). Both the specific mechanisms for this blood flow coupling and the nature of the released vasoactive substances are still under active investigation, although there are compelling data for the candidacy of adenosine, K⁺, lactate (pH), and nitric oxide (3–5). Nevertheless, it is clear that increased neuronal activity requires increased ion pump activity, additional ATP generation, and thus an increase in energy substrates to that area (6). This energy cascade is the link between all functional imaging (including fMRI, PET, and SPECT) and changes in local neuronal activity. However, the increase in blood flow that occurs is not precisely matched to energy expenditure or replacement demands. Rather, what appears to happen is that the released vasoactive substances induce a degree of vasodilation that is greater then the metabolic demand of the tissue, as if the system were saying “here’s more then you need—take what you want”. As such, the local oxygen supply will now exceed the oxygen extraction, leading to the somewhat counterintuitive relative decrease in local deoxyhemoglobin (7). The relevance of this “over-perfusion” of oxygenated blood for fMRI contrast comes from an observation by Linus Pauling, who first reported that deoxyhemoglobin is paramagnetic and oxyhemoglobin is diamagnetic (8). As such, the body produces what is essentially its own MR contrast agent; washing out of this agent by enhanced blood flow leads to a decrease in proton (mostly water) spin dephasing and, using the appropriate pulse sequence, an increase in the MR signal. Thus, echo planar pulse sequences, the most prominent sequences used, produce a contrast mechanism known as blood oxygen level dependence (BOLD) (9–11).

Because BOLD MR signals are measured in arbitrary units and are generally quantitated as percent change from a contrast state (usually some type of activation versus a no activation or “rest” state), early fMRI studies presented stimuli in an alternating on-off block design fashion. Activation is quantitated using a cross correlation procedure such that the input vector (the temporal on-off sequence of the task) is correlated with the output vector (the BOLD signal), making the implicit assumption that brain activity exactly follows the temporal order of the stimulus (a neurobiological assumption that may, in fact, not be accurate some or all of the time) (12). To increase the power of the analysis, the on-off pattern must be repeated multiple times (generally at least 5) within a scan series. Whether the brain processes information in such a tight, inflexible input/output fashion is not known on a systems level, although single cell data have clearly demonstrated various patterns of responses that are not directly driven by the stimulus (e.g., rapidly adapting cells that cease firing shortly after onset of a constant stimulus) (13). More recently, so-called event-related paradigms have become popular, with BOLD responses measured following a single, brief stimulus presentation. However, once again, many individual repetitions of the event must be presented in an irregular temporal pattern to gain sufficient statistical power (14).

Pharmacological fMRI

More recently, fMRI has begun to be used to explore the sites and mechanisms of active of various pharmacological agents within the brain. With appropriate design considera-

Signal processing strategies and control procedures necessary with pharmacological fMRI experiments

As noted, changes in BOLD signal are generally measured relative to some resting baseline state. As such, most non-pharmacological sensory, motor, and cognitive experiments use block design or repeated brief event-related stimuli. Because of the pharmacodynamic and pharmacokinetic properties of virtually all drugs and the practical time limitations of a given scan session (generally about 2 h), it is difficult, if not impossible, to administer a drug repeatedly within a scan session and assume a constant brain response—even with rapidly absorbed intravenous injections. The situation is even more complicated when a drug is administered using the slower absorption rate routes of oral and intranasal administration. The fMRI response to such non-alternating events presents signal processing challenges because cross-correlation analysis is inappropriate for such long duration non-alternating events. As such, several alternative strategies for the determination of drug-induced brain activation have been proposed.

Breiter et al. (15) used the Kolmogorov-Smirnov non-parametric test to compare the average signal before a cocaine injection to that immediately thereafter. More recently, Marotta et al. (16) used a Student’s t-test between specific time points before and after a cocaine injection in rats after first parsing their data into positive and negative signal changes. By recursively analyzing different postinjection time points, they were able to identify the most sensitive time of drug action. Although these strategies have successfully identified drug sites of action, much of the strength of fMRI as an analytical tool is its ability to continuously measure neuronal activity, and, in
the case of a non-constant input (e.g., the variable effects of a drug over time), the fMRI BOLD signal possesses a temporal richness that is ignored by these methods.

A drug's pharmacokinetics, or the time-related changes in drug concentration within the bloodstream or other body compartment, is measurable, predictable, and known for most commonly used drugs. Similarly, the time course of a drug's physiological actions, such as change in heart rate, can be measured and quantitated. As such, an input function based upon the single-dose pharmacokinetic and/or pharmacological effect time course can also be used for signal detection. Several analytical strategies have been developed to take advantage of drug pharmacokinetic properties. However, to use such a function, at least three assumptions are necessary to posit. The first is that the concentration of the drug in the brain is directly proportional to its concentration in the circulation. Short-acting lipophilic drugs such as nicotine and cocaine have a rapid onset (distribution phase) and a relatively short duration of action (elimination phase) consistent with this assumption (17). Second, it is assumed that brain activity, as reflected by changes in fMRI BOLD signal, is directly and proportionately driven by a drug's concentration at its receptors in the brain. This assumption reflects the law of mass action and is consistent with mechanisms of drug action (18). A third assumption is that the BOLD signal accurately reflects the effects of the drug on neuronal elements. This final aspect may be the most problematic in that as discussed, the BOLD signal is an indirect measure of neuronal activity that is sensitive to changes in blood flow, blood volume, and local oxygenation. Thus, the observed fMRI signal may also reflect, in whole or part, indirect actions of a drug, either directly upon the cerebrovasculature, on the neuronal-vascular coupling mechanisms that leads to local vasodilation, or on autonomic nervous system alterations that could alter these factors (e.g., respiration-induced changes in pCO₂ or changes in heart rate or blood pressure). Control procedures and convergence with results obtained using other techniques in other species can often help disentangle these potential confounds.

For example, regional, heterogeneous fMRI activation seen after pharmacological stimulation would suggest an action upon specific, neuronal receptor based mechanisms. In contrast, a drug acting directly upon the vascular smooth muscle would be expected to produce global, homogeneous effects on a BOLD image. Likewise, drug-induced alterations in heart rate, blood pressure, or respiration would be hypothesized to induce global signal changes. Alterations of these autonomic parameters might be expected to globally alter changes in blood flow—even if they occurred rapidly before full autoregulatory mechanisms were able to maintain constant cerebral blood flow. Changes in respiration rate and/or depth have the potential to change pCO₂ levels that would, in turn, cause changes in vasodilation and blood flow. Again, this would be expected to globally alter BOLD signal. Careful on-line monitoring of ANS parameters can obviate these issues.

Marotta and colleagues (16) applied many of these caveats to their observed cocaine-induced fMRI signal changes. In addition to these ANS and/or vascular drug effects, demonstration of an intact neurovascular coupling system under the influence of the drug is an important control because drug administration could directly interfere with coupling independent of neuronal or vascular activity. Coupling integrity can be determined with simple or complex finger tapping or visual stimulus presentation (19). Constant activation in motor and/or visual cortex before and after drug administration would suggest that the drug did not interfere with coupling processes between neural activity and changes in blood flow (20). Additionally, a drug might directly interfere with the ability of the vascular smooth muscle to dilate. This too can be directly tested. Measuring changes in BOLD activity while breathing mild CO₂ mixtures (generally 5–7% in air) before and after drug administration (or other cognitive, motor, or sensory manipulations) can demonstrated the level of 'spare' vasodilatory capacity of the system (21) and can be used to follow changes in tonic flow-induced changes in BOLD signal (22). Such CO₂ changes in flow would allow one to eliminate a drug effect directly upon vascular smooth muscle.

Finally, alternative pulse sequences that are susceptible to changes in blood flow (e.g., QTiPPS. FAIR) and the use of contrast agents to measure changes in cerebral blood volume can also help separate vascular from neuronal effects (23–25). More details of these and other control procedures and their interpretation have recently been reviewed (26).

Based on the assumption that a drug-induced BOLD effect truly reflects a neuronal site of action and not a vascular, ANS, or coupling mechanism, two novel signal detection procedures to analyze drug-induced fMRI signals have been recently proposed. The first procedure is based upon a binary decision-making model in which the waveform in any given voxel must meet all of a set of criteria based upon that drug's pharmacokinetics to be considered activated (27). The second procedure is based upon a nonlinear curve fitting of each voxel's fMRI signal waveform to a generalized pharmacokinetic differential exponential equation with constraints based upon a drug's characteristic pharmacokinetics. Activation decisions are then based upon goodness of fit using an F-test statistic against the null hypothesis of a straight line (28). With the later procedure, it is then possible to use such a difference of two exponents model to fit to each voxel's signal and to extract such important pharmacological data as time to peak, time to half signal max, and half return to baseline and area under the curve. Both methods have been successfully used in our lab (29,30). Currently being investigated are the benefits provided by a combination of these two methods to extract brain drug actions.

Specific Examples of Pharmacological fMRI

Effects of IV nicotine on fMRI BOLD signal changes
In one of the first applications of the use of fMRI to study the sites of actions of a psychoactive substance, we administered IV saline and three doses of nicotine (0.75, 1.5, and 2.25 mg) in a population of dependent smokers while acquiring BOLD fMRI. Drug-induced changes in fMRI signal were extracted using the binary decision algorithm (27). BOLD time course data
from six contiguous voxels taken from the anterior cingulate region are illustrated in Figure 1. The time of nicotine injection (0.75 mg/70 kg) is indicated by the arrow. Note that in three of the voxels, nicotine caused a rapid increase in BOLD signal that decreased exponentially back to baseline within 15 min. Note too that different rate constants were seen in these putative active voxels, whereas adjacent voxels 3.75 mm away did not demonstrate a drug effect. Together these observations suggest that the changes in signal were due to drug action on neuronal elements and not on cerebral blood vessels or on peripheral autonomic parameters, which might be expected to homogeneously alter BOLD signal. When combined across all 16 subjects, heterogeneous activation was seen in such brain areas as the insula, cingulate, frontal lobe (orbital, dorsolateral, and medial), and portions of the temporal and visual cortex. A number of limbic subcortical regions were also activated including the nucleus accumbens, amygdala, hypothalamus and limbic thalamus (29). The average peak fMRI signal intensity increased above pre-drug baseline levels between 2.5 and 3 min after nicotine injection, and returned back to 50% of baseline about 4.6 min after the peak. These data suggest that nicotine produces a regionally selective increase in brain activity that is temporally consistent with the observed behavioral actions of the drug. Although nicotine was administered in a cumulative dosing paradigm in this experiment with successively higher doses of nicotine administered every 30 min, an approximate dose-response profile was evident. Figure 2 (see page 9A) illustrates this effect from averaged data across the experiments. An inverted U dose-response profile is illustrated with the medium dose causing more activity then both the low and high nicotine doses, suggesting that rapid tolerance (tachyphylaxis) developed after the second injection. This phenomenon is more fully discussed.

Data from the described experiment have recently been reanalyzed using the non-linear curve fitting model discussed (28). A one-way ANOVA was performed on the percent area under the curve (AUC) as a measure of the magnitude of drug effect on a per-voxel basis to locate brain regions showing a significant effect of dose (this was not testable in the original binary analysis). Four significant frontal regions were found: the anterior cingulate, right orbital frontal, left dorsolateral frontal, and insula cortex (Figure 3, see page 9A). In addition, significant DOSE activation was observed only in the right caudate/NAcc.

More recently, we have applied fMRI BOLD imaging to determine the sites, time course, and distribution of acute nicotine tolerance (tachyphylaxis) in the brain. In these experiments, we administered two IV injections of nicotine into subjects (0.75 mg/70 kg over 20 s) separated by either 15, 60, or 120 min. Preliminary analysis revealed that although BOLD signal responses were unchanged after the three longer time points, apparent rapid tolerance was seen when a second nicotine injection was separated from the first by 15 min (30). Figure 4 illustrates a BOLD time course from four contiguous voxels in one such experiment. Note the significant decrease in BOLD signal magnitude following the second injection in two of the voxels, but not in the others, suggesting that tolerance may not be an all or nothing neuronal phenomena but rather may be regionally specific. This conclusion awaits further experiments and analyses.

**Acute administration of IV cocaine**

Using a cumulative-dosing, within-subjects design, experienced crack-cocaine addicts were each given four cocaine injections: saline, 10, 20, and 40 mg/70 kg over 1 min. The resulting fMRI time-series data were fit to a pharmacokinetic model using nonlinear regression and the resulting %AUC measure was analyzed by ANOVA on a per-voxel basis. Three significant frontal regions were found: anterior cingulate, right orbital frontal, and left dorsal lateral frontal cortex (Figure 5, see page 9A). In addition, dose-significant activation was also observed in right caudate/nucleus accumbens. The DLF, Nacc, and OFC activation are consistent with published findings (15), but, importantly, the cingulate activation, which was the most robust, was not noted in that report. The activation driving this signal is negative, indicative of a decrease in neuronal activity in this region. The similarity between these data and the nicotine data, which were analyzed in the same manner, is particularly noteworthy.

**Control procedures**

We performed two series of studies to examine cocaine’s potential cerebrovasoactive properties in humans. In the first experiment, two experienced crack-cocaine users were scanned in two 20-min runs using the QUIPSS pulse sequence (23), which simultaneously measures both BOLD and rCBF in a...
single brain slice. During each scan, subjects were injected over a 1 min period with 40 mg/70 kg IV cocaine 4 min into the scan. The first scan involved no task; the second, bilateral finger tapping. An average 13% decrease in task induced BOLD activation was seen 5 min after cocaine injection. This is comparable to the 10% decrease reported by Gollub et al. (20) in visual cortex, suggesting a small global cocaine vascular effect. Nonetheless, task-induced (finger tapping) BOLD changes were clearly evident in the presence of cocaine (Figure 6), suggesting that neuronal-vascular coupling mechanisms remain intact after cocaine administration. These changes were seen concomitant with an insignificant 0.6% decrease in BOLD baseline and a 5% decrease in QUIPS measured CBF, consistent with those previously reported (20).

We have recently begun a study using dynamic contrast enhancement (gadolinium bolus) following an acute cocaine injection. This method, developed by Østergaard et al. (25), allows one to directly measure CBV and calculate CBF for each subject using a singular value decomposition (SVD) technique. All scanning occurred on a Bruker Biospec 30/60 3Tesla scanner (SE-EPI sequence, TR = 1400, TE = 80). Four injections of Gadodiamide (Omniscan®, Nycomed Amersham) were administered to each subject: a loading dose of 0.086 mg/kg and three injections (0.17 mg/kg) during functional scans at baseline and at 2 and 12 min after an IV cocaine injection (40 mg/70 kg). Preliminary analysis based on seven subjects at a threshold of $p < .01$ revealed no global changes in either CBV or CBF at either time point after cocaine, nor any significant regional alterations. At a $p < .05$, a few significant clusters were seen, notably in the anterior cingulate. However, with only seven subjects, insufficient data have yet to be collected to calculate the appropriate threshold level. Nevertheless, the QUIPS, CBV/CBF, and BOLD data taken together with the previous data of Breiter et al. (15) and Gollub et al. (20), support the ability to use BOLD fMRI to measure task-induced changes in brain activity in the presence of cocaine.

Conclusions

Since its inception less than a decade ago, fMRI has been used predominately to non-invasively measure changes in local brain activity following changes in sensory, motor, or cognitive tasks. Only more recently has it been applied to study the acute effects of drug administration. This new field, sometimes called pharmacological fMRI (phfMRI) presents enormous opportunity to determine the sites and mechanisms of drug action in the intact human brain. However, with this opportunity comes additional methodological considerations and design specifications because fMRI does not directly measure changes in neuronal activity. Rather the signal must be transduced via various biophysical, hemodynamic, and physiological mechanisms and steps before a MRI signal can be detected. Nevertheless, data are presented illustrating the effects of acute nicotine and cocaine administration in human subjects as well as various control procedures required to assure data integrity. Using such a strategy, an entirely new class of experiments is now possible, which should shed new insights into human psychopharmacology and play an increasing role in drug discovery and treatment efficacy.

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References

Figure 3. Dose-response activation pattern following three injections of nicotine (0.75, 1.5, and 2.25 mg/70 kg) delivered in a cumulative dosing paradigm ($n = 14$). Data were first non-linear fit to a pharmacokinetic model followed by a voxel by voxel one-way ANOVA across nicotine dose. Note the localized cingulate, frontal, and basal ganglia, including NAcc activation that changed specifically as a function of dose.

Figure 5. Dose-response activation pattern following three injections of cocaine injections (10, 20, and 40 mg/70 kg) delivered in a cumulative dosing paradigm ($n = 7$). Data were first non-linear fit to a pharmacokinetic model followed by a voxel by voxel one-way ANOVA across cocaine dose. Note the anterior cingulate, inferior frontal (orbitofrontal) cortex and nucleus accumbens activation.