Noninvasive Methodology (NMR)

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Abstract

Neuroimaging with MRI provides a noninvasive means to assess drug effects in vivo. In addition to the discovery of potential markers of psychopathology, MRI methods can be used to test existing and novel compounds. The assessments can be of metabolite levels, task-based brain activation, brain connectivity, drug-related activation, and quantitative perfusion. These methods are pharmacodynamic in nature and can also be used to describe pharmacokinetic–pharmacodynamic relationships. They complement emission tomography assessments of brain penetration and dose-occupancy relationships and can extend or even be a substitute for these methods when ligands are not available, with particular value when the desired outcome is intermediate markers of function. Limitations and challenges of these methods are intrinsic to the measurement, such as vascular artifacts, but

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they can be overcome with additional assessments.

Purpose and Rationale

Neuroimaging with nuclear magnetic resonance (NMR) technology, commonly referred to as magnetic resonance imaging (MRI), is a noninvasive method to record from the brain in vivo. In this respect, it differs from intracortical recording techniques – which require surgery and necessarily measure highly localized activity – and emission tomographic techniques which require the injection of radiotracers. MRI has better spatial resolution than surface-based brain functional measurement methods such as electroencephalography and near-infrared spectroscopy, and MRI is able to measure from both deep and superficial structures. The potential role in drug discovery and evaluation of NMR technology is in three main areas:

1. Discovery and validation of neuropharmacological mechanisms associated with pathophysiology
2. Developing imaging markers associated with dysfunction as targets for drug evaluation
3. Testing compounds using imaging markers to determine their pharmacodynamic effects

These methods can be used to answer key questions, principally including, dose-response evaluation of the *functional* effects of compounds and mechanistic validation of compounds. The utility of MRI can be at any phase of drug discovery and development. Preclinical imaging can be used in both discovery and evaluation, particularly when used in combination with animal models of dysfunction. There are strengths in preclinical studies in the ability to test multiple doses of drugs, with pharmacokinetic ranges that may not be possible in humans. Moreover, preclinical MRI can be combined with other techniques to provide comprehensive assessments and control for potential confounds such as measurement specific artifacts (Coimbra et al. 2013; Jonckers et al. 2015). While remaining valuable as a methodology, good examples of translational viability from animal models into successful human trials are limited. When using markers of function, such as functional neuroimaging with MRI, drugs can potentially be tested very early in the developmental pathway in humans, and the focus of this chapter will be on human studies. Such studies will typically be in Phase 1b or early in Phase II. Examples of potential utility include (i) the testing that a compound aimed at improving cognitive dysfunction mediated by a specific brain circuit, actually modulates these circuits in healthy volunteers, giving an early indicator of the potential for efficacy before the drug is administered to patients, (ii) testing that a compound reverses the effects of a drug known to model a component of a disorder, providing evidence of a specific pharmacological mechanism in vivo, and (iii) testing the effects on specific brain circuits in patients with known impairment in such systems, giving an early indicator of the potential for efficacy in the target population.

Procedures

There are five principal methods of imaging with MRI to evaluate drug effects, which are depicted in Fig. 1. These methods offer the ability to perform multimodal studies, in that the different methods are sensitive to different aspects of a drug effect, and can be deployed in the same study sessions. Importantly, these methods can also be tailored to each study to ensure that the pharmacodynamic measures capture the desired window within the pharmacokinetic profile (Deakin et al. 2008; Paloyelis et al. 2016; De Simoni et al. 2013). They each have limitations and advantages; for example, all the methods in Fig. 1 (except MRS) rely on the concept of neurovascular coupling (Mathias et al. 2018); when a brain area is more physiologically and electrically active, then there is a cascade of changes which result in more blood flow being delivered to the active area. As another example, task-based fMRI, typically collected using blood-

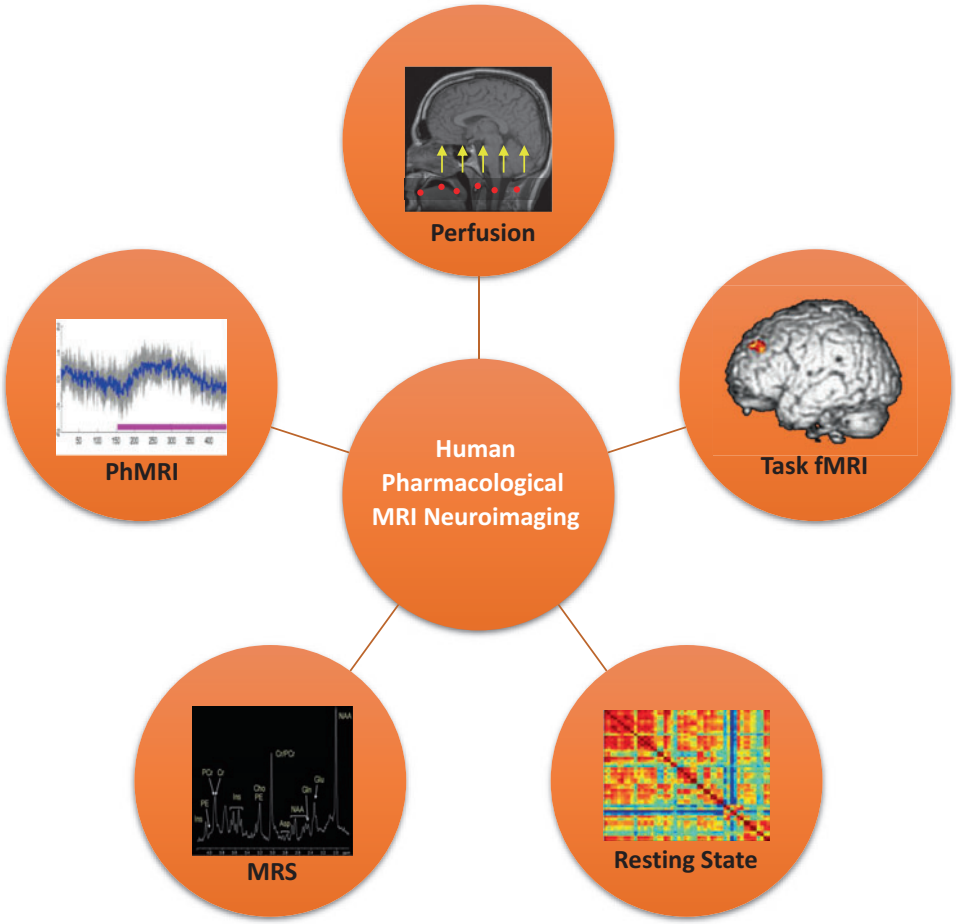

Fig. 1 Five principal methods for measuring drug effects with MRI. Perfusion imaging or cerebral blood flow mapping can be used to quantify drug effects, as is particularly useful in comparing across sessions and correcting within session for baseline differences on the vasculature; task-based fMRI is useful when the effect on the brain activity or connectivity during specific contexts is required; resting state imaging is useful to investigate network level effects on connectivity between regions in a context independent state; MRS is useful in measuring specific metabolite levels (e.g., glutamate); phMRI is a method to track the main effects of typically rapidly acting drugs within a session

oxygen-level dependent (BOLD) imaging, is non-quantitative but can be applied with reasonable temporal (2s) and spatial (~2–3mm) resolution.

Perfusion Imaging

Perfusion imaging with MRI can be achieved through the use of a contrast agent such as gadolinium, but for research purposes, it is the advent of the endogenous contrast agent, allowing non-invasive data acquisition that has seen this method grow in use for pharmacological imaging studies (Zelaya et al. 2015).

Arterial spin labelling (ASL) is the technique of choice for perfusion imaging, in which two types of image are acquired, label and control. The label image is acquired after encoding of the inflowing blood, and the control image will have the same acquisition parameters, but different encoding. The difference between these two images is regionally weighted by the perfusion in the brain tissue, often referred to as the regional cerebral blood flow (rCBF). A separate image

with the same acquisition parameters but sensitive to the proton density (an Mzero image) is required for quantification (Wong et al. 1997). There are two main forms of ASL:

(i) Pulsed ASL (or pASL) involves the labelling of arterial blood by inverting signal over a relatively wide volume (~100 mm) below the area of data acquisition, typically in the vicinity of the carotid arteries. This is achieved with tailored pulses where the B1-field profile is homogeneous over a wide region (Wong et al. 1997). After a short delay, the imaging data is collected. If this is implemented with a single-shot acquisition, such as echo-planar imaging, then the time for volume acquisition can be similar to functional magnetic resonance imaging (see below). The control image can be collected using double inversion of the arterial blood and collected interleaved with the label images. These "control-label pairs" can be treated as a timeseries, although a single pair is insufficient to provide a robust and reliable perfusion map. The timeseries of pairs can also be separated into blocks to produce a series of perfusion-weighted volumes with significantly enhanced contrast-to-noise ratio (CNR) over the individual pairs.

(ii) Continuously labelled ASL (CASL) or "pseudo-continuously" or "pulsed-continuously" labelled ASL (pCASL) has superior labelling efficiency to pASL. This is achieved by arterial blood being labelled by flow-driven inversion of arterial blood in the presence of a magnetic field gradient, applied in the direction of the moving blood into the brain. A labelling plane is used which leads to a rotation of the longitudinal magnetization vector about the effective field, which in turn changes the orientation of the spins by 180° as they cross the labelling plane (Dai et al. 2008). This method is extremely effective at labelling a substantial amount of arterial blood, which allows for the collection of image data using multishot acquisition techniques, which can give whole brain coverage.

ASL carries a number of advantages for pharmacological imaging (Zelaya et al. 2015).

1. Determine the average effects of a drug on rCBF across the brain, referred to as "global effects."
2. Quantification of the changes induced by a drug on rCBF. The most common technique for functional brain imaging, BOLD, is non-quantitative and dependent on the cerebral metabolic rate of oxygen utilization, blood flow, and volume, whereas perfusion imaging reports on rCBF. Indeed, ASL could be used to "calibrate" BOLD imaging.
3. Assessment of drug effects over multiple sessions. These sessions may be separated by seconds, minutes, hours, days, or longer. It is the quantitative and noninvasive nature of the method that allows multiple assessments over time, which may be not be possible when using alternative methods. Emission tomography may be limited by the half-life of some tracers and radiation dosimetry restrictions. Electroencephalography (EEG) may be restricted by the number of times the electrode cap can be removed and refitted without significant artifacts. BOLD imaging is limited due to low frequency fluctuations within sessions and the nonquantitative nature confining comparisons across sessions to interaction effects.
4. The determination of context-independent drug effects acquired over a number of minutes. These so-called tonic effects offer the advantage when assessing interactions between drugs and the state of the system, notably including pain states and affective states.
5. The determination of context-dependent drug effects on functions/processes which change at very low frequencies. For example, learning paradigms, where the learning occurs over a period of minutes when BOLD contrast data are dominated by low frequency fluctuations. The use of control-label pairs markedly reduces sensitivity to the sources of low frequency noise (e.g., scanner temperature fluctuations).

ASL has very good test-retest reliability (intraclass correlation coefficients >0.80) (Li et al. 2018) making a stable measurement, useful for within-subjects studies, where a drug can be compared to placebo or other active controls. Indeed, ASL methodology is sensitive to a variety of drugs. With fast-acting compounds such as fentanyl, pulsed techniques have been used to chart the rapid changes in the brain (Zelaya et al. 2015). With oral drugs, cASL methods have been used to both describe and discriminate different compounds. For example, different antipsychotic drugs are poorly discriminated by their efficacy in patients with few exceptions, but cASL has demonstrated a difference between the older dopamine D2 receptor antagonist haloperidol and the partial agonist aripiprazole in healthy volunteers (Handley et al. 2013). Aripiprazole had more effects in the cortex, while haloperidol produced a greater increase in rCBF in the ventral striatum. These localized effects are important for understanding potential differences in regional functionality between drugs for the same indication. Another example is from a study of methylphenidate and atomoxetine, both indicated as a treatment for attention deficit hyperactivity disorder, but with known differences in their pharmacology. ASL has been used to demonstrate the cortically and subcortically distributed changes in rCBF that allow the drugs to be discriminated from each other and placebo (Marquand et al. 2012), with methylphenidate have more prominent effects in a set of regions including subcortical structures and atomoxetine having more prominent effects in a set of cortical regions.

It is only relatively recently that there have been attempts to harmonize ASL methods across scanner manufacturers with recommendations for multisite studies (Alsop et al. 2015). Indeed, the number of studies using ASL to describe drug effects is low compared to BOLD fMRI. The tension between optimization and harmonization is present in individual studies, although sharing of data across centers is a drive to standardize the acquisitions as much as possible to address questions that are beyond individual studies. This has been successfully applied to analgesic compounds to demonstrate shared and distinct regions in the brain for their functional effects (Duff et al. 2015).

Task-Based fMRI

Task-based neuroimaging with MRI is almost exclusively implemented with BOLD contrast (Huettel et al. 2014). This method relies on functional hyperemia, that is the arterial delivery of oxygenated blood at a level that exceeds the need in the target region. The venous outflow from an active region will necessarily have a higher level of deoxygenated hemoglobin, but the hyperemia leads to a paradoxical decrease in the relative concentration of deoxyhemoglobin. Because deoxyhemoglobin is paramagnetic, the local distortions in the magnetic field are reduced in more active regions increasing the MRI signal that is sensitive to spin-spin relaxation times (T2* and T2*-weighted signal). When combined with a fast readout, whole brain images of the deoxygenated hemoglobin sensitive contrast can be collected with a temporal resolution of about 2 s and a reasonable spatial resolution, with voxels of approximately 3 mm isotropic. The advantages of the BOLD method are the rapid acquisition of whole brain images, sensitivity to deep brain structures, and standardized processing pipelines that are publicly available. The typical signal change with task activation can be as high as 3%, usually in primary sensory cortices, but is typically fractions of a percent. A disadvantage of the BOLD contrast is that following neuronal activity the signal change peaks a number of seconds later before slowly returning to baseline. This is known as the hemodynamic response function and needs to be factored in to any data modelling for task-based fMRI. Another challenge to BOLD imaging is the influence of movement which must be accounted for in the analysis pipeline. Susceptibility artifacts result from areas of the imaging field of view that alter the local gradients in the magnetic field leading to loss of contrast. Air-tissue boundaries produce loss of contrast in orbitofrontal cortex and the temporal pole, often extending into the basal forebrain. This can result in additional loss of signal in the ventral portions of the striatum and the amygdala. This

potential for artifacts and data loss emphasizes the need for excellent quality control processes in data processing in order to account for scans with loss of contrast, or problematic movement. In some cases, scan volumes or entire scan runs will need to be removed from the analysis. Building such controls into the analysis pipeline is crucial to ensure decisions about data quality and inclusion are made independent of the actual analysis (Schwarz et al. 2011).

Timeseries Correlations and Resting State BOLD

Resting state BOLD imaging involves the acquisition of BOLD contrast timeseries data without the use of a task. Participants typically are asked to lie still in the scanner with their eyes open or closed and not to engage in any particular cognitive activity. The most common use of resting state BOLD is to derive connectivity metrics from the data. Connectivity is a term used to describe the correlation, or coherence, in signals from different brain regions. If regions correlate, they are considered functionally connected. The correlation between any two defined regions can be examined at the level of a region of interest (usually derived from a predefined atlas) or at the voxel level. The connectivity described can be a summary of the entire timeseries, or index dynamic variations in connectivity over time. An advantage of resting state imaging is the simplicity with which it can be implemented, requiring little or no training of the subject or experimenters. A challenge is the potential for artifacts in the correlation of timeseries, such as noise in the BOLD contrast data from the scanner (e.g., low frequency fluctuations) or from the subject (e.g., physiological noise from the pulsatile nature of blood flow, or from respiratory noise). A method to account for these artifacts must be included in the data analysis. A common method is to band pass filter the data.

Sophisticated data processing and analysis methods can be applied to resting state BOLD data to extract network or topological parameters. Independent component analysis can be used to extract components in the data which are separated at spatial or temporal scales. These have two important uses. The first is to derive separable networks that have been linked to distinct functional domains (Smith et al. 2013) and the second is a means to identify and remove components of the data that are related to noise, such as movement or respiration (Pruim et al. 2015). Graph theory is applied to resting state BOLD timeseries data and can be used to calculate the correlation of each brain area to all others, the pathways between any two areas, the efficiency of the system in terms of connection pathways, and other metrics such as small word topology (Bullmore and Sporns 2009).

Connectivity analysis can also be applied during the performance of cognitive tasks. The correlation of the timeseries BOLD data can be hypothesized to alter during changes in cognitive demands (Friston et al. 1997).

Magnetic Resonance Spectroscopy

MRS is used to study metabolite levels in brain tissue (for review, see Buonocore and Maddock 2015). While protons can align along the applied magnetic field, 31P and 13C MRS can also be utilized, and 23Na and 19F are options for MRS investigation, but additional equipment may be needed. At the core of MRS is the fact that the magnetic field a particular atom experiences is affected by its local chemical environment, specifically the magnetic field from nearby motion of electrons. The consequence of experiencing slightly different applied fields due to the varied chemical environment is that the atoms resonate at slightly different frequencies. This “chemical shift” effect can be used to detect different environments of relevant nuclei. It is the chemical shift that gives rise to a MR frequency spectrum consisting of nuclei which resonate at different frequencies, depending on their local environment. The frequencies are not related to the exact concentration of nuclei and depend on the exact magnetic field strength. Therefore, MRS peaks are usually expressed in dimensionless units (parts per million, ppm), with reference to a

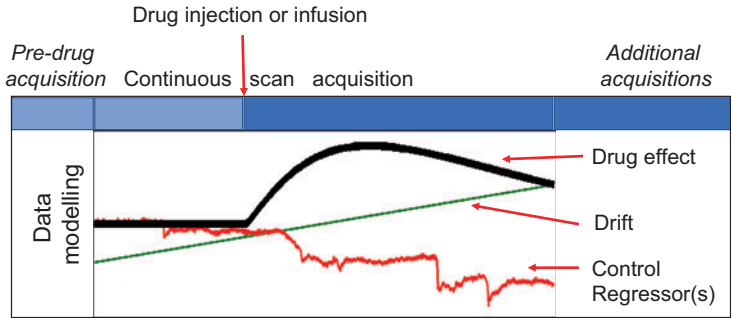

Fig. 2 An example of a phMRI experiment implementation. The blue bar represents the scan acquisition which may involve scans both before and after the phMRI scan. An example modelling approach is shown below for the phMRI scan only. Here regressors for the main drug effect (thick black line) and common confounds of drift (green line) and movement (represented by a single red line, can include multiple regressors) are shown (based on method used in De Simoni et al. 2013)

specific molecule. For proton MRS (1H-MRS), water is commonly used as a reference at 4.7 ppm. Proton MRS is of particular interest in neuroscience, because it can be used to measure the important central nervous system amino acids glutamate and glycine. The main inhibitory neurotransmitter GABA can also be assessed with more elaborate acquisition and analysis.

PhMRI

Pharmacological MRI or phMRI is a method used to describe the changes in the MRI timeseries following compound administration (Breiter et al. 1997). Any timeseries data acquisition method can be combined with compound administration in phMRI. In rodents, the use of a blood-pool contrast agents such as super-paramagnetic iron oxide particles can be used to collect cerebral blood volume timeseries data (Mandeville 2012). These use the same T2* imaging sequences and fast acquisition as BOLD imaging. PhMRI methods in humans have almost exclusively used BOLD contrast. Participants are normally at rest in the scanner when acquisition begins and after sufficient volumes have been acquired to define a stable baseline, the drug is administered without a break in the data acquisition. The expectation of phMRI is that following drug administration, a rapid change in MRI signal can be detected. Due to practical considerations with pharmacokinetics and difficulties with multiple administrations of a drug, this technique is limited to only one timeseries per participant per session.

The main challenge with this method is how to describe the effect of the drug on the MRI signal. One option is to compare the MRI signal before and after the drug administration (Breiter et al. 1997). However, this does not capitalise on temporal variation. Instead, an input function can be used, derived from the profile of the drug plasma levels. This will identify brain areas where the neuroimaging signal matches that of the plasma drug level over many minutes. The implicit assumption here is that the plasma and brain pharmacokinetics are matched and that the functional effects of the compound are temporally matched to the pharmacokinetics. Alternative input functions can be used to enhance the interpretation of the findings, such as the profile of subjective effects (Breiter et al. 1997). Mathematical models of the profile of predicted drug effect derived from pilot, or independent are useful when other input functions are not available or considered inappropriate (Fig. 2).

Critical Evaluation of MRI Approaches in Drug Development

There are a number of questions that NMR methods can be used to address when used in drug development.

Defining and Confirming the Marker for Assessment of a Drug Effect

These markers may be the level of particular metabolites in a specific brain region, a task-related activation, or connectivity change, or a localized difference in perfusion. For example, glutamate is elevated in patients with schizophrenia (Merritt et al. 2016; Schwerk et al. 2014) and MRS would be a viable methodology to test compounds for their ability to reduce glutamate levels in vivo. Hyperperfusion has been noted and replicated in patients at risk of psychosis (Allen et al. 2017) and is therefore a candidate target for interventions to reduce such risks. Amygdala reactivity to negative affective stimuli is elevated in patients with depression and a reduction this response has been used as a target to study antidepressants (Godlewska et al. 2012).

There are significant challenges here that speak to the entire effort of those aiming to describe the neural correlates of brain disorders. The first challenge is the definition of the patient groups (Insel and Cuthbert 2015). The diagnoses are often based on clinical presentation (and in some cases additional tests), which do not define the patients by their abnormal glutamate, perfusion or brain reactivity, etc. That is, using the first example above, there will be individuals with elevated glutamate without schizophrenia and patients with schizophrenia with apparently normal MRS-measured glutamate levels. Moreover, the existence of differences which are sensitive to specific subgroups may lead to dilution of effects unless these subgroups are taken into account. These subgroups may be relatively easy to define, such as first-episode vs. chronic patients, or treatment-responsive vs. treatment-resistant, or prove difficult to define, such as patients with similar symptoms, but different etiologies, or patient groups that separate based on the biological data collected, so-called biotypes (e.g., Drysdale et al. 2017). Such stratifications may or may not be relevant for later assessment of potential treatments and represent an area of significant research need.

Despite these challenges, there are examples of robust, evidence-based neuroimaging markers in use for testing drug effects. Many patients with schizophrenia show elevated presynaptic dopamine markers (Mccutcheon et al. 2017), altered brain connectivity during working memory tasks, and differential activity during reward processing; many patients diagnosed with major depression show hyperreactivity in the amygdala (Leppanen 2006) and altered activity in the anterior cingulate and frontal cortex that may be predictive of treatment response (Fonseka et al. 2018).

Important contributions from the literature with existing drugs will be to provide clear demonstrations that changes to these imaging markers leads to improvements in patient outcomes.

Provide Supportive Evidence for the *Functional* Mechanism of Action of a Drug on a Pharmacological or Disease-Relevant Mechanism

When the effects of a drug are tested on the fMRI signal, it can provide evidence for mechanisms of interest. For example, a drug with potential to enhance reward-related activity in the brain can be tested using a paradigm sensitive to reward processing. An example of this is the emerging evidence for the dopamine antagonist, lurasidone, to act as an antidepressant. A group of individuals were scanned during the performance of the monetary incentive delay (MID) which can be used to ascertain the brain regions involved in response to a reward predicting cue and later in response to actually receiving the reward. Such anticipatory and outcome events have been associated with overlapping brain regions. Increased activity of the key ventral striatum region responsive to reward anticipation was improved by lurasidone, but only in those with higher levels of anhedonia (Wolke 2018). Here the mechanism is captured by the brain regions responsive and the task phase.

The change in fMRI signal indicates support for the hypothesized reward mechanism being modulated by the drug. While this is a valuable goal in proof of concept experimental studies, understanding if the effect size of the fMRI signal change is clinically meaningful would provide a stronger platform for translating findings for

patient benefit and potentially aid outcome measure selection and effective dose selection. In other words, if a change in brain activity is observed, is that predicted to lead to clinical benefit? There are many examples of treatment studies in which fMRI changes are accompanied by symptomatic improvement. For example, in patients with psychosis and other psychiatric disorders such as depression studies show that activation, connectivity and multivariate patterns can normalize with treatment effects (Abbott et al. 2013; Hart et al. 2012). When studies have reported brain activation effect sizes, existing treatments do not fully reverse but instead reduce impairments (Snitz et al. 2005; Williams et al. 2015). For example, responders to antidepressants show a two-third reduction in amygdala activation abnormality compared to no change in nonresponders (Williams et al. 2015) and frontal cortex impairments reduce by a similar amount after successful antipsychotic treatment in patients with schizophrenia (Snitz et al. 2005). While these studies require replication, they are important in defining the goals of these proofs of concept studies such that they can be powered appropriately.

Proof of concept fMRI studies can include either healthy volunteers or patient groups. The decision about which group to focus on depends on a number of factors. Healthy volunteer studies can be conducted faster than patient studies, and the group is theoretically more homogeneous, without potential underlying variation in disorder related processes. In addition, healthy volunteers can be recruited for pharmacological modelling of a potential disease mechanism, against which novel treatments can be assessed.

Ketamine is an example of a model for the glutamatergic system contributions to schizophrenia (Krystal et al. 1994). When given as an infusion to healthy volunteers, it produces a rapid increase in rating scale scores of both positive and negative symptoms of schizophrenia and cognitive impairment (Javitt et al. 2018). Using the phMRI methodology (see above), ketamine produces a robust and reliable neuroimaging response (Deakin et al. 2008; De Simoni et al. 2013). This neuroimaging response can be considered as an assay for the acute disturbance of glutamate transmission and is thus suitable for testing the effect of compounds which may act to attenuate this disturbance, such as lamotrigine and risperidone (Doyle et al. 2013). This assay has been used to test the effectiveness of two group 2 metabotropic glutamatergic receptor agonists (mGlu2 agonist prodrug LY2979165 and the mGlu2/3 agonist prodrug LY2140023). Both compounds were able to attenuate the phMRI ketamine response (Mehta et al. 2018), providing evidence in vivo in humans that the drugs are able to reduce an acute glutamatergic disturbance (as modelled by ketamine). The use of multiple doses of each compound was able to inform on dose response relationships, with only the highest doses of each compound used showing statistically significant attenuation of the ketamine response.

Direct investigation in patients does not require pharmacological modelling of the disease or disorder and thus task-related fMRI, resting state fMRI or perfusion can be used to assess the effects of a drug relative to a placebo or other comparison condition. While the imaging methods have been addressed above, additional considerations of existing treatment, treatment variation and drug-drug interactions must also be addressed. The advantage of these investigations is that they provide direct evidence in the target group of modification of brain mechanisms relevant to the pathology. A series of studies in ADHD suggest that psychomotor stimulant medication normalizes the task-related fMRI impairment (Hart et al. 2012). This represents a candidate assay in ADHD to assess putative novel treatments. Positive results in these studies can provide key input into decisions about larger trials in patient populations and may even inform on assessments for inclusion and target dose ranges.

Drug Comparisons

Different compounds may have similar clinical efficacy, despite having variations in precise mechanisms of action. Antipsychotic drugs are a case in point as they cannot be easily separated in

terms of clinical efficacy to reduce positive symptoms of psychosis. Neuroimaging can be used to demonstrate that the circuits modulated by these compounds do not fully overlap (Handley et al. 2013). Another case is that of medication for ADHD, which includes the archetypal stimulant drug methylphenidate and the non-stimulant atomoxetine. Methylphenidate is an inhibitor of the dopamine and noradrenaline transporter, whereas atomoxetine is selective for the noradrenaline transporter. Arterial spin labelling and task-related fMRI have both been used to show clear discrimination of these medications in healthy volunteers where each participant receives each drug (Marquand et al. 2012). Perfusion mapping showed a preferential response in widespread areas including the caudate and putamen to methylphenidate and more cortical regions for atomoxetine. Task-related fMRI has also been used to derive candidate markers for superior response to each of these compounds in youth with ADHD, with caudate response suggested as a marker for superior response to methylphenidate and motor cortex as a marker for superior response to atomoxetine (Schulz et al. 2017).

The value of testing different compounds, that have proven clinical utility, is the potential to use their responses to define core signatures of efficacy and identify candidate markers for superiority of response to individual compounds. The inclusion of novel compounds in such designs during phase 1b/II can allow both early assessment against a core response signature and also assessment of discrimination from existing treatments *outside* of this core signature (e.g., in other brain networks). These functional "off-target" effects can then be tested for their potential predictive value in clinical efficacy or side effects. In summary, neuroimaging markers can be used to compare different compounds to demonstrate that they operate through difference functional mechanisms or show that despite difference pharmacological profiles there is overlap in the functional effects.

Confounds and Limitations

Functional MRI methods are indirect methods used to infer changes in neuronal function. They are reliant on a process known as neurovascular coupling. This involves neurons, the vasculature and glial cells, in particular astrocytes. Projections known as astrocytic feet modulate vasoconstriction and vasodilation, and these processes are mediated and moderated by a range of neurochemical cascades (Petzold and Murthy 2011). Receptors on the smooth muscle or the astrocytes can also modulate vascular responses. Assessment of the potential alterations in neurovascular coupling and the vascular response following drug administration would be included in optimized protocols. The confounding effects can manifest as (i) changes in baseline perfusion following the drug, which may alter the amplitude of activations during tasks (Cohen et al. 2002), and this can be assessed by including an additional scan with arterial spin labelling, (ii) changes in the vascular reactivity, which may also alter the amplitude of activations, and this can be assessed via non-neuronal methods for vasodilation, such as breathhold-induced hypercapnia (Kannurpatti et al. 2014), and (iii) changes in the variance of the fMRI contrast similarly representing changes in vascular reactivity, which can be correlated with activations or included as a confounding factor in the analyses (Kannurpatti et al. 2014). The increased use of these methodologies will lead to a growth in the number of examples where these additional control steps refine the results and can help define drug classes and neurotransmitter systems where assessing such confounds is imperative. The global signal across the brain can also be affected by physiological noise from normal breathing and cardiac motion, which can be assessed with bellows and pulse monitors. Fluctuations in breathing and heart rate may have direct effects but also alias to lower frequencies. While this can be dealt with in part by filtering the data to include frequencies between ~0.009 Hz

and ~0.1 Hz, these effects can also be included in the modelling of the data by calculating a basis set from the measured respiration and heart rate monitors (Murphy et al. 2013).

Movement is a major confound in functional neuroimaging with MRI and must be accounted for in the data modelling. During data collection, movement is limited by ensuring participants are comfortable and securing the head with straps and foam. Care must be taken that this is balanced against participant comfort otherwise there is a risk that the participants may not tolerate the entire scanning protocol resulting in missing or incomplete data. Prior to data collection, participants can be acclimatized to the neuroimaging environment by the use of a mock-up scanner. Although movement during scans cannot be eliminated, predefining standards for acceptable movement can ensure that artifactual results or reduced sensitivity due to movement is mitigated. Exploratory analyses can be added to the protocol to ensure that any drug effects are not explained by movement.

Multimodal Neuroimaging

FMRI can be combined successfully with other methods, most notably electroencephalography and positron emission tomography. While there are no clear examples of such combinations enhancing the use of MRI methodology in drug development, advances in these methods and the availability of new hardware create an opportunity. For example, EEG and fMRI have been combined in many studies, but for pharmacological studies, there are two main uses that that could provide benefit. The first is to contribute to understanding potential neurovascular artifacts in the fMRI data from the addition of a drug, by providing information on the neuronal activity (e.g., Arthurs et al. 2004), although considerable work is required on the underlying models that link the EEG and fMRI outcomes. The second is to use one method to enhance the analysis of the other. For example, using fMRI to narrow the solutions to the inverse problem in EEG and define potential sources of signal and EEG-informed fMRI analyses, where the EEG response to single conditions can be included in the fMRI model. An example of a single trial model is where the EEG derived P3 response to trials in an oddball paradigm were included in the fMRI analysis as a marker of attention to the task. By informing the fMRI model – in a trial-specific manner – from the EEG response the sensitivity to nicotine modulation of attentional responses in the brain was dramatically enhanced (Warbrick et al. 2012).

Other methods such as emission tomography or postmortem receptor or gene expression data can be combined with MRI-based outcomes to link the fundamentally hemodynamic responses to molecular targets (Dukart et al. 2018). Such methods are important in the development of our understanding of the molecular mechanisms behind the MRI changes with drugs, but as yet do not have a role in supporting MRI for drug development.

Conclusions and Future Directions

The multimodal nature of functional MRI allows for assessment of different aspects of pharmacological modulation in the brain, including changes in task-related activation, functional connectivity between brain regions and network topology, metabolite levels, drug-related activation as assessed using phMRI and quantitative perfusion. The key advantages and challenges of these methods are recapitulated below along with comments on future directions:

(i) MRS can be used to provide evidence of the neurochemical changes induced by drugs but are limited to the metabolite levels that MRS is sensitive to with poor temporal (minutes) and spatial (cm) resolution. Dynamic changes in metabolite levels using, so-called functional MRS (Jelen et al. 2018) will be a significant advantage allowing testing of the responsivity of the system and the role of treatment in potentially normalizing abnormalities. Newer methods that allow whole brain assessment rapidly with good signal to noise ratio will be important developments.

(ii) Task-related fMRI can be used to provide evidence of context-dependent modulation of brain function and thus identify areas of greater and smaller sensitivity and indicate the neural mechanisms underpinning the drug effects. The implementation of such methods requires expertise in the specific task for delivery during the scan and appropriate analysis of the data. Participants may have to be dropped because of poor performance and those who are cognitively challenged, lack motivation or are otherwise unwell may not be able to engage with the tasks. Typically, a very small number of cognitive domains can be assessed because of the time required to collect sufficient data while maintaining participant engagement and comfort. Methods to reduce data acquisition time will be important to enhance the participant experience and allow the protocols to be more efficient or contain more measurements.

(iii) Resting state imaging has proved extremely versatile as a technique for assessing pharmacological effects of compounds, demonstrating relatively local changes in specific connections (i.e., specific pathway) and topological reorganization (i.e., widespread network effects). It has been used to validate assays for drug development (e.g., Joules et al. 2015 for ketamine). Its great advantage is that it provides task-independent descriptions of drug effects, but it lacks functional specificity and it is not clear if a drug effect observed during a resting scan translates into a functional effect in the same brain areas.

(iv) Scanning at rest is also the basis of phMRI which is used to measure the effect of a fast-acting drug on the fMRI signal. It is particularly well suited to study drugs which are injected and is a translatable technique as the same methods can be used in small animal imaging. In drug development, in humans, this method has been used to develop an assay of glutamatergic dysfunction against which novel drugs can be tested (Mehta et al. 2018). The translational value is an advantage of this method (Coimbra et al. 2013), which accompanies its growing use in human studies (Javitt et al. 2018). Being constrained to only fast-acting drugs does however limit the number of drugs that can safely be used in this assay.

(v) Perfusion imaging can be used to track the effects of injected compounds with the phMRI methodology and also to quantify regional cerebral blood flow in order to compare drug effects across sessions. The signal to noise ratio is lower than that of BOLD fMRI, but the sensitivity and reliability is very good, with effects of many different drug classes demonstrated to date. There are two main uses of perfusion imaging – to assess the baseline state of the system, with particular value in understanding the vascular contributions to task-based and resting state fMRI changes, and to quantify the effects of compounds directly.

Overall, these methods can be used as described above, by themselves, or in combinations within the same imaging sessions to answer questions on neuropharmacological mechanisms of psychopathology, development of assays and describing pharmacodynamic effects, and pharmacokinetic–pharmacodynamic relationships of compounds. When testing novel compounds, these methods are fundamentally pharmacodynamic in nature and can be applied in healthy volunteers or patient groups. They complement emission tomography data which can be used to confirm brain penetration and dose-occupancy relationships to specific targets and can extend or even stand in for these methods when ligands are not available or multiple targets are involved in the desired effects providing intermediate markers of functional outcome.

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