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BOLD functional MRI in disease and pharmacological studies: room for improvement?

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Abstract

In the past decade the use of blood oxygen level-dependent (BOLD) fMRI to investigate the effect of diseases and pharmacological agents on brain activity has increased greatly. BOLD fMRI does not measure neural activity directly, but relies on a cascade of physiological events linking neural activity to the generation of MRI signal. However, most of the disease and pharmacological studies performed so far have interpreted changes in BOLD fMRI as "brain activation," ignoring the potential confounds that can arise through drug- or disease-induced modulation of events downstream of the neural activity. This issue is especially serious in diseases (like multiple sclerosis, brain tumours and stroke) and drugs (like anaesthetics or those with a vascular action) that are known to influence these physiological events.

Here we provide evidence that, to extract meaningful information on brain activity in patient and pharmacological BOLD fMRI studies, it is important to identify, characterise and possibly correct these influences that potentially confound the results. We suggest a series of experimental measures to improve the interpretability of BOLD fMRI studies. We have ranked these according to their potential information and current practical feasibility.

First-line, *necessary* improvements consist of (1) the inclusion of one or more control tasks, and (2) the recording of physiological parameters during scanning and subsequent correction of possible between-group differences. Second-line, *highly recommended* important aim to make the results of a patient or drug BOLD study more interpretable and include the assessment of (1) baseline brain perfusion, (2) vascular reactivity, (3) the inclusion of stimulus-related perfusion fMRI and (4) the recording of electrophysiological responses to the stimulus of interest. Finally, third-line, *desirable* improvements consist of the inclusion of (1) simultaneous EEG–fMRI, (2) cerebral blood volume and (3) rate of metabolic oxygen consumption measurements and, when relevant, (4) animal studies investigating signalling between neural cells and blood vessels.

Keywords: Functional magnetic resonance imaging (fMRI); Blood oxygen level-dependent contrast (BOLD); Disease; Pharmacological fMRI; Electroencephalography (EEG); Physiological confounds

1. The trouble with stand-alone BOLD fMRI studies

Blood oxygen level-dependent (BOLD) signal is the most widely used image contrast in functional magnetic resonance imaging (fMRI) of the human brain [1,2]. BOLD-based fMRI has been used extensively in the past 15 years to investigate diseases in the brain [3]. Results from those studies have been often used to suggest pathophysiological mechanisms of disease. More recently, BOLD fMRI has been also used to investigate the pharmacological modulation of brain activity [4], aimed at elucidating mechanisms of drug action.

Although BOLD fMRI does not measure neural activity per se but relies on a cascade of physiological events linking neural activity to the MRI signal, most of the disease and pharmacological studies performed so far have tended to assume that the observed changes in BOLD fMRI signal faithfully represent "brain activation." This brain activation is tacitly assumed to refer to neuronal activity, the component of brain activity that is responsible for information transmission and processing. In most of these studies the complex influences that the disease or the drug under investigation is likely to have on the different physiological events linking neural activity to the MR signal are ignored.

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It is imperative that researchers investigating disease conditions or drugs using BOLD fMRI are aware of the existence of these influences, and of the strategies to identify, characterise and, when possible, correct them.

In this paper, (1) we provide evidence for the necessity of an experimental demonstration of this assumption in both patient and pharmacological studies, and (2) we suggest a series of experimental measures that need to be performed in order to extract meaningful information on brain activity in patient and pharmacological fMRI studies using BOLD as contrast mechanism. We acknowledge that this field is constantly evolving. Some of our suggestions for improving the interpretability of fMRI data in such studies can be implemented now, while others have value and practicality that have yet to be tested.

1.1. The BOLD signal

In an ideal experimental situation, we want to be able to infer changes in neural activity from changes in BOLD signal. However, it is mandatory to keep in mind that BOLD as a measure of neural activity relies upon the intact signalling between neurons or glia and blood vessels, as well as the maintenance of a consistent level of vascular reactivity.

In the context of fMRI, we define neurovascular coupling as the relationship between a change in neuronal activity and the haemodynamic response that is reflected by a BOLD signal change. Any pharmacological or disease-induced modulation of neurovascular coupling will confound our interpretation of BOLD signal changes as neuronal in origin. We now explore in more detail how such a confound may arise.

We consider the change in BOLD signal as arising from a four-stage process, the first three of which could be influenced by the presence of disease or a drug (Fig. 1): (1) neuronal activity, (2) signalling to the blood vessels that control cerebral blood flow (CBF) and (3) vascular reactivity or responsiveness. We regard the final stage, (4) the transduction of modified cerebral physiology into a change in BOLD signal, as a physical process governed by the properties of the MRI scanner and pulse sequence that are obviously unaffected by the presence of disease or drug in the patient. Note that there may, however, be exceptions to this assertion; for example, if additional bulk head motion were induced by the drug or disease the sensitivity to detect a change in BOLD signal would be compromised.

1.1.1. Neural activity

The relationship between BOLD signal and neural activity has been investigated by combining fMRI with electrophysiology in humans and animals [5,6]. In the normally functioning brain, the BOLD signal is an indirect measure of neural activity. Recent investigations have shown that the BOLD contrast appears to reflect most strongly the input and the intracortical synaptic processing of a given brain area rather than its spiking output [6]. However, there remains an ongoing debate over the contributions of different types of neural activity to the BOLD signal, e.g., pre-synaptic vs. spiking activity, not

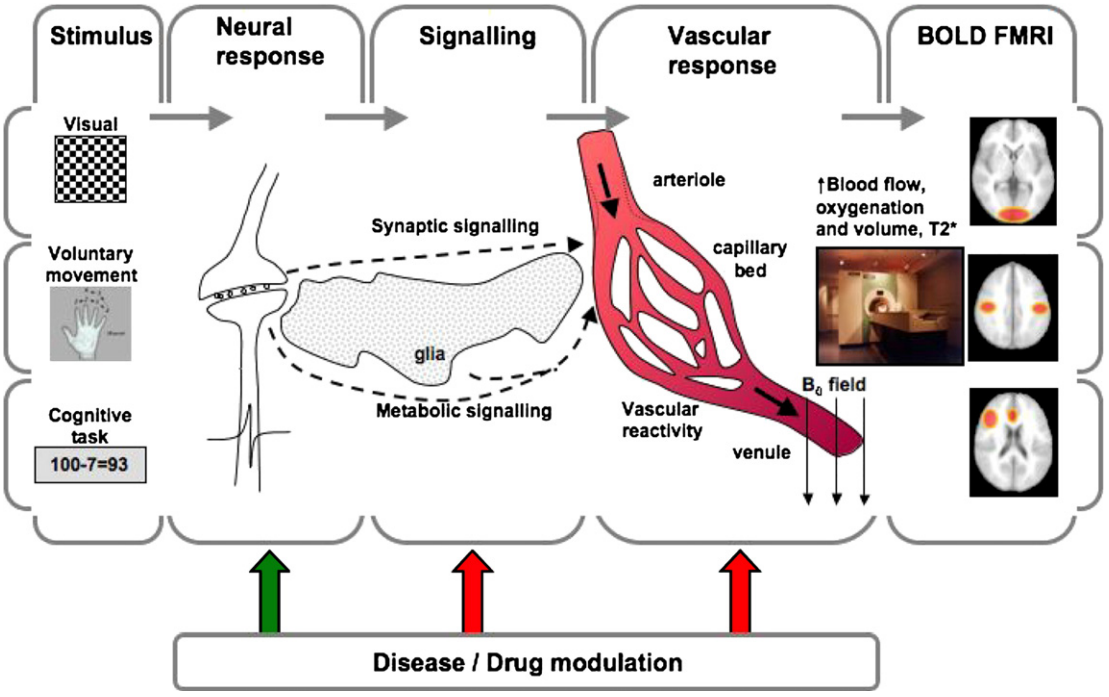

Fig. 1. Schematic illustration of the generation of the blood oxygen level-dependent (BOLD) signal including the stages at which a disease or drug may have an influence. Although BOLD FMRI studies primarily aim at disclosing disease/drug modulation of the neuronal (synaptic and spiking) activity, unwanted effects on the synaptic and metabolic signalling that controls the blood flow responses, as well as on the vascular reactivity and physiological factors that determine the BOLD contrast, can confound the observed results.

least because in many cases presynaptic activity will correlate with spiking activity [7,8]. Fig. 1 illustrates schematically the physiological and physical processes underlying the generation of the BOLD signal.

Already at the level of neural activity we must consider one possible confound in interpreting the possible difference in task-induced BOLD signal increases observed between patients and control or between drug and placebo. This confound consists of disease or drug-induced changes of the baseline neural activity. It has been convincingly demonstrated that the size of BOLD signal increase in response to a task depends on the starting, resting neural activity in the brain before the task is introduced [9]. This is not generally measured in fMRI studies. If the resting, baseline neural activity is modified by the disease or the drug under investigation, this may lead to misinterpretations (either over- or underestimation) of the true modulatory effect of the disease or drug. For example, this confound could be of particular importance in the study of anaesthetic or sedative compounds that are known to modify the baseline energetics [9], or in Alzheimer's disease, where there is evidence of a change in the resting state of the brain [10].

1.1.2. Signalling

An increase in neuronal activity generates a metabolic demand with an increased requirement for nutrient and oxygen supply. The mechanism or combination of biochemical mechanisms that signal to the blood vessels (principally arterioles) to increase CBF remains to some degree unknown. Two principal routes of signalling have been suggested. A local increase in CBF may arise as a consequence of increased local energy consumption or oxygen consumption (metabolic signalling) [11,12]. In the second route, the CBF response is controlled by neurotransmitter signaling [13]. It is likely that several agents work together to produce this CBF increase, and the importance of each agent may differ across brain regions. Candidate chemicals to mediate this signaling include NO, K, vasoactive neurotransmitters and neuropeptides [14]. Modulation by disease or pharmacological agents of the functioning of any of these agents may alter the neurovascular coupling characteristics.

1.1.3. Vascular reactivity

Even if the regulation of CBF remains unaffected by disease or drug administration, they may still modify the reactivity of the cerebral vasculature. Here we refer to "vascular reactivity" as the collected processes resulting in the fMRI-measured haemodynamic response. We use this term to describe all the events between the signalling and the final physiological changes of tissue that give rise to a change in BOLD signal. This includes the local vascular reserve or tone, i.e., the local capacity to modify CBF, the physical properties of the cerebral blood vessels (largely the veins) important in the BOLD signal and the properties of the tissue as they are often described in mathematically formulating a description of the BOLD response, e.g., cerebral blood volume (CBV). We briefly survey the components of this vascular reactivity that are likely to be important.

The amount of deoxyhaemoglobin in any given image voxel, the primary determinant of BOLD signal (see below), is dictated by the venous blood volume, blood flow and blood oxygenation (dependent on arterial oxygenation and oxygen consumption, $CMRO_2$). Pharmacological or disease factors that modify the responsiveness or even the baseline values of these parameters are consequently likely to modify BOLD contrast even in the absence of any modulation of neuronal activity.

Some experiments have investigated the influence of increased baseline CBF through breathing elevated concentrations of CO_2 (hypercapnia) or administration of acetazolamide. Both CO_2 and acetazolamide are vasoactive substances that are thought not to modify $CMRO_2$. While early results have been somewhat conflicting, with some studies suggesting an enhanced BOLD response with increasing baseline CBF [15], more recent studies have led to an emerging consensus that there is a reduced BOLD response to a stimulus with an increase in baseline CBF [16,17]. However, it is likely that the size of this effect will depend on the nature of the stimulus and the brain region under examination.

The CBV is akin to a gain factor in determining the BOLD signal from each image voxel [18]. Any influence of a drug or disease on the local CBV will strongly modify the observed BOLD signal changes. Extending this, the passive or active elastic properties of the venous vessels to modify their volume in response to an activation-induced change in blood flow will also affect the degree of BOLD signal change observed. It is conceivable that many agents or diseases that act on the vasculature could interfere with the biochemical–mechanical properties of vessels.

The effect of baseline blood oxygenation on BOLD response contrast is less well characterised. This could be reduced during respiratory depression, e.g., opioid analgesia, or increased with oxygen therapy in patients. Hypoxia may reduce stimulus-induced BOLD contrast while hyperoxia may increase it [19,20]. BOLD signal can also be subject to systemic physiological influence, for example, anaesthesia-induced reductions in arterial blood pressure [21]. Assuming an intact cerebral autoregulation, reduced blood pressure may increase venous blood volume and reduce BOLD signal. In the case of disrupted autoregulation, oxygen delivery may be additionally impaired, which could itself depress the neuronal activity of interest.

1.1.4. Generation of BOLD signal

The physical principle underlying the BOLD signal change is a decrease in R_2^* (increase in T_2^*), the transverse relaxation rate constant (time) relevant in a typical gradient-echo echo-planar imaging experiment. This process is reviewed comprehensively by Buxton [22].

The increased CBF demanded by the neuronal activity results in a local vasodilation. The resulting fractional

increase in CBF, while coupled to the increased metabolic demand, is typically at least a factor of two larger than the fractional increase in metabolic oxygen consumption [23–25]. This apparent oversupply of oxygen can be explained by the diffusion limitation model [18]. This model assumes that increasing the flux of oxygen across the capillary wall requires an increase in the oxygen gradient from capillaries to mitochondria. It is therefore necessary to raise the partial pressure of capillary oxygen by reducing the fraction of extracted oxygen and this is achieved by a large increase in blood flow. This results in an increased concentration of diamagnetic oxyhaemoglobin and most importantly a decreased quantity of paramagnetic deoxyhaemoglobin on the venous side of the local vasculature. The net result is a decrease in the local spatial differences in magnetic field inside and around the blood vessels (principally veins) producing a reduced intra-voxel dephasing of NMR signal and an increase in T_2* and fMRI signal intensity.

In summary, changes in BOLD signal can faithfully inform us of changes in neural activity *if* and *only if* the intermediate steps in the signal transduction from neurons to the fMRI scanner are not greatly altered (Fig. 1). When the experimental manipulation we are interested in modifies these intermediate steps, any information about experimental modulation of neural activity is confounded (Fig. 2). This potential for confounds exists particularly in disease and pharmacological studies. We now describe some examples of how such confounds may present themselves in a typical disease study or pharmacological study.

1.2. The typical study

Perhaps surprisingly, many papers presenting patient or drug fMRI studies (including some of our own) do not explicitly address these potential confounds in the interpretation of their results. It may well be that such potential confounds are negligible, but they do need to be considered

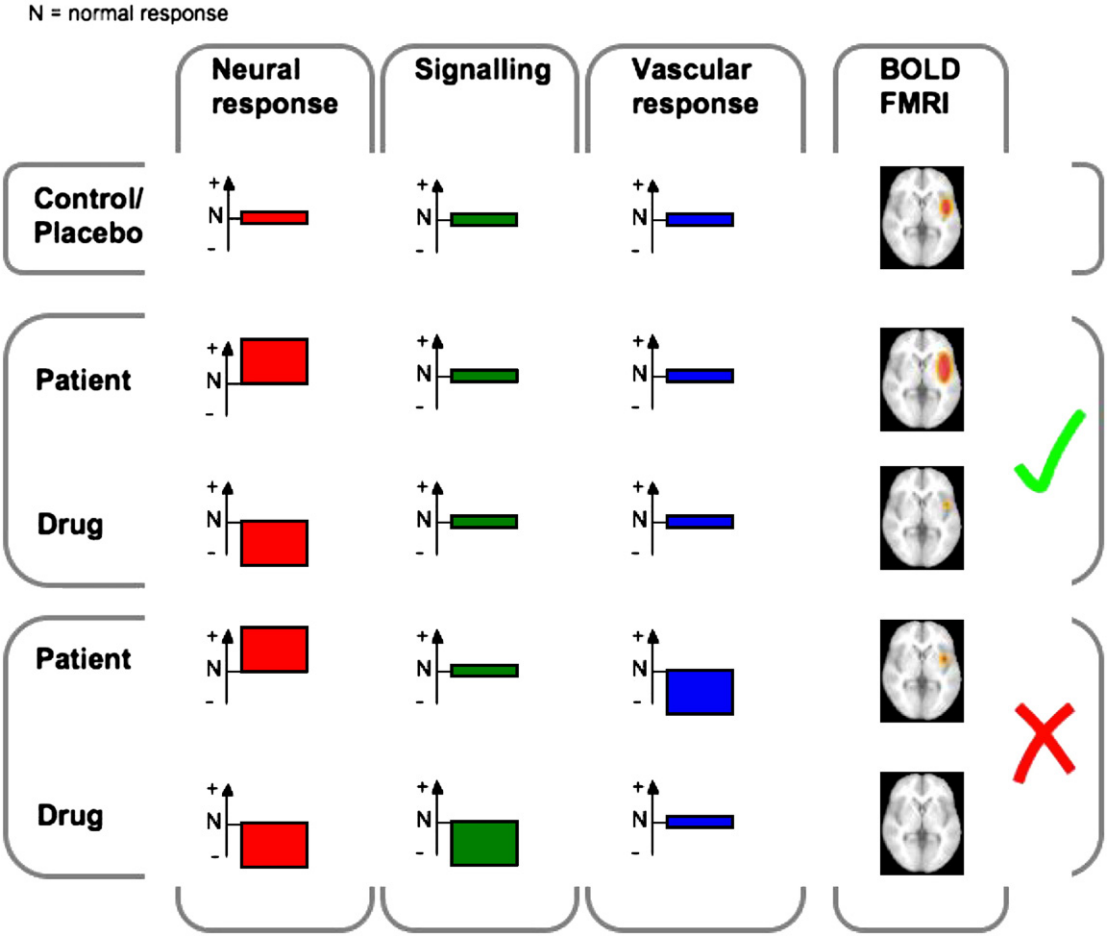

Fig. 2. The effect of potential confounding influences on the generation of BOLD FMRI signal. The schematic graphs illustrate the normal (N), elevated (+) or reduced (−) levels in the processes leading to the measured BOLD FMRI signal. These processes include the neural activity, which is what BOLD FMRI studies primarily aim at disclosing, as well as the confounding factors of the signalling to the vasculature and the vascular responsiveness. The first row (upper panel) illustrates a schematic BOLD FMRI activation map in a control group or under placebo administration. Rows 2 and 3 (middle panel) illustrate the situation in which the change in neural activity induced by disease or drug is "correctly" reflected in the final statistical map. Rows 4 and 5 (lower panel) illustrate situations in which the change in neural activity induced by the disease or drug is "incorrectly" reflected in the final statistical map because of the intervening confounds of a changed neurovascular signalling or vascular responsiveness.

and characterised to allow valid conclusions, especially when the drug or disease under investigation is likely to affect the cascade of signalling and vascular events upon which the BOLD signal relies. Examples of such patient studies include studies on multiple sclerosis [26,27] and stroke [28,29].

The aim of this opinion paper is not to provide an exhaustive criticism of the existing literature and to discredit existing results as they stand at the time of execution of the studies. In contrast, our aim is to encourage the adoption of experimental strategies that will improve the interpretability of future BOLD–fMRI studies.

An example of a pharmacological fMRI study of the nociceptive system in which we have only included a limited battery of investigations includes Wise et al. [30] and Iannetti et al. [31]. While we did discuss the potential confounds of vascular and systemic action of the pain-relieving drugs under investigation, the evidence for a specific effect of these compounds relies upon the combination of the presence of modulation of the BOLD response to noxious stimuli with the lack of modulation of the BOLD response to a visual control task, thus suggesting no global vascular confound induced by the drug. As discussed below, we have now begun to add corroborative studies to this area of investigation which have so far supported our initial results [32].

In the typical disease BOLD fMRI study, such as may be found in the literature, the brain responses to a sensory stimulus, a motor or a cognitive task are collected in a cohort of patients (typical number ranging between 8 and 15) and in group of age-matched controls. Average maps of significant increase of BOLD signal in response to a task of interest (e.g., voluntary movement or sensory stimulation) are generated for each group. These group maps are compared either in a qualitative fashion, or more rigorously using appropriate tests to detect and localise statistically meaningful differences. These statistical maps of differences or regional differences in BOLD signal are usually read by the authors as differences in brain activation between patients and controls, and their interpretation eventually leads to neurophysiological inferences on the effects of the disease or the drug under investigation.

The approach for pharmacological fMRI studies is rather similar. Often a crossover study is performed if healthy volunteers are under investigation. Volunteers undergo scans in which either drug or placebo is administered in separate sessions. The results of these sessions are compared to reveal regional differences in BOLD signal. There have been two differing approaches to pharmacological fMRI studies. The first approach is to seek regional changes in BOLD signal induced by administration of the drug [33] in the absence of a specific task of interest. The second and more commonly adopted approach is to seek the modulation by a drug of specific task-induced activity. The task is usually chosen to be relevant to the expected clinical action or mechanism of the drug. Hence this more targeted approach is often preferred.

The interpretation in neurophysiological terms of the differences between patients and controls or drug and placebo is allowed only when the physiological events happening downstream of the neural activity (i.e., signalling and vascular reactivity) are similar between the groups. This case is represented in the middle panel (Row 2) of Fig. 2, where the larger BOLD signal recorded in the patient group is actually due to an increase in the level of their neural response to the task of interest. Similarly, when signalling and vascular reactivity are not affected by a pharmacological agent that is investigated (e.g., an analgesic agent), the reduction in BOLD signal compared to control/placebo can be correctly interpreted as reduction of neural activity in response to the task of interest (in the same example a noxious somatosensory stimulus) (Fig. 2, middle panel, Row 3).

However, many things can go wrong in this process. It is possible (and sometimes highly likely) that the disease or the drug under investigation changes either signalling or haemodynamic response, or both. For example, if the disease under investigation induces a reduction in the vascular reactivity, inhibiting the haemodynamic response to neural activity, the BOLD signal would not be representative of "brain activation" in the patient group, and an actual disease-induced increase in neural activity would be underestimated or undetected (Fig. 2, lower panel, Row 4). Similarly, if a drug (e.g., an analgesic drug), besides inhibiting the neural response to a task of interest (in the same example a noxious somatosensory stimulus), also reduces the efficiency of the signalling between neurons/glia and blood vessels, the net result will be a reduction of stimulus-induced BOLD signal response compared to placebo that, if the drug effect on the haemodynamic response is not accounted for, will erroneously lead to an overestimation of the effect of the drug on neural activity (in the same example to an overestimation of its analgesic effect) (Fig. 2, lower panel, Row 5). Note that the possible modulations of signalling and vascular reactivity processes can occur in both directions (increased or decreased efficiency).

It is worth noting that, when exploring specific diseases and pharmacological agents, modulation of both signalling and vascular reactivity may be expected.

Diseases that are increasingly investigated using BOLD fMRI, like multiple sclerosis, stroke and brain tumours, are known to change, sometimes dramatically, the baseline CBF and the CBV [34–38]. These changes can influence the stimulus-induced BOLD response discussed earlier, introducing serious confounds that it is imperative to identify, characterise and correct.

Besides the direct confounding effect that a disease can exert on signalling and haemodynamic response, in patient studies other disease-related confounding effects can be present, and their occurrence should be always considered when designing the experiment and interpreting the results. Patients enrolled in BOLD fMRI studies are often undergoing drug treatments which may themselves introduce the

confounds that have been discussed earlier for experimental drug studies in healthy volunteers.

2. Suggestions for improving BOLD fMRI studies

The strategy for improving BOLD fMRI studies should aim to assess the contribution of all the physiological events that relate the BOLD response to the neural activity, as discussed above. It is currently not possible to quantify completely all of these contributions in humans. Here we suggest a series of practical experimental approaches that are promising to characterise and sometimes correct possible confounds, and that we hope will undergo improvement, refinement and replacement with more effective controls over time as fMRI develops.

2.1. Including a control task

In most disease or drug studies the implicit hypothesis on entering an fMRI study is that the disease or drug modulates only specific brain functions. We suggest therefore that a control task, based on this assumption of specificity, must be introduced. The control task should explore the function of a system which is not expected to be modulated by the disease/drug under investigation (e.g., visual stimulation while investigating motor function recovery after stroke, or auditory stimulation or voluntary motor tasks while investigating analgesic drug modulation of pain-related brain responses), in order to assess possible global modulations of signalling and/or vascular reactivity induced by the drug/disease.

In addition, because some signalling/vascular effects can be local (i.e., drugs binding to spatially discrete areas of the brain), a control task that activates brain tissue within the main regions engaged in the task of interest would be even more effective in excluding local signalling and vascular confounds happening downstream of neural activity.

Since control tasks can be easily performed in clinical and simple experimental settings, and these tasks provide already useful information about the meaningfulness of the experiment, this must be included in any experiment investigating disease or drug efficacy in normal subjects. Many of the existing pharmacological studies have included such control tasks (e.g., Ref. [31]).

How should the results of the control task be assessed? The group results of the control task in patients and controls should be statistically compared [39]. It is important to use a liberal threshold for detection of disease/drug effects on the control task in order to avoid underestimating between-groups differences. If there is a significant difference between patients and controls or between drug and placebo, this suggests a more generalised effect of the disease/drug on brain activity or a global vascular confound, for example, systemically mediated. This seriously reduces the ease of interpreting the disease/drug effect on brain activity as being specifically related to the main task of interest. If such a global confound is uncovered it becomes necessary to investigate the potential sources using the approaches described below.

When the result of the control task is not modulated by the disease or the drug (i.e., the control task is "successful"), this suggests a global confound is not present and does help to support a hypothesis about a specific disease/drug effect on the task of interest. However, a "successful" control task should also always be interpreted with caution, because it does not exclude confounds that are spatially restricted or restricted to the particular population of neurons engaged in your main task of interest.

2.2. Assessing the baseline brain perfusion

There is evidence that changing the baseline brain perfusion can modulate the stimulus-induced increase in BOLD signal [16]. Hence, it would be wise to assess perfusion in the brain areas investigated in the disease/drug experiment [40]. Without the use of injected tracers, the most popular MR-based methods for measuring perfusion are known as arterial spin labelling (ASL) [22]. Practical limitations of ASL currently prevent routine perfusion measurement over the whole brain during a single scan, restricting measurement to a slab typically 30 mm in thickness. ASL MRI pulse sequences to assess brain perfusion are becoming more and more available in research centres, and their inclusion in experiments investigating disease or drug efficacy in normal subjects is highly recommended. As well as indicating a potential regional confound in the interpretation of BOLD signal changes, local perfusion changes could yield interesting information by themselves, indicating either a local metabolic effect (neuronal or not neuronal) or a purely vascular effect of the drug or disease under investigation.

How should the results of a brain perfusion measurement be assessed? The perfusion values in patients and controls should be statistically compared on a regional or voxel-wise basis in a similar manner as would be performed for BOLD fMRI. Again, a liberal statistical threshold would avoid erroneously discounting an influence on perfusion. If a difference between groups (for patient studies) or between conditions (in drug studies) is detected, the difference could be included as a regressor in the second-level analysis of the BOLD fMRI study. Namely, the relevant perfusion value would be included in the group analysis as a factor influencing the amount of observed BOLD activity. This approach is rather blunt as it does not address the mechanism by which the change in perfusion could alter the observed stimulus-induced BOLD response of interest. Alternatively, more focussed biophysical models of the effect of changing perfusion baseline could be applied and are currently under investigation.

2.3. Stimulus-induced CBF response

The measurement of CBF (or perfusion) can offer advantages over the BOLD signal when performing fMRI. While CBF measurements generally have a lower

contrast-to-noise ratio than the BOLD signal, there is evidence that the perfusion response to a stimulus-induced change in brain activity is less sensitive to the change in baseline blood flow than is the BOLD signal [17]. At a simple conceptual level this may arise from the fact that the BOLD signal is a complex mixture of changes in CBF, CBV and the rate of metabolic oxygen consumption ($CMRO_2$). MR pulse sequences for the simultaneous measurement of BOLD and CBF are becoming available [41,42], opening the possibility to more routinely measure flow and metabolism changes using a signal modelling approach [24], and overall improving our confidence in the interpretation of neuronal brain activity with fMRI.

CBF-based fMRI also makes it possible to measure changes in perfusion over a longer time scale than BOLD fMRI. The noise in the BOLD signal time course increases greatly at low frequency, thus making studies which aim to track slow changes in brain activity impossible. Perfusion measurements do not suffer this limitation, allowing it to be used to track changes with long-duration experiment variables, like, for example, learning [43].

2.4. Assessing vascular reactivity

Vascular reactivity is here defined as the ability of blood vessels to react with contraction/dilation following the signalling mechanism (Fig. 1). For the purposes of this discussion we do not include the actual signalling process which leads to such a change. Vascular reactivity defined as such can be practically assessed with the administration of specific agents. The most commonly used in neuroimaging have been carbon dioxide (CO_2) and acetazolamide [44–46]. As a practical example, in order to assess vascular reactivity a subject is put into the scanner during the acquisition of BOLD-sensitive images, and he/she is periodically supplied with elevated concentrations (~5% in air) of CO_2 in a block-designed manner. CO_2 inspiration induces a robust vasodilation that in turn increases cerebral perfusion without an increase in cerebral oxygen consumption and therefore the BOLD signal.

Assuming that a respiratory challenge is possible in a group of patients, the same challenge would be performed in the patient and control group. For a drug/placebo study, the CO_2 challenges would be performed once during the placebo session and then again during the drug dosing session [32].

How should we evaluate the results of a vascular reactivity assessment? The BOLD response to CO_2 is statistically compared between patient and control group (for disease studies) or on and off the drug within the same subject (for drug studies) [32]. If a difference between groups (for patient studies) or between conditions (in drug studies) is detected, we suggest that the difference in BOLD signal reactivity is included as a factor in the general linear model and used to linearly scale, within-subject, the BOLD response measurements in the response to the actual experimental task (e.g., voluntary movement or sensory stimulation). This approach is analogous to a procedure of BOLD signal "normalisation" using hypercapnia [47]. Once again, this approach would benefit from refinement to extend it from a simple normalisation to the inclusion of a biophysical model that takes into account the mechanisms by which a measured change in vascular reactivity could influence the neuronally related BOLD response. Besides allowing us to model out possible vascular reactivity effects, the spatial information about the presence (or lack) of altered vascular reactivity is useful as a marker of local drug/disease action.

It is worth noting that, besides the magnitude of the vascular response, its temporal dynamics could be also different in patient populations or during drug administration [48]. In the simplest case this information can be obtained by looking at the peristimulus plot for a short vasodilatory challenge. A more advanced approach would be to deconvolve the haemodynamic response from the BOLD time series [49]. As a consequence of this, it would then be possible to account for the difference in haemodynamic response by using an appropriately modified HRF to analyse the subsequent BOLD activation studies.

2.5. Assessing cerebral blood volume

As discussed earlier, local *cerebral blood (venous) volume* (CBV) strongly influences the degree of local BOLD signal change, with a greater CBV producing greater BOLD signal change. Therefore, although a disease/drug may not modify the reactivity of the vessel walls, a change in resting CBV can modify the degree of BOLD signal change observed. This biophysical effect on the MR signal would be revealed by a CO_2 challenge. A measure of CBV would therefore provide mechanistic information about what might be causing an apparent change in vascular reactivity as measured from the BOLD signal. However, routine measurement of CBV relies on the use of injected contrast agents and may therefore be undesirable as an adjunct to human fMRI studies. Noninvasive alternatives (e.g., VASO, vascular space occupancy) are now becoming available [50].

2.6. Assessing the rate of cerebral oxygen metabolism ($CMRO_2$)

BOLD signal changes result from changes in CBF, blood volume and the local rate of oxygen consumption. As shown in Fig. 1, oxygen metabolism is upstream of the vascular response. Therefore, $CMRO_2$ may be a more faithful, or less confounded, measure of changes in neuronal activity in disease or drug studies than the raw BOLD signal. However, the measurement of $CMRO_2$ is experimentally challenging. The current approach with MR is to calibrate the BOLD signal change using an iso-metabolic CO_2 challenge to increase CBF and to invoke a model to describe the BOLD signal response [23,25] This approach has been demonstrated in a recent study of indomethacin [51], but is likely to require further testing and refinement before it becomes universally

adopted in disease or drug studies not, least because of the assumptions that must be made in the BOLD signal modelling procedure [18] and because of the need for a CO_2 challenge which could prove difficult in patients.

2.7. Neuronal electrophysiology (EEG/MEG)

The recording of EEG or MEG from the scalp allows direct sampling of summated post-synaptic potentials or associated magnetic fields, and it can provide information about the neural processing of the experimental stimulus (e.g., visual-evoked potentials in response to flickering checkerboard). The stimulus to induce a detectable EEG/MEG response should be the same as used in the primary BOLD–fMRI experiment. EEG and MEG responses, either traditional scalp evoked potentials or more advanced time-frequency decomposition, should be analysed and significant differences between patients and control, or between on and off drug, should be disclosed. In addition, source analysis of multi-channel data can be important in relating EEG/MEG sources to regions of activity measured using fMRI.

How should the results of a control EEG/MEG experiment be assessed? If there is a good correspondence between the disease/drug modulation in the two imaging modalities, this supports the hypothesis that the difference disclosed with the BOLD technique has a significant neuronal component. If there is no correspondence between the two modalities (e.g., lack of change in evoked potential amplitude/time-frequency modulation but significant differences in BOLD brain maps), the aforementioned controls for potential vascular or signalling confounds become even more important, and a careful investigation of the cascade of events downstream of neural activity is required.

It is necessary to keep in mind that there is a caveat of any EEG/MEG-BOLD comparison. This caveat is that, even if a good correlation between BOLD and electrophysiological measures has been observed [52], they sample at least partly different aspects of neuronal activity. This is particularly true when the EEG/MEG response is measured as stimulus-evoked potentials or fields (EPs/EFs). When long-lasting stimuli are applied, the EP/EF responses capture only the first part of the neural activity in response to the stimulus, while BOLD integrates neural activity over a much longer time scale [53]. So there is need to be careful about matching the stimuli used to assess BOLD changes and those used in conjunction with electrophysiological measures.

2.8. Simultaneous neuronal electrophysiology (EEG–fMRI)

Recent technical advances have made it possible to collect good quality data during truly simultaneous BOLD-fMRI [54,55]. This has the potential to improve the comparison between electrophysiological measures of brain activity and the BOLD haemodynamic response. However, it is important to understand clearly when this rather complex setup is really necessary. We believe that simultaneous EEG–fMRI, is really useful and provides physiological information, otherwise impossible to obtain, only in the following selected cases.

1. In *epilepsy*, where abnormal spiking activity occurs spontaneously and unpredictably, the simultaneous collection of EEG data is crucial to define when the abnormal events occurred and to analyse the MRI time series accordingly [56,57].
2. In the evaluation of *ongoing brain rhythms* (including *sleep*), where increases/decreases in power at different frequency occur in an unpredictable fashion, the simultaneous collection of EEG data is crucial to define when these modulations occurred as well as their size, to inform the analysis of the MRI time series [58,59].
3. In the evaluation of stimulus-induced *brain activity that is not stationary* the availability of simultaneously collected measures permits the assessment of physiologically meaningful between-trial variations of electrophysiological responses and their relationship with other parameters (e.g., stimulus intensity, psychophysical and fMRI responses), thus increasing the power of statistical analysis and allowing within-subject comparisons [60].
4. In the evaluation of brain activity in *neuropsychological experiments*, where the experimental design introduces time-dependent effects such as habituation or learning.
5. In the evaluation of *drug effects*, when an important within-subject between-sessions variation in the response to the drug is expected, the simultaneous collection of EEG and fMRI data would eliminate the variance in the drug effect introduced by multiple sessions. As an example, the potential development of tolerance to opioids could make the comparison of EEG and fMRI brain responses recorded in separate sessions difficult.
6. In selected cases where a *large effect of the scanner environment* on patient or volunteer behaviour has been demonstrated. For example, the effect of psychoactive drugs (e.g., ketamine) is rather different in the noisy and potentially more stressful scanner environment compared to the tranquillity of an EEG recording room [61]. This issue is particularly relevant for patients whose brain responses in the scanner may be much more sensitive to those factors.

2.9. Monitoring and correcting for physiological parameters

Changes in systemic physiological parameters (e.g., heart rate, breathing rate) can influence the fMRI signal both globally and locally. These have the potential to introduce additional “physiological noise” into the fMRI experiment, reducing the significance of stimulus-induced BOLD signal

changes. Physiological noise arises in part from breathing-induced and cardiac pulsation-induced motion [62] and changes in arterial carbon dioxide tension [63,64]. Furthermore, easily measurable physiological parameters such as heart rate, breathing rate, arterial oxygen saturation (measured peripherally, SpO_2) and systemic blood pressure may modulate these noise sources and exert their own direct or indirect influence on the BOLD signal.

It is possible to correct for a large part of physiological noise in a BOLD fMRI time series using a technique which removes, after a process of linear regression, the component of those signal fluctuations that correlate with the physiological processes. The approach which has gained the greatest popularity to perform this correction is known as RETROICOR [65]. In its typical form, this accounts for breathing- and cardiac-related noise. However, the inclusion of the other easily measurable parameters to a linear regression analysis at the single-subject level (or group level for differences between patients and controls or placebo/drug) could help to account for such non-neuronal influences on the BOLD signal.

2.10. Animal studies

Disease or drug influences on neurovascular signalling are the most difficult to assess in humans. The need to probe signalling systems pharmacologically restricts in most cases their investigation to animals.

For a class of compounds or for each specific disease area under investigation, it could become necessary to establish a strategy of complementary animal studies to investigate influences of that disease or drug on the signalling eventually leading to BOLD response, particularly in the event that the other investigations discussed earlier indicate a modulation of the signalling process.

3. Recommendation

In this final section, we aim to give a practical "guide" to make the results of patient and drug BOLD studies more reliable and interpretable. We have considered the balance between the importance of the information yield by the additional experiments and the current practical feasibility of implementing them. These recommendations are summarised in Fig. 3 and divided into three broad categories: *necessary*, *strongly recommended* and *desirable*.

The first line of improvements that we consider *necessary* to draw any conclusion from a patient or drug BOLD study consists of the inclusion of (1) one or more control tasks, and (2) the recording of physiological parameters during scanning and the subsequent correction of possible differences between patient and control group (for disease studies) or between on and off the drug within the same subject (for drug studies). These are first-line recommendations largely because their easy implementation and the usefulness of information that they can yield in revealing global confounds, thus helping the correct interpretation of the study results.

The second line of improvements that we *highly recommend* to make the results of a patient or drug BOLD study more interpretable includes (1) the assessment of baseline brain perfusion, (2) the assessment of vascular reactivity, (3) the inclusion of perfusion stimulus-related

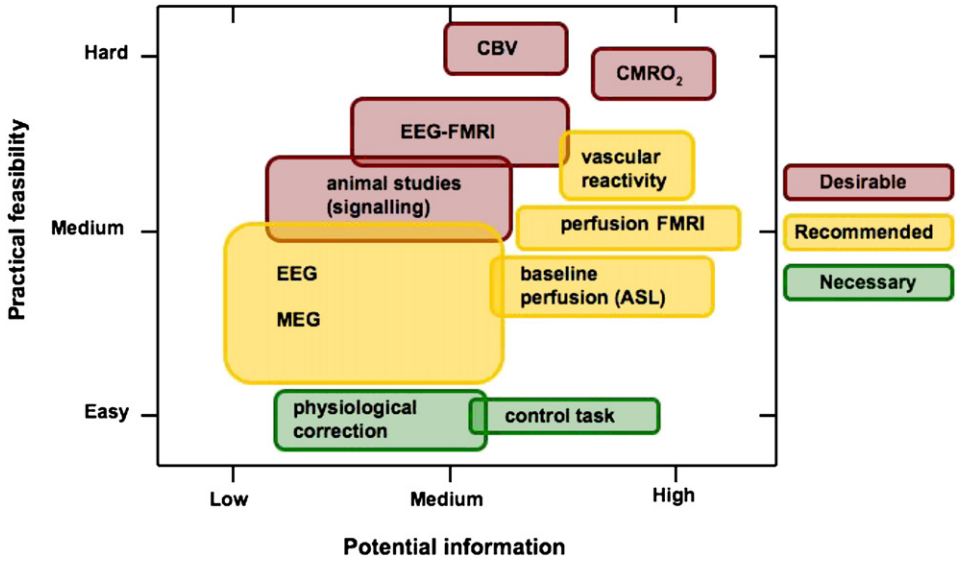

Fig. 3. Comparison of experimental measures for improving the investigation of the sources of modified BOLD signal change in disease/drug studies. The *x*-axis indicates the potential usefulness of the information provided by the experimental measure. The *y*-axis indicates the current practical ease of implementation of each technique. Note that the positioning of each technique both along the *information* and *feasibility* axes will depend on the design of the proposed FMRI study and the cohort of volunteers or patients to be examined. The *necessary* investigations are those which can be easily carried out with little modification in a typical FMRI study. The *recommended* studies offer additional information largely to attempt to disentangle potential vascular confounds. The *desirable* studies are experimentally more demanding but have high potential for avoiding or quantifying a signalling or vascular confound.

fMRI and (4) the recording of electrophysiological responses to the stimulus of interest. These are second-line recommendations because these additional experiments require more experimental effort than first-line improvements and are more specific in the information that they may yield (i.e., they aim at elucidating the potential vascular mechanisms of a confounding influence).

As a third line of improvement, we suggest adding (1) a simultaneous EEG/fMRI recording, (2) CBV and (3) $CMRO_2$ measurements and, when relevant, (4) animal studies specifically investigating the signalling steps. These additional experiments are extremely *desirable* and can potentially yield a lot of information, but they imply highly complex and not-standardised experimental setups.

Lastly, it is important to keep in mind that the perfect list of corroborative experiments does not exist. For each disease or drug investigated using BOLD fMRI, researchers should define the best strategy to identify, characterise and correct possible confounds, by carefully considering all the possible issues related to the specific disease or drug under investigation.

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