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Interpreting BOLD: towards a dialogue between cognitive and cellular neuroscience

Catherine N. Hall[1,†], Clare Howarth[2,†], Zebulun Kurth-Nelson[3,†] and Anusha Mishra[4,†]

[1]School of Psychology, University of Sussex, Brighton, UK
[2]Department of Psychology, University of Sheffield, Sheffield, UK
[3]Max Planck UCL Centre for Computational Psychiatry and Ageing Research, University College London, London, UK
[4]Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK

CNH, 0000-0002-2316-7714

Cognitive neuroscience depends on the use of blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) to probe brain function. Although commonly used as a surrogate measure of neuronal activity, BOLD signals actually reflect changes in brain blood oxygenation. Understanding the mechanisms linking neuronal activity to vascular perfusion is, therefore, critical in interpreting BOLD. Advances in cellular neuroscience demonstrating differences in this neurovascular relationship in different brain regions, conditions or pathologies are often not accounted for when interpreting BOLD. Meanwhile, within cognitive neuroscience, the increasing use of high magnetic field strengths and the development of model-based tasks and analyses have broadened the capability of BOLD signals to inform us about the underlying neuronal activity, but these methods are less well understood by cellular neuroscientists. In 2016, a Royal Society Theo Murphy Meeting brought scientists from the two communities together to discuss these issues. Here, we consolidate the main conclusions arising from that meeting. We discuss areas of consensus about what BOLD fMRI can tell us about underlying neuronal activity, and how advanced modelling techniques have improved our ability to use and interpret BOLD. We also highlight areas of controversy in understanding BOLD and suggest research directions required to resolve these issues.

This article is part of the themed issue 'Interpreting BOLD: a dialogue between cognitive and cellular neuroscience'.

Introduction

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Authors for correspondence:
Catherine N. Hall
e-mail: catherine.hall@sussex.ac.uk
Clare Howarth
e-mail: c.howarth@sheffield.ac.uk

[†]These authors contributed equally to this study.

1. What do we know about BOLD?

(a) An increase in the positive BOLD signal in adults generally represents a net increase in neuronal activity

This relationship between the BOLD signal and neuronal activity is extensively discussed in previous reviews [1–2] and other papers in this issue [3–5]. Box 1 provides an introduction to the origin of the BOLD signal.

(b) Neurovascular coupling usually links neuronal activity to increases in blood vessel diameter

Release of neurotransmitters such as glutamate induces dilation of cerebral blood vessels by triggering the release of vasoactive signalling molecules from both neurons and glia (LeCrux and Hamel, and Uhlirova *et al.* in this issue; [3,4,7]). Much is now known about these signalling pathways, but their variability across brain areas and circuits is likely to lead to differences in neurovascular coupling properties and therefore also to variations in the relationship between BOLD and neuronal activity across different brain regions and experimental conditions (discussed below).

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Box 1. What is the BOLD signal?

The brain has only limited storage for the energy substrates oxygen and glucose. Although the extra energy use associated with cognitive tasks utilized for BOLD studies is typically less than 10% [6], when there is an increase in energy demand by active neurons, a local increase in blood flow is required to supply the extra glucose and oxygen required. This is achieved through neurovascular coupling. Work over the past two decades has attempted to define the signalling mechanisms that give rise to neurovascular coupling (see the main text, Lecrux and Hamel [3] in this issue and [7]), which is exploited by the BOLD fMRI technique to visualize neuronal activation. BOLD contrast is dependent on the level of deoxyhaemoglobin in the blood [8]. The iron in deoxyhaemoglobin is paramagnetic. This causes inhomogeneities in the magnetic field, which dephase proton 'spins' and thus decrease the MR signal. When neural activity increases, oxygen extraction from the blood increases, resulting in an increase in the amount of deoxyhaemoglobin in the blood and a faster dephasing. This effect alone would decrease the MR signal. However, within a few seconds, neurovascular signals increase blood flow and volume, bringing in more oxygenated blood and washing out deoxyhaemoglobin. Thus, there is a net decrease in deoxyhaemoglobin, resulting in a more homogeneous magnetic field in which transverse relaxation occurs more slowly and the BOLD fMRI signal increases. Hence, BOLD signals reflect the net increase in blood oxygenation following neural activity, and represent the sum of the effects of oxygen consumption (which decreases BOLD) and blood flow increase (which increases BOLD). The resulting functional imaging signal is assumed to reflect neuronal activity. As these two effects of neural activity are in opposite directions, however, alterations in signalling between the brain and the vasculature can potentially seriously confound BOLD interpretation. For example, if neural activity fails to increase blood flow, BOLD could decrease instead of increase. Indeed, during development, there is an extended period when the effect of the increase in energy expenditure outweighs the effect of any increase in blood flow, and the BOLD signal is negative [9]. Such potential confounds of the BOLD signal are discussed further in the main text and in Lake *et al.* [10] in this issue.

(c) BOLD is a better indicator of processing within an area than the output of the area

Where synaptic activity and action potentials do not correlate, BOLD signals better reflect synaptic activity. This concept has been demonstrated elegantly by studies in both anaesthetized and awake primate cortex [11,12] and anaesthetized rat cerebellum [13,14] and is highlighted in the articles by Lohrenz *et al.* [15], and Freeman & Li [16] in this issue. Lohrenz *et al.* report that striatal dopamine release, which reflects prediction error, can become dissociated from BOLD signals under certain conditions. Presumably, such dissociations occur when competing inputs (e.g. the glutamatergic input from prefrontal cortex), rather than dopamine release alone, drive striatal synaptic activity and energy use. Energy budgets of both the cerebral cortex and cerebellum imply that synaptic activity consumes more ATP and oxygen than action potentials [17,18], suggesting an evolutionary drive for vascular responses to be more closely correlated with local synaptic activity than with spiking output. In this issue, Freeman & Li [16] further illustrate this correlation using binocular interaction in the visual cortex *in vivo*. O_2 usage is strongly correlated with both synaptic input and spiking output during excitatory binocular facilitation, whereas during inhibitory binocular suppression, O_2 levels only reflect synaptic input and are dissociated from the spiking output. This is probably because the output no longer correlates with the degree of synaptic activation, and therefore with net O_2 use.

(d) BOLD can be used to glean robust information about functional localization within the brain

This is true, in particular, when spatially localized groups of neurons are concurrently active (e.g. sensory cortical maps; [19] and Knutsen *et al.* [20] in this issue), and the testing conditions can be well controlled. Increases in magnetic strength further allow increases in the spatial resolution of these maps (discussed below and in papers by Turner [5] and Ugurbil [21] in this issue).

(e) Early BOLD signals are more reliable reporters of neuronal activity than are late BOLD signals

Vascular responses propagate outward from their site of initiation [22,23], which means that late BOLD responses are less spatially localized to the initial site of neuronal activity than the first few seconds of the positive BOLD response (discussed below, and by Uhlirova *et al.* [4] in this issue and in [24]).

2. Cellular mechanisms underlying BOLD: how our incomplete understanding limits our ability to interpret BOLD fMRI

That increased neuronal activity leads to increased blood flow has been known for over 100 years [25], but the relative contribution of different parenchymal and vascular cells, and of different synapses, to neurovascular coupling remains an area of active research (see [7] and [26] for recent summaries).

(a) Synaptic activity is linked to cerebral blood flow via glutamate release

As the majority of neurons within the cortex are excitatory [27] and glutamate has been known for a long time to evoke vascular dilation [28], neurovascular coupling research has primarily focused on glutamate-evoked blood flow changes. This glutamate-mediated neurovascular coupling neatly links the most energetically expensive component of synaptic transmission, ion flux through post-synaptic glutamate receptors [17,29,30], with increased energy supply. Glutamate evokes vascular responses by stimulating neurons and astrocytes to produce vasodilatory second messengers and by raising the extracellular K+ concentration, as has been comprehensively reviewed (Uhlirova *et al.* [4] in this issue; [7]). In summary, glutamate-evoked calcium signals

in excitatory and inhibitory neurons can dilate blood vessels via production of nitric oxide (NO) [31], prostaglandins [32] and vasoactive peptides [33]. Neuronal activity has also been shown to stimulate astrocytes to release K^+ [34] and vasodilatory arachidonic acid metabolites such as prostaglandin E_2 and epoxyeicosatrienoic acids [34–38] to dilate vessels (reviewed in [7]). However, the extent to which astrocytes contribute to functional hyperaemia *in vivo* remains open to debate [39–42]. Rather than triggering a rapid vascular response, synaptic activity-evoked astrocyte calcium increases have been proposed to mediate sustained vascular responses to prolonged stimulation [40,43,44]. Furthermore, astrocytes also appear to play a role in maintaining the resting tone of the vasculature [45–47], which could affect the size of the blood flow response following neuronal activity [48].

(b) Vascular mediators of neurovascular coupling—smooth muscle cells and pericytes

Contractile cells on blood vessel walls respond to neuron or astrocyte-derived second messengers by contracting or relaxing, thus constricting or dilating vessels and altering cerebral blood flow (CBF). Classically blood flow was thought to be regulated solely by rings of smooth muscle on penetrating arterioles, but recent work has suggested that capillary blood flow can also be regulated by virtue of the contraction and relaxation of pericytes [49,50]. While these findings are somewhat disputed [51,52], this is primarily due to differences in the definitions of capillaries, arterioles, smooth muscle cells and pericytes (see [53] and Uhlirova *et al.* [4] in this issue). In fact, the studies broadly agree that cerebral blood vessels from large to small calibre (down to 4 µm) and even up to four branches from diving arterioles can be regulated by neuronal activity [50,52] due to the relaxation of vascular mural cells which express contractile proteins, including those that have a 'bump on a log' morphology and processes extending along and around the blood vessels [52,54]. Current evidence suggests that pericytes on capillary regions at branching orders more than four from penetrating arterioles are less contractile and therefore less likely to actively contribute to neurovascular coupling ([52]; Uhlirova *et al.* [4] in this issue).

Capillary-level blood flow regulation explains how individual cortical columns can be mapped from the haemodynamic response (e.g. whisker barrels [55]), despite the territory fed by individual arterioles not mapping onto these columns [56]. Regulation of blood flow at the capillary level therefore probably sets the spatial resolution of the BOLD signal [57], at least at early time points after stimulation (see below).

(c) Upstream propagation of vasodilation

It is now well established that in the brain vascular responses propagate outward from their site of initiation [23], similar to what has been observed in the periphery [22]. The cells propagating this signal may vary, depending on the strength of stimulation, with evidence supporting signal propagation along both the vascular endothelium [58] and through the astrocytic syncytium [59]. This means that, in the case of dilation, the positive BOLD signal spreads over an increasingly larger volume of tissue over time and therefore provides progressively less information as to the initiation site and the neurons that were originally activated. This has been demonstrated using intrinsic optical imaging (IOS) of haemodynamic changes in rodent barrel cortex [55], which correlate with the positive BOLD response (Knutsen *et al.* [20] in this issue), and using *in vivo* two-photon imaging of individual blood vessels in cat visual cortex [60]. In the first study, IOS changes could discriminate between two independently stimulated whiskers 1.5 s after stimulation onset, but not after 4 s, suggesting a spread of dilation to neighbouring regions over time. In the second study [60], individual arterioles dilated not only to stimuli that activated the cortical column surrounding the arteriole, but also to stimuli that activated adjacent cortical columns, despite a lack of neuronal activity in the area immediately surrounding the blood vessel: i.e. the vascular response occurred over a larger tissue volume than the neuronal response. Thus, uncoupling between neuronal and vascular responses exists at the level of cortical columns. Furthermore, pial vessels responded to a wider range of stimuli than diving vessels, presumably due to summation of dilations from several diving vessels fed by the same surface artery [60]. Propagation of vascular dilation away from the site of neuronal activity therefore gradually reduces the spatial resolution of the BOLD signal, which is greatest in the capillary bed shortly after stimulus onset.

(d) Central neuromodulatory pathways

The cerebral vasculature is innervated by nerve terminals from subcortical regions that can release vasoactive neurotransmitters such as acetylcholine (from the basal ganglia), serotonin, dopamine (from the ventral tegmental area) and noradrenaline (from the locus coeruleus) [61–64]. Changes in emotional and arousal states can alter the release of these neurotransmitters, potentially resulting in altered vascular tone independent of a change in synaptic activity [65]. Furthermore, the release of neurotransmitters from monoaminergic and cholinergic subcortical afferents can evoke both a neural and CBF response concurrently. For instance, the release of acetylcholine from afferents originating in the basal forebrain [66,67] alters cortical neuronal activity, but also concurrently evokes increases in CBF [68]. The vascular response appears to be partly mediated by local somatostatin- or neuropeptide Y-positive or GABAergic interneurons [69] and/or astrocytes [67]. Similarly, vasoconstrictions evoked by noradrenaline released by locus coeruleus neurons are thought to be important in optimizing neurovascular coupling [70], but direct effects of this pathway on both excitatory and inhibitory cortical neurons resulting in increases in cortical CBF have also been demonstrated [71]. It is not yet established whether the subcortically-evoked changes in cortical activity and blood flow always occur in the same direction—does neurotransmitter release from these afferents always increase both neuronal activity and blood flow, or do the neuronal and vascular responses sometimes uncouple? Even when they respond in the same direction, is the relationship between neuronal activity and the vascular response causal or correlative? If it is purely correlative, e.g. due to parallel signalling from the subcortical region to cortical neurons and to the vascular cells, then the relationship may fall apart in pathological conditions or during different arousal states, thus complicating our interpretation of BOLD.

(e) How does this limit our interpretation of BOLD?

As so many different cell types and second messenger pathways are involved in neurovascular coupling, it should come as no surprise that the properties of neurovascular coupling vary across brain areas, developmental stages and experimental conditions. Direct evidence for regional differences in the relationship between neuronal activity and BOLD has been demonstrated in rodents by simultaneously recording electrical and vascular activity. For example, increased neuronal activity during seizures is associated with positive BOLD and increases in CBF in the cortex, but negative BOLD and decreased CBF in subcortical structures such as the striatum, despite equivalent LFP sizes [72]. The mechanisms for such variation are unclear, but are likely to involve differences in both signalling pathways and vascular anatomy [73]. For example, the involvement of NO varies between brain areas, both in terms of the magnitude of its contribution and whether it acts to mediate or modulate neurovascular coupling (as discussed in [74]). There also appear to be regional differences in the involvement of metabotropic glutamate receptor (mGluR) subtypes, as the mGluR5 antagonist MPEP decreases stimulation-evoked BOLD responses more in rat subcortical regions than in the neocortex [75]. Furthermore, many enzymes involved in neurovascular coupling are developmentally regulated [9], and neuronal activity elicits vascular constriction, and CBF decreases [76–78] corresponding to negative BOLD responses in infants [79,80]. The mechanisms underlying neurovascular coupling in a given situation will vary depending on expression patterns of key enzymes and receptors across different brain areas, as well as the local and global circuit dynamics engaged by a given task. For example, different subtypes of inhibitory interneurons presumably alter neurovascular coupling not only by the release of different vasoactive molecules, but also by regulating the activity of the local network (Lecrux and Hamel [3] in this issue; [74]), affecting glutamate release and second messenger production in excitatory neurons and astrocytes. As discussed above, the activity of these circuits and the reactivity of blood vessels may also be affected by global brain state as expressed in different levels of monoaminergic and cholinergic tone.

In short then, variability in neurovascular coupling mechanisms means that the BOLD signal evoked by a given net increase in neuronal activity not only could vary in magnitude across different experimental conditions, ages, brain areas and brain states, but also could reflect different balances of circuit activity. How all these factors interact to generate the BOLD signal from changes in underlying neuronal activity has only been well characterized during primary sensory processing, mostly in anaesthetized animals. Much further work is required to understand exactly how the neurovascular coupling relationship varies across the brain and therefore what exactly BOLD can inform us about underlying patterns of neuronal activity.

(f) Changes in neurovascular coupling and MRI signals due to disease processes

In a number of neurological conditions, including stroke, glioma, subarachnoid haemorrhage, cortical spreading depolarizations, brain injury, hypertension and neurodegenerative diseases such as Alzheimer's and Parkinson's disease, neuro-glio-vascular coupling may be altered or dysfunctional [81–84], altering the relationship between neuronal activity and changes in blood oxygenation. In this case, a decreased BOLD response to a task (when compared with healthy brain) may reflect a decrease in neural response, a decrease in neurovascular coupling, or both.

Neurodegenerative diseases may affect BOLD responses in various ways. There may be altered brain levels of neuromodulators/vasoactive molecules in the brain (e.g. the changes in dopamine levels that occur in Parkinson's disease) which could either directly impact BOLD signals or modulate the cellular and molecular mechanisms underlying neurovascular coupling. Reactive astrogliosis, which occurs in almost all neurological diseases [85,86], is characterized by changes in the morphology and protein expression patterns of astrocytes [87,88]. In particular, there are deficits in proteins involved in calcium dynamics, neurotransmitter sensing and K^+ buffering, all of which are important in neurovascular signalling, and dysfunction in astrocyte-mediated neurovascular coupling in disease has been reported [89].

Pathological changes affecting BOLD responses are not restricted to changes in neurovascular coupling. Pathology can result in changes to magnetic resonance parameters, for example oedema and changes in diffusion, which will affect the number of water molecules and hence protons in the region of interest [90,91], can generate artefacts or, when interpreted correctly, could serve as an important biomarker (Lake *et al.* [10] in this issue). Furthermore, in the case of cerebrovascular pathology, the BOLD response may be altered in the absence of neuronal dysfunction, such as after acute injury, when a complete remapping of the vascular response may occur (see Lake *et al.* [10] in this issue). Our lack of knowledge of how neurovascular coupling is altered in disease and pathology impacts our ability to accurately interpret BOLD data.

(g) Regional differences in BOLD signal magnitude

To further complicate interpretation of BOLD signals, regional differences in the size of BOLD responses have been observed in different brain areas. In the hippocampus, for example, the relative difficulty in observing task-related activations may be due to cognitive factors (i.e. greater hippocampal activation at rest), vascular factors (a decreased capillary density in some subregions compared with neocortex) or altered patterns of neurovascular coupling ([73] and discussed above). Notably, human neocortical BOLD signals are expected to vary by as much as 40% across different cortical regions, simply due to the varying orientation of the vasculature relative to the magnetic field in the highly folded cortical structure ([92], further refined by Gagnon *et al.* [93] in this issue). It will be important for future experiments to further investigate the regional variations in signal magnitudes and the underlying reasons for these, and to build these findings into fMRI analysis methods.

3. How can advances in fMRI/BOLD experimental design and analysis strengthen inferences of neuronal activity from BOLD signals?

(a) Model-based analyses

BOLD generally reflects neuronal activity and is reasonably co-localized with the site of neuronal activation. Based on this understanding, early studies used BOLD to identify brain

areas or networks where activity is modulated during a particular function [94,95]. Reassuringly, there is an extremely high correspondence between response properties measured in fMRI and those measured from invasive electrical recordings or from homologous brain areas in non-human primates, for a very wide range of properties including visual object selectivity, orientation tuning, motor somatotopy and many others. fMRI has thus enabled a very detailed study of functional specialization, including those more difficult to study in non-human animals such as emotion, psychiatric disorders, abstract reasoning, autobiographical memory, meta-cognition, complex social cognition, language and consciousness [96]. However, such research concerning functional specialization failed to address questions of mechanism.

A key advance in going beyond early designs was to postulate formal models of brain computation, and test whether properties of these models are encoded in the brain (Lohrenz *et al.* [15] in this issue; [97,98]). By revealing *where* these quantities are encoded, we additionally gain linkages to other levels of neuroscience. For example, if a reward prediction error is sent from the midbrain to the striatum, we can form hypotheses about how plasticity at synapses in the striatum should work. As more aspects of the model can be linked to plausible neural computational mechanisms, and competing models eliminated, we can conclude whether our model is an accurate explanation of how the brain computes.

Many experiments in model-based neuroimaging have used psychological models with very few parameters that incorporate particular structural hypotheses. A recent, more open-ended approach is to use models with many parameters that are learned from large amounts of data, perhaps in a manner analogous to the brain. In particular, the success of deep neural networks in many engineering problems [99] has generated interest in these networks as models of the brain (Kriegeskorte *et al.* [100] in this issue). For example, deep neural networks trained on natural images can explain cell firing properties in monkey inferotemporal cortex better than any other existing models [101]. These methods are now beginning to be applied to fMRI data [102,103].

(b) Multivariate analyses

In a similar vein, there has been growing use of multivariate methods (Turner [5] in this issue), which trade spatial specificity for increased sensitivity, by looking for a signal in the pattern of activation across multiple voxels. These methods allow precise identification of *what* is encoded, in addition to some (albeit reduced) information about where it is encoded. By giving up spatial specificity, a resulting benefit is a minimized impact of co-registration errors between the unique brains of each participant. Unlike univariate methods, multivariate methods have the potential to capture information that exists only in the relationships between different voxels. This is important given that distributed representations are probably a central feature of information encoding by the brain.

A popular variety of multivariate analysis, called representational similarity analysis (RSA), goes a step further in abstraction by disregarding information about what particular patterns over voxels actually encode the signal of interest, and only considering the similarities between different patterns [104]. Thus, neural recordings made in different modalities can be analysed in a common space, for example to combine the spatial resolution of fMRI with the temporal precision of magnetoencephalography (MEG) [105]. Neural recordings can even be analysed in a common space with non-neuronal sources of information, such as subjective reports [106] or the output of the deep neural networks mentioned above. RSA is particularly well suited to be combined with model-based approaches like deep neural networks, which have many free parameters. Although the neural networks are too complex to map directly onto brain imaging data, the similarity structure of the neural network output can be compared directly with the similarity structure of BOLD data.

(c) Towards insulating cognitive interpretations from the physiological details of neurovascular coupling

The use of model-based neuroimaging and multivariate analyses enables our conclusions to be quite robust against the details of neurovascular coupling. For example, a trained neural network closely matches the similarity structure of BOLD recorded in some parts of the brain. From this, we may conclude that the brain is likely to compute in a similar way to this neural network. Crucially, this conclusion does not depend on whether BOLD arises from spiking or local field potentials. Effectively, methods whose interesting mechanics are at a more abstract level protect us, to a greater or lesser degree, from the underlying physiology compared with older analysis methods. For example, if multivoxel pattern analysis (MVPA) can discriminate between two remembered stimuli during memory retrieval based on the patterns of BOLD activation during encoding, we can be relatively confident that these patterns of activation relate to the neural signature rather than changes in vascular reactivity. It is worth noting that use of these analyses may minimize the impact of variations in neurovascular coupling created by disease states on the generation of false positives, but diseases could still increase the false negative rate (for example, by reducing the ability of neurons to activate a vascular response).

(d) fMRI adaptation

In contrast to multivariate methods that probe the encoding of stimuli or model quantities across multiple voxels, fMRI adaptation can reveal encoding at a within-voxel level. When a stimulus is shown twice in succession, the BOLD response to the second presentation is often decreased compared with the first presentation. Such a decrease can also be observed if the second stimulus is related but not identical to the first stimulus, which is called cross-stimulus suppression. This effect is thought to arise at least in part from the diminished response of individual neurons to the second stimulus (Barron *et al.* [107] in this issue).

This is particularly important because multivariate methods depend on a heterogeneous distribution of neuronal response properties across voxels. If every voxel contains 50 cells encoding stimulus A and 50 cells encoding stimulus B, it will be difficult to decode A versus B even using the most sensitive multivariate analysis [108]. fMRI adaptation bypasses this problem by relying on the sensitivity of individual neurons to repeated activation (Barron *et al.* [107] in this issue), and has yielded a wealth of new insights in cognitive neuroscience [109–112].

Interestingly, although fMRI adaptation aims to address similar questions of fine-grained representation as MVPA,

cognitive interpretations of adaptation are much more likely to be affected by physiological variation in neurovascular coupling. The underlying mechanism driving the adaptation is still not fully understood (as discussed by Barron *et al.* [107] in this issue) and it is unknown how neurovascular coupling is affected by repeated stimulus presentation. The relationship between neuronal and vascular responses could be impacted by neuromodulators or other factors, which might produce false positives. Any such influences might also be heterogeneous across the brain. This is an important area for further study.

(e) High field strength

Meanwhile, developments on the hardware side have increased both imaging resolution and our confidence in the results, by enabling single subject analyses. As discussed by Turner [5] and Ugurbil [21] in this issue, high field MRI (7 T and above) has many advantages over the lower field strengths which have predominated to date. High field MRI provides improved signal to noise, which can be traded for temporal or spatial resolution. The resulting smaller voxel sizes allow the resolution of structural details that were not previously visible, and the observed cortical microstructure can allow identification of different brain regions in each subject's brain. The ability to compare structure with function in individual subjects eliminates the need for both spatial smoothing of BOLD signals and comparison with standardized brains, allowing finer details of functional organization to be resolved. Higher field strength favours detection of signals from capillaries [5,21] (in this issue). In combination with improved spatial resolution, this has enabled detailed imaging of cortical layers [113–115] as studies are now more able to detect the local, capillary-level vascular changes that better map onto sites of neuronal activity. As discussed above, however, propagating dilation from the capillary bed can reduce this spatial resolution over time.

(f) Functional connectivity and resting state fMRI

fMRI has facilitated, both in humans and animals, the study of functional interactions between distant brain areas [116–118]), something that was challenging to achieve with unit recordings. Initially, in studies focusing on functionally-evoked neural activity, spontaneous fluctuations observed in fMRI signals were regarded as noise (Mitra and Raichle [119] in this issue). However there is increasing evidence that brain areas displaying correlated spontaneous fluctuations represent the same regions activated together during task-related fMRI ([120,121], reviewed by Uğurbil [21] in this issue) and therefore constitute functional networks known as 'resting state networks' (RSNs). For example, fMRI signals in the posterior cingulate cortex, medial prefrontal cortex and angular gyrus fluctuate together during spontaneous activity and play a coordinated role in imagination, episodic memory and theory of mind. More recently, temporal lags between propagating fMRI signals during resting state have also been investigated to identify the direction of connectivity between brain areas co-activated closely in time [119]. Our understanding of RSNs, both in space and time, is expected to improve as higher field strengths with better signal-to-noise ratios are employed, e.g. by making it possible to detect more components with possibly new functional significance [122].

Understanding network organization also provides clues about the structure of computations in the brain. For example, some brain regions, called hubs, are functionally connected to many other regions, suggesting they may coordinate computations at a broader scale [123,124]. This interpretation is further supported by distinct patterns of expression in hub regions of genes supporting metabolism and inter-cortical connections (Vertes *et al.* [125] in this issue). Such studies can therefore assist investigations into disruptions of functional connectivity in neurodegenerative and psychiatric disorders [117].

The spatial and temporal relationship of RSNs is assumed to arise from the activation of neural connections between brain regions and recent data demonstrate that electrophysiological recordings can predict RSNs in the human brain to some extent [126]. However, it is unclear whether these patterns of neural activity solely reflect functional connectivity, or could be influenced by the vasculature. For example, regional differences in the haemodynamic response could contaminate RSNs [127–130], while concurrent modulation of neurons and the vasculature in different brain regions by ascending monoaminergic and cholinergic projection systems could create apparent connectivity in the absence of direct functional connections when these systems are differently engaged (discussed above; [71,131]), for example during regulation of arousal states [132,133]. Although it is possible that vasomotion intrinsic to the vasculature might distort RSN signals [134], functional connectivity patterns have recently been successfully separated from vasomotion by applying low band-pass filters [135]. Furthermore, neurons tend to generate random spontaneous activity during 'rest' (meaning the absence of task-evoked activity) due to their intrinsically excitable nature [136], giving rise to small haemodynamic responses. Such fluctuations might represent actual functional connections between neurons [137], or may be due to spontaneous noise (which nevertheless may be computationally beneficial due to stochastic resonance [138]). More studies employing multi-modal approaches, both in animal models and humans, are required to test how reliably fMRI-based RSNs reflect the underlying spontaneous neural activity and the nature of the relationship between them.

(g) Multi-modal imaging approaches

Inferences from BOLD experiments can be confirmed or augmented by using a multi-modal imaging approach. fMRI results have been replicated using electroencephalography (EEG) or MEG (often with spatial specificity through source localization algorithms) while magnetic resonance spectroscopy (MRS) has been used to add detail of neurotransmitter contributions to circuit-level mechanisms [110]. In addition to applying MRS, Barron *et al.* [110], applied transcranial direct current stimulation while studying fMRI adaptation to test, at the cellular population level, hypotheses based on BOLD experiments. Performing multi-modal MRI enhances the information we can obtain from a study involving only BOLD. For example, by combining BOLD and arterial spin labelling, allowing measurement of blood oxygenation and cerebral blood flow, respectively, the coupling between blood flow and brain energy metabolism can be calculated [139]. As the amplitude of the BOLD response is dependent on baseline physiology, i.e. oxygen extraction fraction, cerebral blood volume and haematocrit levels, it is difficult to interpret changes in BOLD in situations where physiology may be variable. This has led to the development of calibrated BOLD fMRI [140] in which a calibration scan

(typically involving a hypercapnic breathing challenge) is applied with the aim of accounting for physiological variations (Blockley *et al.* [141] review the different methods of performing this calibration).

Although in general we must rely on using non-invasive methods (such as fMRI) in human subjects, it has been possible in some patient groups to undertake invasive recordings. Microelectrode recordings of single neurons in the human brain have allowed access to cell response properties, supporting inferences of some BOLD experiments (e.g. the existence of grid cells in entorhinal cortex; [142]), while invasive chemical recordings of neurotransmitters such as dopamine and serotonin have expanded hypotheses based on BOLD results to implicate specific neurotransmitters in cognitive processes (Lohrenz *et al.* [15] in this issue; [143]). Such invasive recordings can, of course, only be obtained, with consent, from patients undergoing neurosurgery for other reasons, so such methods are understandably not widely available as a tool for research.

While in humans we are mostly restricted to estimating neural activity from an indirect, non-invasive measurement such as the BOLD signal, in animal studies the use of invasive techniques allows us to concurrently measure neural activity and haemodynamics. This approach enables a thorough investigation of neurovascular coupling and the mechanisms which relate neural activity to the BOLD signal. A wealth of information has already been acquired from detailed, invasive animal studies [144] and our understanding of how BOLD fMRI signals relate to neural signals is set to increase as newly developed techniques, such as genetically encoded fluorescent proteins and improved hardware, are increasingly coupled with existing imaging approaches, such as wide-field optical mapping techniques (Ma *et al.* [145] in this issue). The key is to use these data from animal studies to aid our interpretation of non-invasive signals in humans. In this issue, Uhlirova *et al.* [4] propose that by developing models that estimate non-invasive imaging signals from the population activity of specific neurons, we can establish a 'ground truth' that can be used to estimate neural activity from non-invasive data in humans. Multi-modal imaging is a powerful tool for increasing our understanding of the relationship between neural activity and BOLD fMRI signals, and vice versa.

As discussed above, an exciting development is recent work combining fMRI with information from gene expression databases such as the Allen Institute, in order to understand functional differences between brain regions and networks detected using fMRI (Vertes *et al.* [125] in this issue). Interestingly, these results suggest differences in energy use between different functional nodes, and these hubs may be a useful future target for neurovascular coupling studies, to investigate if neurovascular coupling properties vary with net energy demand of a brain region. Functional changes associated with specific genes can also be investigated by stratifying subjects according to genotype and testing the effect of genetic variation.

Finally, the combination of high resolution gradient echo, spin echo and resting state fMRI have recently been shown to allow automated parcellation of cortex into functionally distinct regions, based on topology, architecture and functional connectivity, and in doing so have identified many novel functionally distinct cortical regions [146]. Parcellating cortex in this manner will allow for neuroanatomically valid spatial smoothing of fMRI data and increased statistical power. If these analyses could be combined with information about regional differences in neurovascular coupling and expected BOLD magnitudes [93,94], the power of these new methods could be increased even further, allowing for increased accuracy and validity in understanding functional specialization across the brain.

4. Future directions to enable better interpretation of BOLD signals

To fully understand the physiological basis of the BOLD signal, and therefore be able to optimize experimental design and interpretation, we need a complete understanding of the processes that generate it at the molecular, cellular, vascular and voxel level. While, as discussed above, we are still some way from this complete understanding, there are certain areas where research should be focused to best increase our ability to confidently interpret BOLD:

— To account for regional differences in BOLD signals, bottom-up modelling of BOLD responses based on vascular anatomical networks could be extended to human vascular anatomy. Incorporation of predicted differences in signal strength due to vascular anatomy alone could then be incorporated into fMRI analysis software.
— Physiological research on the properties of neurovascular coupling should investigate the cell types responsible, modulation by neurotransmitter projection systems (and therefore brain states), and how consistent these properties are across different brain regions, from neocortex to subcortical structures.
— fMRI analysis tools could allow more flexibility for modulation of the haemodynamic response function, to allow the effects of variations in neurovascular coupling properties on the BOLD response amplitude and time course to be reflected in fMRI analyses.
— Multi-modal experiments in humans and animals should investigate the physiological basis of phenomena such as resting state BOLD and fMRI adaptation that are increasingly being used to infer cognitive functions, but are understudied at the physiological level.

5. Conclusion

Recent research has gone some way to elucidating the cellular mechanisms that underlie the BOLD signal, revealing a multitude of cell types and signalling pathways that coordinate the vascular response to neuronal activity. These pathways link blood flow increases primarily with increased synaptic activity and, by defining the spread of the vascular response from the site of neuronal activity, set the minimum spatio-temporal resolution that could be achieved with BOLD.

Within these pathways, however, there is a huge potential for variation in the properties of neurovascular responses between different brain regions, at different developmental stages, in conditions of altered local or global circuit dynamics, or during disease states, as reflected in the observed uncoupling of neural activity and BOLD in subcortical structures and alterations in predicted BOLD between human neocortical areas. Caution is therefore required when interpreting BOLD signals that could be affected by such factors, particularly

using experimental paradigms (such as resting state fMRI and fMRI adaptation) that have been less studied using multi-modal methods that allow measurement of both neuronal and vascular responses. Future research should characterize these multi-modal responses across the wide variety of paradigms currently used in fMRI research, to establish when BOLD does and does not provide useful information about underlying neuronal activity.

Developments in fMRI analysis and hardware are increasing the potential for fMRI/BOLD to inform us about cognitive processes. Model-based fMRI analyses partly mitigate the effect of uncertain underlying physiology by testing whether a given predicted neuronal response is present in the spatial or temporal BOLD signature, while increases in magnetic field strength both reduce the requirement for spatial smoothing and allow prioritization of the signal from the blood vessels closest to the site of neuronal activity.

If we can incorporate our increasing understanding of cellular processes and the limits they impose on BOLD signals into fMRI analysis methods, we will enhance the power of these analyses and thus enable greater understanding of the cognitive processes that are monitored using BOLD. Continued dialogue between cellular and cognitive neuroscientists, as initiated by the 2016 Royal Society Theo Murphy meeting, will be critical to achieving this ambition.

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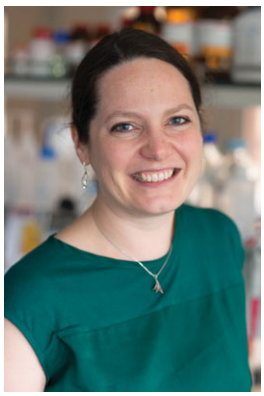
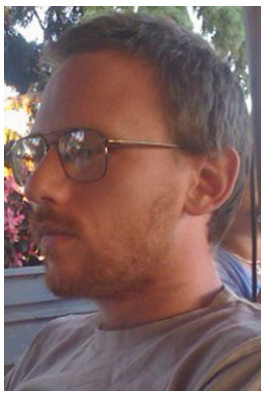
Guest Editor profiles



Catherine N. Hall is interested in how the brain balances energy supply and demand. During her PhD (with John Garthwaite, UCL), she studied nitric oxide (NO) consumption by brain tissue. As a post-doctoral researcher (with David Attwell, UCL), she investigated when NO impacts brain oxygen consumption and how much oxygen is required by different components of neuronal transmission. She then studied how NO and other signalling molecules interact to control the brain's energy supply by regulating the tone of capillary pericytes. They found that capillaries dilate before arterioles in vivo, and constrict and die after ischaemia. This suggests pericytes may initiate the vascular response to neuronal activity, but could contribute to hypoperfusion and delayed neuronal damage after stroke. In 2014 she became a Senior Lecturer at the University of Sussex, and now studies how neurovascular coupling varies during different brain states and at the onset of conditions such as Alzheimer's disease and obesity.

Clare Howarth undertook a PhD at University College London on the Wellcome Trust 4 year PhD program in Neuroscience. Working with David Attwell, she discovered a new mechanism for the control of brain blood flow at the capillary level and produced the first energy budget for the cerebellum. Following her PhD, she was awarded a Sir Henry Wellcome Postdoctoral Fellowship which enabled her to work with Brian MacVicar (University of British Columbia), applying two-photon microscopy to brain slices, and with Nicola Sibson (Oxford), using in vivo MRI and optical imaging techniques to elucidate the role of astrocytes in regulating cerebral blood flow responses to hypercapnia. In October 2013 she moved to the University of Sheffield, where she was recently awarded a Sir Henry Dale Fellowship by the Wellcome Trust and Royal Society to investigate the role of astrocytes in neurovascular coupling in health and aging.



Zebulun Kurth-Nelson did an experimental PhD at the University of Minnesota studying signalling between glia, neurons and blood vessels. He then did a theoretical postdoc about reinforcement learning and decision making, also at Minnesota. When he got tired of winter, he moved to University College London for a second postdoc in experimental neuroimaging, with a focus on understanding the neural representations - especially at fast timescales - that underlie model-based decision-making.

Anusha Mishra started her research career in Kristen Harris's lab at the Medical College of Georgia studying 3D electron micrographs of the brain. She then did her PhD with Eric Newman at the University of Minnesota where she investigated changes in retinal neurovascular coupling in pathology and discovered a drug that reverses the loss of this response in diabetic animals. She is doing her postdoctoral training in David Attwell's lab at University College London, where she has been studying the contribution of astrocytes to cortical neurovascular signalling at the capillary and arteriole level in health and.

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