

## ALTERATIONS IN THE BOLD FMRI SIGNAL WITH AGEING AND DISEASE: A CHALLENGE FOR NEUROIMAGING

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Functional magnetic resonance imaging (fMRI) has rapidly emerged as a powerful tool for studying brain function, despite the fact that it measures neuronal activity indirectly, through the blood-oxygen-level-dependent (BOLD) signal. The BOLD signal depends on neurovascular coupling — the processes by which neural activity influences the haemodynamic properties of the surrounding vasculature. Although the exact mechanisms that underlie neurovascular coupling are not completely understood, there is empirical evidence that these mechanisms might be altered in normal ageing and disease. So, interpretation of BOLD fMRI studies of individuals with different ages or pathology might be more challenging than is commonly acknowledged.

Functional MRI has rapidly emerged as a powerful non-invasive technique for studying brain function with superb spatial and temporal resolution. It is an essential tool for studies that seek to determine the mechanisms of sensorimotor processes or high-level complex behaviour. It also has great potential to provide insight into the neural mechanisms that underlie normal development, ageing and neurological and psychiatric disorders. However, despite the exponential increase in published papers that have used fMRI to explore brain-behaviour relationships in normal or diseased individuals, little is known about the physiological basis of its basic unit of measurement, the BOLD signal.

The BOLD signal depends on the blood-flow mediated relationship between neural activity and the concentration of deoxyhaemoglobin in the surrounding microvasculature. When a neural event occurs anywhere in the brain, there is a subsequent increase in local blood flow<sup>1</sup> resulting in a decrease in the concentration of deoxygenated haemoglobin in the microvasculature that surrounds the activated region<sup>2</sup>. This change leads to an increase in the BOLD signal<sup>3-5</sup> that reflects the ratio of non-paramagnetic oxygenated haemoglobin to paramagnetic deoxygenated haemoglobin<sup>6,7</sup>. Neural activity alters this ratio by influencing several factors including cerebral blood flow (CBF), cerebral blood

volume (CBV) and cerebral blood oxygen consumption (CMRO<sub>2</sub>)<sup>8-10</sup> (FIG. 1). So, the BOLD signal is an indirect measure of neural activity. The process by which neural activity influences the haemodynamic properties of the surrounding vasculature is referred to as neurovascular coupling.

Recently, there has been much interest in the relationship between neural activity and the BOLD signal. The few studies that have examined this have generally supported a roughly linear relationship (for a review on the topic, see REF. 11). These studies showed that, up to some limit, the BOLD response to successive neural events could be predicted from the arithmetic addition of the responses to single neural events, considering the time delay between the events<sup>12</sup>. It is to be hoped that similar studies in monkeys that simultaneously record neural activity and the BOLD signal will provide even deeper insight into the boundary conditions of this linearity<sup>13</sup>. Notwithstanding, the parameters of this relationship might vary between brain regions<sup>14,15</sup> and between populations. As most fMRI studies are performed on healthy young individuals, little attention has focused on the effects of changes in the cerebrovascular system on the BOLD signal. However, direct comparisons of the BOLD signal made between groups of individuals rely heavily on the assumption of

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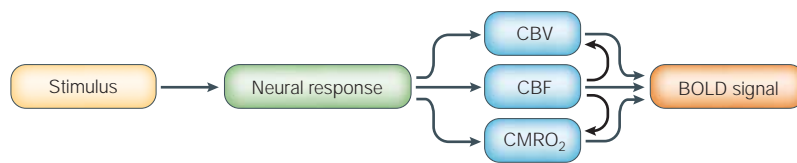


Figure 1 | **Schematic of the transformation of neural activity elicited by a stimulus to a haemodynamic response resulting in a blood-oxygen-level-dependent (BOLD) signal.** The BOLD signal reflects the ratio of non-paramagnetic oxygenated haemoglobin to paramagnetic deoxygenated haemoglobin. Neural activity alters this ratio by influencing several factors including cerebral blood flow (CBF), cerebral blood volume (CBV) and cerebral blood oxygen consumption (CMRO<sub>2</sub>). Modified, with permission, from REF. 9 © (2002) Cambridge University Press.

comparable neurovascular coupling, and any alteration in the cerebrovascular dynamics (such as reduced vascular reactivity or vascular pathology) could affect neurovascular coupling. So, interpretation of fMRI studies of individuals with altered cerebrovascular dynamics might be difficult.

In this review, we first present some of the main mediators that transform neural activity into a haemodynamic response, to provide a foundation for understanding the mechanisms of neurovascular coupling, and therefore the potential points of failure of the system. We show how alterations in neurovascular coupling will influence the interpretation of changes in the BOLD signal. Such alterations can also occur if the dynamics of the vascular system itself are affected. Therefore, we next review changes in cerebrovascular dynamics that have been observed in normal ageing and neurological disease, as well as empirical observations that reveal the effect of these changes on the BOLD signal. Understanding the mechanisms of neurovascular coupling and the impact of altered cerebrovascular dynamics on the BOLD signal allows us to highlight the implications for the design and interpretation of fMRI studies that compare different populations.

#### Mediators of neurovascular coupling

In 1890, Roy and Sherrington concluded that “the chemical products of cerebral metabolism contained in the lymph which bathes the walls of the arterioles of the brain can cause variations of the caliber of the cerebral vessel: that in this re-action the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with the local variations of functional activity”. Although this conjecture has been refined by a century of research, its basic premise remains valid. Recent research, however, implicates new mechanisms for this ‘functional hyperaemia’ or neurovascular coupling<sup>16</sup> (FIG. 2).

**Local metabolites.** The dilatory effects of the local metabolites K<sup>+</sup> and H<sup>+</sup>, which increase in the extracellular fluid during a series of neuronal action potentials, are well known<sup>17</sup>. K<sup>+</sup> diffuses quickly out of terminals during the rapid repolarization stage of the action potential, and therefore increases in the vicinity of active neurons. Either by simple diffusion, or mediated by astrocytes<sup>18</sup>, the elevated K<sup>+</sup> concentration affects adjacent RESISTANCE VESSELS. *In vitro*, small increases in

extracellular K<sup>+</sup> cause dilation of resistance arterioles. This effect is mediated by the opening of several kinds of K<sup>+</sup> channel on the membrane of arteriolar smooth muscle cells<sup>19–22</sup>, leading to their hyperpolarization and subsequent relaxation. The vasodilatory effect of increased concentrations of H<sup>+</sup> is also mediated, at least in part, by the opening of K<sup>+</sup> channels<sup>23</sup>. Opening of distinct classes of K<sup>+</sup> channels might be responsible for distinct stages of vasodilation. For example, a voltage-dependent K<sup>+</sup> channel might be necessary for the initial transient vasodilation, whereas a second, ATP-dependent channel is responsible for the sustained dilation during an ongoing increase in extracellular K<sup>+</sup> (presumably related to sustained neuronal firing<sup>19</sup>). The trigger for opening the latter channel is a low energetic state of the smooth muscle cell<sup>21</sup>, apparently due to increased activity of the Na<sup>+</sup>/K<sup>+</sup>-ATPase pump, which is driven by the increased extracellular K<sup>+</sup> concentration. However, other causes of ATP depletion, such as uncompensated increases in oxygen extraction by active neurons, might lead to the opening of the ATP-dependent K<sup>+</sup> channel and cause vasodilation.

Chronic cerebral ISCHAEMIA (and a consequently low energetic state of the arteriolar smooth muscle) might be compensated for by sustained dilation distal to partially occluded vessels<sup>20</sup>. This might provide adequate tissue perfusion but, importantly for fMRI studies, could hinder the additional flow increase that is responsible for the BOLD signal, or at least reduce the dynamic range of this response (see also REF. 24, which discusses reduced reactivity of the cerebrovascular circulation owing to chronic vasodilation secondary to CAROTID STENOSIS). So, although neuronal activity might be normal, the difference in blood flow and oxygen concentration between the resting and the active state (which is the dependent variable in fMRI studies) might be limited, potentially leading to erroneous conclusions regarding the underlying neural activity in the examined task. In addition, there is accumulating experimental evidence that the function of all of the types of K<sup>+</sup> channel that are responsible for vasodilation can be altered by the main risk factors for cerebrovascular disease, including hypertension, diabetes and hypercholesterolaemia<sup>22</sup>. Presumably, this will affect the transformation of neural activity into changes in blood flow.

**Nitric oxide.** An important soluble candidate for mediating neurovascular coupling is nitric oxide (NO)<sup>25–28</sup>. Initially, the vasodilatory effects of NO were related to endothelial production of this compound, but it seems that a neuronal form is more likely to be involved. The increase in CBF that accompanies the neuronal response to rat whisker stimulation is inhibited by a specific inhibitor of neuronal NO synthase (NOS)<sup>29–31</sup>, and studies of knockout mice lacking neuronal NOS confirmed that endothelial NO does not play an important part in neurovascular coupling<sup>32</sup>. Although NO’s participation in neurovascular coupling is widely accepted, its exact mechanism of action is not clear. As NO serves as a retrograde messenger in synapses, one source of NO could be leakage from synaptic clefts and

#### RESISTANCE VESSELS

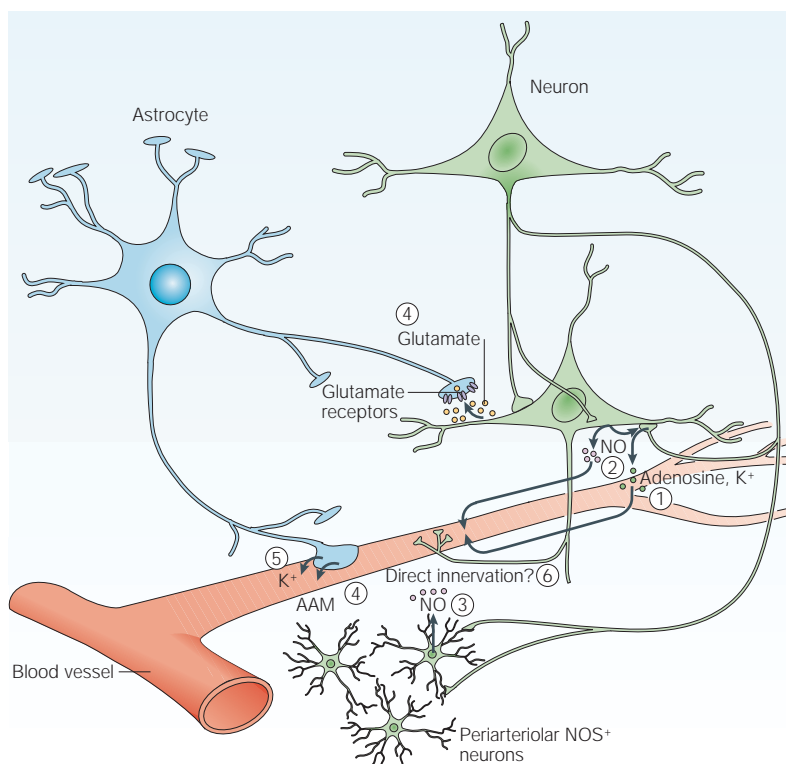
Arterioles and small arteries.

#### ISCHAEMIA

A condition of insufficient oxygen delivery to a tissue.

#### CAROTID STENOSIS

The condition in which one or both carotid arteries (the arteries in the neck that supply blood to the brain) is narrowed or blocked. Can lead to stroke secondary to decreased blood flow in the brain region that it supplies.



**Figure 2 | Some of the suspected mediators of neurovascular coupling.** 1. Local metabolites such as  $K^+$ , protons and adenosine passively diffuse from active synapses to the membrane of smooth muscle cells in the walls of nearby arterioles. 2. Nitric oxide (NO), acting as a retrograde messenger, leaks from active synapses to the walls of nearby arterioles. 3. NO is also locally produced by specialized networks of periarteriolar neurons. 4. Glutamate leaking from active excitatory synapses binds to glutamate receptors on the endfeet of astrocytes. This leads to the formation of vasodilatory arachidonic acid metabolites (AAM) (leukotrienes, prostaglandins and epoxyeicosatrienoic acids), which are secreted by endfeet that intimately surround nearby arterioles. 5. Astrocytes also participate in the re-uptake of  $K^+$  (a potent vasodilator) from the vicinity of active synapses, secreting the surplus at endfeet at the wall of the arteriole ('siphoning'). 6. Smooth muscle cells in the arteriolar wall might also be directly innervated by axon collaterals that control vascular tone. Some of these mechanisms are better established than others (see text).

diffusion from active synapses to arterioles. However, as in the case of  $K^+$ , diffusion might be an inefficient and less controllable mechanism. As capillaries are much closer than arterioles to pyramidal neurons<sup>33</sup>, diffusion might be adequate to provide the pyramidal neurons with nutrients from capillaries, but ineffective in delivering metabolic messages to the blood-flow gate at the terminal arteriole. Alternatively, NO could be produced by specialized peri-arteriolar neurons that are activated by direct stimulation from collateral axons, or by leaked neurotransmitters from active synapses<sup>34</sup>. In support of this notion, NOS-containing neurons lie close to pyramidal cells and vary in density consistent with the variation of microvasculature density<sup>33</sup>. In addition, a network of fine nerve fibres and punctate staining for NOS is found around arterioles, indicating NOS-containing terminals<sup>28,30</sup>. Lovick *et al.*<sup>33</sup> propose an NO-producing neuronal network that provides a link between active pyramidal cells and relatively remote terminal arterioles. Whether the control of CBF by NO is implemented through simple diffusion or through specialized networks (neuronal or glial) should determine the degree

to which the activity of clusters of neurons — which are incidentally located closer or further away from terminal arterioles — will produce similar amplitudes and temporal profiles of CBF changes, observable in functional neuroimaging. Another factor in this complex equation is the non-uniform distribution of NO-producing neurons in different cortical areas<sup>35</sup>.

It is also not known whether NO is a direct mediator of enhanced CBF or has only a modulatory ('permissive') role. That is, does NO quantitatively determine the vascular response, or is it necessary for other mediators to exert their effect? In the rat cerebellum, a decrease in CBF response owing to inhibition of NOS could not be overcome by providing NO indiscriminately, indicating that NO acts as a direct mediator of local CBF control. By contrast, Lindauer *et al.*<sup>36</sup> reported that in rat somatosensory cortex NO had a modulatory effect, so that application of NO restored the CBF response after inhibition of NOS. This finding also highlights the fact that neurovascular coupling processes might vary across the brain.

**Glutamate and astrocytes.** Recent studies provide strong evidence for a chain of events whereby glutamate, leaking from active synapses, activates glutamate receptors on astrocyte membranes, leading to elevation of intracellular  $Ca^{2+}$  concentration, which in turn leads to the production and release of eicosanoids (products of arachidonic acid), which are potent vasodilators<sup>37–41</sup>. Both in a slice preparation and *in vivo*, interruption of any stage of this cascade severely reduces the CBF response, without affecting the neural response<sup>39</sup>. Astrocytes might also affect CBF by delivering  $K^+$  from the vicinity of active synapses to the arteriolar wall ('siphoning')<sup>18</sup>, by production of lactate during glutamate recycling<sup>42</sup> or by production of endothelial NO<sup>43</sup>, although the importance of these mechanisms is not clear.

The role of astrocytes in neurovascular coupling is relevant to the interpretation of fMRI studies in certain populations. First, almost any brain insult (such as stroke or trauma) results in some gliosis (largely astrocytic), which might disrupt the orderly neuronal-astrocytic-vascular coupling that is present in the normal brain<sup>44</sup>. Therefore, reduced CBF response in gliotic areas might not linearly reflect reduction in neural activity, as in intact brain areas. Second, the expression of different neurotransmitter receptors on astrocytes seems to vary during development<sup>45</sup>, indicating that different mechanisms might be active throughout life — affecting the observed activity-induced CBF changes. Third, some common medications, including the non-steroidal anti-inflammatory drugs that inhibit the cyclooxygenase pathway of arachidonic acid (for example, aspirin<sup>39</sup>), might alter neurovascular coupling (BOX 1). Fourth, if neurovascular coupling relies on the interaction of specific neurotransmitters with astrocytes, then specific neurological diseases that diminish or enhance the activity of one or more neurotransmitters (such as Parkinson's or Alzheimer's disease) might affect neurovascular coupling beyond their direct effect on neuronal activity.

## Box 1 | The effects of comorbidities and medications on neurovascular coupling

'Healthy' elderly subjects can have clinically silent vascular pathology such as lacunar infarcts. In addition, white matter lucencies (leukoariosis) are common findings on computerized tomography and magnetic resonance imaging (MRI) scans of healthy elderly subjects, and are sometimes found without other evidence of vascular pathology. Most areas of leukoariosis are believed to be associated with small vessel disease, and microscopic evaluation of these regions reveals arteriolar hyalinization and arteriosclerotic changes<sup>95,96</sup>. The severity of leukoariosis has been shown to correlate with a reduction in cerebral blood flow (CBF)<sup>97</sup>, cerebral perfusion within the white matter areas<sup>60,98,99</sup> and cerebrovascular response to hypercapnia<sup>100</sup> and acetazolamide<sup>98</sup>.

Healthy elderly subjects or patients with cerebrovascular disease that participate in functional MRI (fMRI) studies might also have other medical conditions such as diabetes, hypertension and hyperlipidaemia, all of which might affect the blood-oxygen-level-dependent (BOLD) signal by altering CBF and neurovascular coupling<sup>101</sup>. These conditions are also risk factors for vascular pathology<sup>102</sup>. Patients with these conditions also typically take prescribed medications. Few fMRI studies strictly screen participants for the use of all medications, especially those not thought to have direct effects on the brain (such as aspirin or other non-steroidal anti-inflammatory agents). Few studies have investigated the effect of medications on CBF<sup>103–105</sup> or the BOLD signal<sup>106,107</sup>. Little is known about other frequently used substances such as caffeine and nicotine, which might have independent vascular or neural effects<sup>108–111</sup>. Caffeine, even at moderate doses, is a cerebral vasoconstrictor that can decrease the resting CBF by 20–30% (REF. 112) and the BOLD baseline signal by 4.4%. It can also result in an exaggerated task-induced BOLD response because of the larger difference between the rest and active states<sup>111</sup>. Complicating the picture is the observation that the caffeine-related increase in BOLD signal response is not linear, not uniform across the visual and motor cortex, and not of comparable magnitude in all subjects<sup>109</sup>.

**Other neurotransmitters.** Glutamate might not be the only neurotransmitter that is involved in neurovascular coupling. Acetylcholine that is produced by rat basal forebrain neurons increases blood flow in specific cortical (mainly frontoparietal) and subcortical areas<sup>46</sup>. This effect might be due to the direct contact of the cerebral microcirculation by the cholinergic terminals of basal forebrain neurons<sup>29–31,46</sup>, or to the presence of cholinergic receptors on NOS-containing neurons<sup>47,48</sup>.

If cholinergic innervation is necessary to maintain blood flow control in specific brain regions, then degeneration of basal forebrain cholinergic neurons, as in Alzheimer's disease, could uncouple neural activity from blood flow (or alter the nature of the coupling) locally as well as in remote regions. Altered CBF responses in such regions could not directly be interpreted as altered neuronal activity. Moreover, the non-uniform cholinergic cortical innervation could mean that there is regional variability in neurovascular coupling in certain circumstances.

Other neurotransmitters might also contribute to the regulation of local CBF, either by direct stimulation of smooth muscle cells in arteriolar walls or through stimulation of other cells. For example, GABA ( $\gamma$ -aminobutyric acid) neurons co-localize with intraparenchymal blood vessels in a manner that indicates a possible interaction with vascular smooth muscle, directly or mediated by astrocytes<sup>47</sup>. The GABA neurons seem to be local rather than ascending fibres (for example, from the basal forebrain). Undoubtedly, other neurotransmitters with known vasoactive properties (including adrenaline, dopamine, serotonin and vasoactive intestinal peptide) are also involved in the control of local CBF. Any disease state or age-related change that affects these neurotransmitter systems could also alter neurovascular coupling, and consequently the BOLD response, in a manner that is independent of its effects on neural activity.

## Altered cerebrovascular dynamics

Although cerebrovascular pathology is found in many medical, neurological and psychiatric disorders, extensive research has shown that the cerebrovascular system undergoes important changes in multiple components in a continuum throughout the human lifespan, probably beginning as early as the fourth decade<sup>49</sup>. The vascular pathology that is seen in normal ageing can be clinically silent or can lead to vascular compromise resulting in cerebral ischaemia and stroke. These vascular changes are probably the basis for alterations in neurovascular coupling (BOX 2). It is beyond the scope of this review to consider all types of vascular pathology. However, the types of change that are reviewed in the following sections are the most prevalent.

**Ultrastructure.** The compromise to the ultrastructural integrity of the cerebral vasculature in ageing is largely the result of arteriosclerotic changes, principally fibrohyaline thickening of the vessel wall<sup>50</sup>, necrosis of smooth muscle cells<sup>51</sup> and thickening of the basement membrane<sup>52</sup> that gradually increases with age. Although sclerotic changes correlate with the degree of hypertension<sup>50</sup>, age seems to be an independent risk factor<sup>51,53</sup>. It is likely that these changes decrease the elasticity and compliancy of affected vessels, which include capillaries, larger arterioles and cerebral arteries<sup>54</sup>. Venous alterations that accompany ageing, known as periventricular venous collagenosis, are also found in 65% of subjects over 60 years old, and in severe cases can completely occlude veins<sup>55</sup>. There is also an increase in the tortuosity of some vessels with ageing, most notably in the arteriovenous-capillary bed<sup>56</sup>, as well as changes in the density of capillaries and arterioles<sup>57</sup> that have not been observed in venules<sup>58</sup> (FIG. 3).

ATHEROSCLEROSIS can eventually occlude cerebral vessels, and partial recanalization of an occluded vessel might result in post-stenotic compensatory dilation (see previous section). This can lead to reduced vascular

## ATHEROSCLEROSIS

A condition in which fatty material is deposited along the walls of arteries, which then thickens, hardens, and might eventually block the arteries.

## Box 2 | Cerebrovascular alterations

**Potential mechanisms of altered neurovascular coupling:**

- chronic cerebral ischaemia
- chronic cerebral vasodilation secondary to carotid stenosis
- astrocytic proliferation due to neural injury causing gliosis
- developmental changes in expression of neurotransmitter receptors on astrocytes
- alterations of ionic channels on vascular smooth muscle with hypertension, diabetes and hypercholesterolaemia
- effects of medications
- abnormal reduction or enhancement of specific neurotransmitter systems, such as glutamate, acetylcholine or GABA ( $\gamma$ -aminobutyric acid)

**Alterations in vascular dynamics in ageing and neurological disease:**

- ultrastructural changes in cerebral vessels due to atherosclerosis
- increased tortuosity of cerebral vessels
- changes in collateral circulation after recanalization of occluded cerebral vessels
- reduction in resting cerebral blood flow
- changes in vascular reactivity
- lowered resting cerebral metabolic rate of oxygen consumption

reactivity (reduced dynamic range) and possibly redistribution of blood flow in the area surrounding the brain region that is supplied by the occluded vessel, at least in the early period after a stroke<sup>59</sup>. So, even brain regions that are distant from the injured brain region can have altered blood-flow characteristics.

**Resting cerebral blood flow.** Studies that have used functional neuroimaging techniques (such as positron emission tomography (PET), single photon emission computed tomography and gas inhalation contrast computed tomography) have found that ageing is associated with a significant decrease in resting CBF in the cortical and subcortical parenchyma<sup>60–62</sup>.

**TRANSCRANIAL DOPPLER ULTRASOUND**

A non-invasive method that is used to assess blood-flow velocity in intracranial vessels and then make determinations about intracranial haemodynamics, such as cerebral blood flow (CBF).

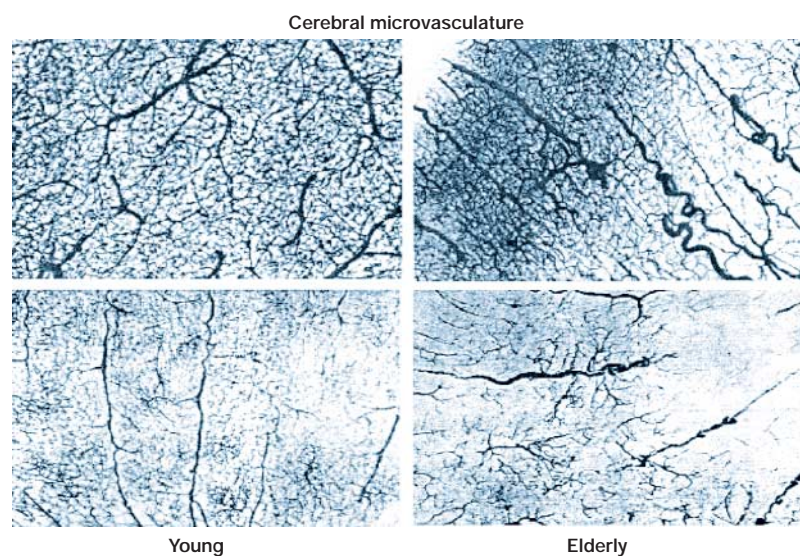


Figure 3 | Ultrastructural changes observed in the cerebral microvasculature of elderly individuals as compared with younger individuals. Note the abnormal 'coiling and looping' (upper right) and 'twists and turns' or 'windings' (lower right) in arteries and venules from elderly individuals<sup>56</sup>.

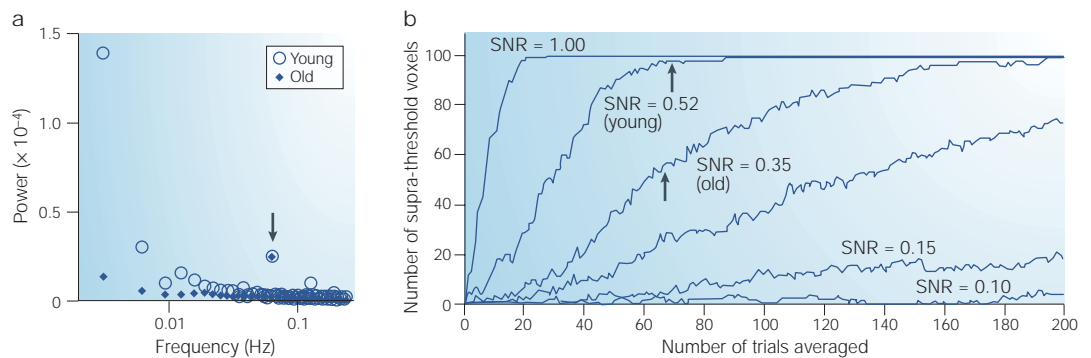
TRANSCRANIAL DOPPLER ULTRASOUND has produced similar findings for blood flow in large cerebral arteries, such as decreases in blood-flow velocity in the middle, posterior and anterior cerebral arteries with advancing age<sup>63</sup>. Baseline CBF might be influenced not only by age, but also by different physiological states such as fluctuating levels of CO<sub>2</sub> (REF. 64).

In measuring the BOLD response, the inactive (resting) state of a given region of interest is as relevant as the active state. This is because the the BOLD signal is not an absolute measure, but rather a relative measure that uses the resting CBF as a baseline. Therefore, differences in resting CBF between populations can have important implications for across-population comparisons.

**Cerebral metabolic rate of O<sub>2</sub> consumption.** The BOLD signal depends not only on the level of oxyhaemoglobin, which is regulated by CBF, but also on the level of deoxyhaemoglobin, which is largely influenced by the CMRO<sub>2</sub>. As mentioned previously, although the haemodynamic effects on the BOLD signal seem to be dominant, increasing neural activity results in increased CMRO<sub>2</sub>, leading to increased levels of deoxyhaemoglobin and a significant decrease in BOLD signal<sup>65</sup>. Ageing is known to influence CMRO<sub>2</sub>. Two PET studies have revealed a significantly lower resting CMRO<sub>2</sub> in cortical and subcortical regions of older subjects compared with younger subjects, which exceeded age-related changes in CBF<sup>66,67</sup>. However, this finding has not yet been extended to consider age-related changes in activity-induced CMRO<sub>2</sub>.

**Vascular reactivity.** There is also an age-associated decrease in the vascular reactivity of cerebral vessels to various chemical modulators, including CO<sub>2</sub> concentration. Normally, increased blood CO<sub>2</sub> results in dilation of cerebral arterioles. Decreased vascular responsiveness to hypercapnia has been observed in aged rats<sup>68</sup> and humans with and without risk factors for atherosclerosis<sup>69–71</sup>. In a study of regional CBF (rCBF) changes monitored with PET, a significant decrease in the total vascular response from a hypocapnic to a hypercapnic state was observed in older adults as compared with young adults<sup>72</sup>. In aged rats, there is a reduced degree of vasodilation in response to cerebrospinal fluid perfusion of the vasodilators adenosine<sup>73</sup>, acetylcholine and bradykinin<sup>74</sup>.

The exact mechanisms of age-related changes in vascular reactivity have not been elucidated, although it is often suggested that they are secondary to a lack of compliance of the ageing vasculature. However, reduced vascular reactivity might result from any of the structural and functional changes described previously. In addition to structural changes, mechanisms that might affect vascular reactivity independent of neural activity include the gliosis that accompanies tissue scarring from stroke or traumatic injury, and disruption of long-range aminergic and cholinergic fibres that innervate the vasculature. So, altered neurovascular coupling can occur close to an injured brain region even in apparently normal tissue<sup>75</sup>. Moreover, age-related changes in vascular reactivity



**Figure 4 | Empirical observations of decreased signal-to-noise ratio in the blood-oxygen-level-dependent (BOLD) signal of older individuals as compared with younger individuals.** **a** | Average power spectrum from motor cortex during performance of a simple reaction time task. The BOLD signal is decomposed into discrete frequency bins (shown as open circles and closed diamonds for young and older subjects, respectively). The part of the signal that correlates with the task has the frequency of the target (which appeared once every 16 seconds), marked with an arrow. Signal in other frequency bins is considered as noise, resulting from physiological processes such as breathing and pulsation, as well as noise from the scanner operation. The plot shows increased noise in elderly subjects, especially in the lower part of the spectrum. **b** | Data from visual cortex during the presentation of checkerboard stimuli. Presented are simulations regarding how different signal-to-noise ratios (SNRs) measured in their experiments with young and older individuals affect the identification of active voxels, for different numbers of averaged trials. For 70 trials (arrows), the lower SNR in the elderly subjects results in a much smaller spatial extent of activation, even if the underlying distributions of neural activity are similar. Panel **a** reproduced, with permission, from REF. 77 © (1999) Elsevier Science. Panel **b** reproduced, with permission, from REF. 83 © (2001) Elsevier Science.

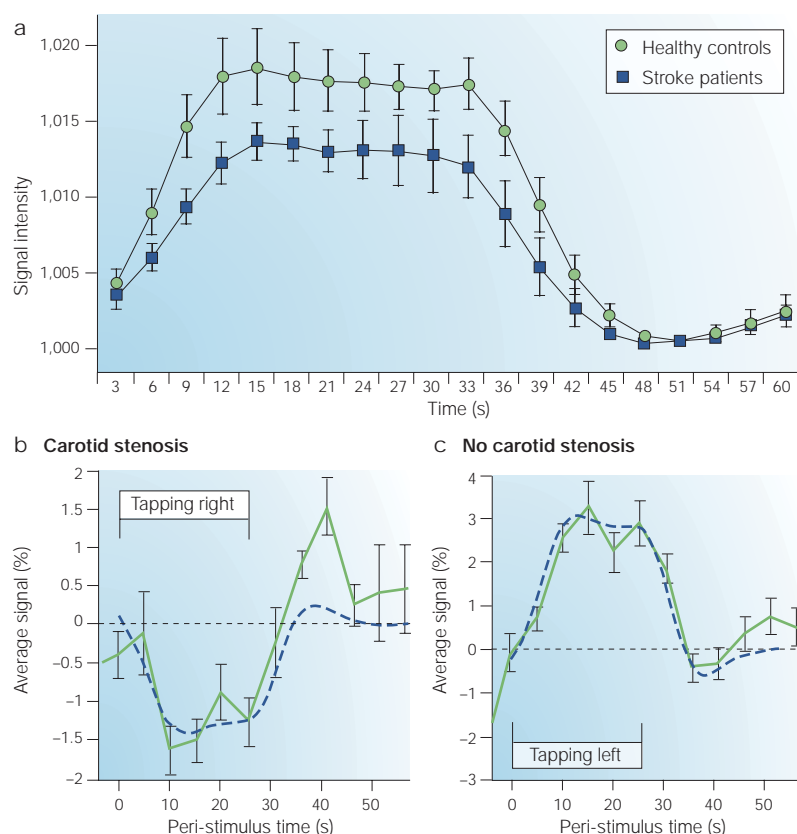
might vary across different brain regions. In a study that compared the resting and stimulus-evoked rCBF in rats, basal forebrain stimulation elicited ipsilateral increases in CBF in both the parietal and frontal cortex of young rats, but only the frontal cortex of the aged rats<sup>76</sup>. This pattern might mirror the non-uniform distribution of atherosclerosis in the brain.

The BOLD signal in ageing and disease  
If alterations in cerebrovascular dynamics affect neurovascular coupling, it follows that certain characteristics of the BOLD signal will differ between individuals with and without such alterations. Evidence for these differences comes from fMRI studies of older individuals and those with cerebrovascular disease.

**Normal ageing.** Several laboratories have begun to study the effect of normal ageing on the BOLD signal. One approach is to study the spatial and temporal characteristics of the BOLD haemodynamic response function (HRF) during a task that is expected to result in equivalent neural activity in younger and older subjects, such as a simple motor<sup>77–81</sup> or visual task<sup>78,82,83</sup>. If there are changes in HRF between groups during a task that is assumed to induce no age-related change in neural activity, then they can be attributed to an alteration in neurovascular coupling. In one such study, we examined the HRF characteristics in the sensorimotor cortex of younger and older subjects during a simple motor reaction-time task<sup>77</sup>. It was provisionally assumed that the neural activity of the two populations was identical on the basis of physiological findings that subjects showed equivalent movement-related electrical potentials under similar conditions<sup>84</sup>. So, we presumed that any changes in BOLD fMRI signal between younger and older individuals in motor cortex would be due to

vascular changes in normal ageing, rather than changes in neural activity. There were several important similarities and differences between age groups. For example, although there was no significant difference in the shape of the HRF or peak amplitude of the signal, older subjects had a significantly decreased signal-to-noise ratio in the BOLD signal when compared to younger individuals (FIG. 4). This was attributed to a greater level of noise in the older individuals that led to a decrease in the spatial extent of the BOLD signal that was considered to be statistically significant. These findings indicate that some property of the coupling between neural activity and BOLD signal changes with age, even for simple motor responses in primary motor cortex.

Other studies have produced similar findings. For example, Hesselman *et al.*<sup>79</sup> also observed a decrease with age in both the signal amplitude and the number of activated voxels within sensorimotor cortex during a finger-tapping task. Similarly, Huettel *et al.*<sup>83</sup> found a decrease in spatial extent, similar amplitudes and increased noise levels of a visually evoked HRF to checkerboard stimuli in the older visual cortex<sup>83</sup>. Using a blocked design, Taoka and colleagues<sup>81</sup> found an age-associated lag in the time taken by the BOLD signal to reach half its maximum level in motor cortex between the start and end of a 10-s hand-grasping task. They proposed that this lag might be attributable to arteriolar changes such as vascular stiffening. A study by Buckner *et al.*<sup>78</sup> found an age-associated regional difference in the BOLD signal between the motor and visual cortex evoked by viewing a large-field flickering checkerboard and pressing a key on stimulus. BOLD signal amplitude was decreased in the visual cortex, in concordance with the findings of Ross *et al.*<sup>82</sup> on a flashlight stimulation task, whereas there was no change in the BOLD signal amplitude in the motor cortex. The authors proposed that these



**Figure 5 | Abnormal blood-oxygen-level-dependent (BOLD) signal in patients with cerebrovascular pathology.** **a** | BOLD signal in motor cortex in healthy controls (circles) and patients (squares) during the performance of a sequential finger-tapping task. Patients show a significantly slower rate of rise and maximum signal intensity change relative to the controls. **b,c** | BOLD responses during a finger-tapping task in motor cortex on the side of carotid stenosis (onset and duration is indicated by the white rectangle) **(b)** versus no carotid stenosis **(c)**. On the side with carotid stenosis there is a normal BOLD signal change, whereas there is an abnormal negative BOLD response on the side with carotid stenosis. The dotted lines represent the fitted response. Panel **a** reproduced, with permission, from REF. 85 © (1999) Elsevier Science; panel **b** reproduced, with permission, from REF. 86 © (1999) Lippincott, Williams and Wilkins.

findings might represent a regional difference in the alteration of neurovascular coupling with age, although they concede that the findings in the visual cortex might be a correlate of regionally reduced neural activity.

**Cerebrovascular disease.** Only two studies have examined the effect of cerebrovascular disease on the BOLD signal. Piniero *et al.*<sup>85</sup> analysed the time course of the BOLD HRF in the sensorimotor cortex of patients with an isolated subcortical lacunar stroke, serving as a marker for cerebrovascular disease, compared with a group of age-matched controls. They found a decrease in the rate of rise and the maximal BOLD HRF to a finger- or hand-tapping task in both the sensorimotor cortex of the hemisphere that was affected by the stroke and in the unaffected hemisphere (FIG. 5). Given the widespread distribution of BOLD signal differences between groups, the change was unlikely to be a direct consequence of the subcortical lacunar stroke. Rather, this change might be a manifestation of pre-existing diffuse vascular pathology. Furthermore, the assumption

**CEREBRAL AUTOREGULATION**  
The intrinsic ability of cerebral vessels to maintain a relatively constant, steady-state CBF despite large changes in arterial blood pressure.

was made that the BOLD change was secondary to an alteration in the CBF, as the other contributing factors to the HRF, the CBV and  $CMRO_2$ , were unlikely to be different between the two groups.

Another fMRI study concluded that severe extra-cranial carotid stenosis in a patient without MRI evidence of an infarct led to neurovascular uncoupling that presented as a negative BOLD signal response during performance of a simple motor task<sup>86</sup> (FIG. 5). Furthermore, this negative BOLD response occurred only in the affected hemisphere and correlated with a severely impaired haemodynamic response to hypercapnia isolated to that hemisphere. Given that there was no reason to suspect an abnormality in neural activity in this patient, the finding was interpreted as a local activity-driven increase in deoxyhaemoglobin, secondary to oxygen consumption, in the absence of an accompanying increase in CBF. Although this was an extreme example of the effect of impaired CEREBRAL AUTOREGULATION on the BOLD response, it serves as an important illustration that extracranial vascular disease can impair this process and alter the BOLD response.

In subjects with altered neurovascular coupling, it will be crucial to assess the dynamic aspects of the BOLD signal, such as scalability, summation and refractoriness, to characterize fully the effects of vascular pathology on neurovascular coupling. For example, it has been shown that there is no age-related effect on the refractoriness<sup>83</sup> or the ability of the HRF to summate<sup>78</sup>.

#### Implications for fMRI studies

fMRI studies that compare the neural responses of elderly or stroke patients with those of young or healthy ones assume that the two groups have comparable neurovascular coupling. However, the presence of pathology that affects neurovascular coupling in one group and not the other, or different pathology in one group than in the other, constrains the conclusions that can be drawn from such comparisons. In this review, we have emphasized the potential effects of neural and vascular pathology on neurovascular coupling. Both direct alterations in the cerebral vasculature and alterations in the complex neurochemical transformation of neural activity to changes in blood flow might affect the measured BOLD response. So, both disorders of vascular structure and disorders that might be considered free of vascular pathology, such as Alzheimer's or Parkinson's disease, could result in altered vascular physiology that is unrelated to changes in neural activity.

It is also important to note that other factors can lead to differences in neurovascular coupling between study groups. As previously mentioned, early developmental changes occur in neurovascular coupling that might affect comparisons of children and adults, and normal ageing is associated with changes in resting CBF and  $CMRO_2$ , even in the absence of gross pathology. The design, analysis and interpretation of any study that includes a subject population with a possible alteration in neurovascular coupling must consider these factors. There are many possible approaches to designing fMRI studies that could limit these potential confounds of the

effects of alterations in neurovascular coupling on the BOLD signal. One simple approach is more careful selection and characterization of the subjects being studied, to reduce unknown variance between subject groups. Studies that include subjects with potential vascular pathology should carefully assess the status of the neurovascular system as well as secondary factors (comorbidities and medications) in their subjects. Most fMRI studies of ageing have not collected images with appropriate pulse sequences for detecting clinically silent white matter lesions (T<sub>2</sub>-WEIGHTED, perhaps using a FLAIR sequence<sup>87</sup>), nor have they reported screening of images for vascular pathology by neurologists or radiologists. Also, the routine screening that is typically performed for medical and neurological conditions could miss symptoms that are consistent with vascular pathology. Often, a subject will not be aware of having vascular risk factors or ever experiencing neurological symptoms, but a medical history taken by a neurologist, which is not typically done in fMRI studies, will reveal episodes or risk factors. Also, future fMRI studies of groups that potentially have vascular pathology might need to consider the use of Doppler ultrasound or magnetic resonance angiography in evaluating the extracranial vasculature for the presence of significant occlusion.

It should be noted, however, that the vascular pathology described in this review is a common feature in the ageing brain and it is possible that age-related cognitive change might result from such vascular changes. We are therefore not recommending the exclusion of all subjects with vascular alterations from investigations into ageing. Rather, we stress the necessity of identifying vascular changes in all older subjects and considering these data when interpreting BOLD fMRI data and behavioural changes.

In BOLD fMRI studies that include subjects with known alterations of vascular dynamics (for example, stroke patients), some outcomes are more difficult to interpret than others. For example, groups might show a similar pattern of brain activation, albeit with lower activation in one group than the other in all (or some) of the same brain regions. Alternatively, there might be a different pattern of activation between groups; that is, brain regions are activated in one group that are not active in the other. If altered vascular dynamics lead to changes in neurovascular coupling causing a reduced BOLD signal in response to an equivalent neural event, then under-activation, as determined by a lower BOLD response, represents the most difficult scenario for disentangling vascular differences from neural differences between populations.

There are several approaches in both experimental design and statistical analysis of fMRI data that might address this potential confound. For example, instead of testing for a main effect between groups on a particular behavioural task, a safer approach is to test for group-by-task interactions. That is, testing for differences in the relative performance between two different behavioural tasks rather than direct comparison between groups on a single task. For example, Mitchell *et al.*<sup>88</sup> compared the BOLD signal in young and older adults on a working

memory task that required subjects to maintain a combination of two types of information (form and spatial features) in memory in one task, and only a single feature in another task. The main goal was not to identify overall differences in levels of neural activation between younger and older adults (a main effect of age), but rather in the relative performance of younger and older adults on memory tasks that require binding and memory tasks that do not (an age-by-condition interaction). In the left anterior hippocampus, only young subjects showed increased activation for the combination condition relative to the single-feature condition. Because the study design used an internal control (the independent variable is a within-group comparison) these results are more likely to be due to an age-related change in neural activity during working memory processing than the result of a haemodynamic change. Correlating changes in BOLD signal with changes in behavioural measures (accuracy or reaction times) will also increase the likelihood that observed group differences are true correlates of changes in neural activity. Last, the use of event-related fMRI designs, in which the BOLD signal that corresponds to particular stages of processing in a trial can be detected, also gives the option to test for an age-by-condition interaction.

Other innovative statistical approaches might also resolve potential confounds in interpretation of data derived from individuals with an altered neurovascular system. For example, Rypma & D'Esposito<sup>89</sup> investigated age-related differences in prefrontal neural activity with random-effects tests of age differences using the mean parameter estimates (the beta values derived from the least-squares solution of a linear model of the dependent data) that characterized the BOLD fMRI signal during each task period. Unlike the common procedure, these parameter estimates were not scaled by the model error term (which would typically be used to obtain *t*-statistics for each voxel), and were less affected by the potential confound of an intrinsic difference between the groups in the BOLD fMRI signal noise. Other approaches, including multivariate statistical techniques such as coherence analyses, are also potentially useful because they are not sensitive to changes in regional differences in the HRF<sup>90</sup>.

It might also be necessary to quantify differences in vascular reactivity and the haemodynamic response for all subjects when population comparisons are being performed, to establish a baseline for each subject and within each group. For example, in addition to the experimental task, each subject could perform sensorimotor or visual tasks to determine the magnitude of regional HRFs. This could be compared with estimates of vascular reactivity independent of neural activity, such as the magnitude of the BOLD response to breath holding (increasing CO<sub>2</sub> concentration), to provide a means of calibrating the BOLD signal.

Finally, combining electrical measures such as event-related potentials or event-related oscillatory activity, which assess neural activity more directly but that lack adequate spatial resolution, with fMRI measurement, might add converging evidence that a given change in

#### T<sub>2</sub>-WEIGHTED

A magnetic resonance imaging (MRI) recording sequence that provides sensitivity to pathologic processes through changes in water content. T<sub>2</sub> is often lengthened for oedema and demyelination.

#### FLAIR

FLAIR (fluid-attenuated inversion recovery) is a T<sub>2</sub>-weighted MRI image that has the advantage of separating white-matter lesions from cerebrospinal fluid-like lesions.

BOLD activity is related to neural rather than neurovascular changes. However, such combined data are rarely conclusive: given multiple foci of BOLD activations (across space), and multiple electrical components (across time), it might be difficult to determine which BOLD signal foci are correlated with a given electrical component. Also, it remains to be determined which of the electrical events that are captured on the scalp are correlated with changes in blood flow (see REFS 91–94 for recent discussion of the brain electrical events that lead to a BOLD response).

### Conclusions

The use of functional neuroimaging has the potential to revolutionize our understanding of the neural basis of cognition, as well as to provide insight into the neural

mechanisms that underlie normal development, ageing and disease. Its high spatial resolution, coupled with its ability to assess correlates of neural activity while subjects are performing cognitive tasks, make it invaluable. However, caution must be taken to avoid misinterpreting the results of BOLD fMRI studies. The BOLD signal reflects the influence of neural activity on CBF, and therefore development-, age- or disease-related changes in resting CBF, vascular structure or neurovascular coupling might influence our ability to attribute BOLD signal changes to alterations in neural activity. Until new methods are developed to more closely link haemodynamic functional imaging with neural activity, care must be taken in all levels of study design, analysis and interpretation to maximize our ability to contribute valid insights to the literature on the neural mechanisms of cognition.

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