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Paradoxical correlation between signal in functional magnetic resonance imaging and deoxygenated haemoglobin content in capillaries: a new theoretical explanation

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Abstract

Signal increases in functional magnetic resonance imaging (fMRI) are believed to be a result of decreased paramagnetic deoxygenated haemoglobin (deoxyHb) content in the neural activation area. However, discrepancies in this canonical blood oxygenation level dependent (BOLD) theory have been pointed out in studies using optical techniques, which directly measure haemoglobin changes. To explain the discrepancies, we developed a new theory bridging magnetic resonance (MR) signal and haemoglobin changes. We focused on capillary influences, which have been neglected in most previous fMRI studies and performed a combined fMRI and near-infrared spectroscopy (NIRS) study using a language task. Paradoxically, both the MR signal and deoxyHb content increased in Broca's area. On the other hand, fMRI activation in the auditory area near large veins correlated with a mirror-image decrease in deoxyHb and increase in oxygenated haemoglobin (oxyHb), in agreement with canonical BOLD theory. All fMRI signal changes correlated consistently with changes in oxyHb, the diamagnetism of which is insensitive to MR. We concluded that the discrepancy with the canonical BOLD theory is caused by the fact that the BOLD theory ignores the effect of the capillaries. Our theory explains the paradoxical phenomena of the oxyHb and deoxyHb contributions to the MR signal and gives a new insight into the precise haemodynamics of activation by analysing fMRI and NIRS data.

1. Introduction

Functional magnetic resonance imaging (fMRI) has been used as a useful imaging technique in brain function research (Turner and Ordidge 2000). fMRI has better spatial resolution than other non-invasive techniques for investigating brain function, and it is also superior in temporal resolution to positron emission tomography (PET). MRI, like PET, reflects haemodynamic changes associated with neural activation. Understanding the haemodynamics of activation in fMRI studies strongly depends on an understanding of the theoretical mechanism of fMRI.

It is believed that an increase in regional cerebral blood flow (rCBF) results in a decrease in deoxygenated haemoglobin (deoxyHb). When deoxyHb decreases, magnetic field homogeneity improves because paramagnetic perturbation due to deoxyHb is reduced and magnetic resonance (MR) signal intensity thus increases. This fMRI mechanism has been described as the blood oxygenation level dependent (BOLD) theory (Ogawa *et al* 1993). The BOLD theory has led to the canonical understanding that signal changes in fMRI are negatively proportional to changes in deoxyHb content.

While deoxyHb content is believed to determine MR signals, MRI does not directly measure changes in deoxyHb content. On the other hand, the optical techniques of near-infrared spectroscopy (NIRS) and optical imaging have separately revealed changes in oxygenated haemoglobin (oxyHb) and deoxyHb during brain activation (Kato *et al* 1993a). These optical studies, together with fMRI measurements, have shown discrepancies with the canonical BOLD theory. An optical imaging and fMRI study of gerbil vibrissa stimulation showed that the MR signal increased even though deoxyHb increased in activation focus and that MR signal changes correlated better with total haemoglobin changes than with deoxyHb changes (Hess *et al* 2000). Spatially resolved optical imaging has revealed that an increase in rCBF does not always cause a decrease in deoxyHb, especially in an activation focus in tissue, and that the time course of greater changes in oxyHb content than in deoxyHb content is similar to the time course of rCBF (Malonek *et al* 1997). In human studies, the MR signal was found to increase without changes in deoxyHb in a motor task experiment (Kleinschmidt *et al* 1996). MR signal changes in fingers due to compression of the arm were consistent with changes in oxyHb rather than changes in deoxyHb (Kato *et al* 2001).

The question that therefore arises is why fMRI shows positive MR signal changes in spite of an increase in deoxyHb, which is a major cause of deterioration in magnetic field homogeneity (Kato *et al* 1993a, Kato and Takashima 2000). This discrepancy has been explained (Hess *et al* 2000) using the BOLD signal model of Buxton *et al* (1998), which utilizes the intravascular contribution in fMRI signal changes. This intravascular contribution, however, becomes less dominant at higher magnetic field strengths (Gati *et al* 1997). It is thus difficult to extend the explanation by the BOLD signal model of Buxton *et al* to higher magnetic field strengths. Why MR signals correlate with diamagnetic oxyHb but not with deoxyHb is another question.

To answer these questions, we developed a new theory of the relationship between MR signals and changes in the two haemoglobins. It is based on two equations from the first simulation of the BOLD effect (Ogawa *et al* 1993): one for the small vessels and another for the large vessels. The physical background of the difference between the two equations comes from the influence of the diffusion of water in a range of capillary sizes (Hajnal *et al* 1991). The effect of diffusion relaxes B_0 inhomogeneity around the blood vessels. When the blood vessel dilates, the relaxed area surrounding the vessel also increases. This increase in relaxed effect tends to result in an increased MR signal. The relative volume of the relaxed area to the blood vessel volume is large for the small vessels. Therefore, the relaxed effect is conspicuous for the small vessels. MR signal changes due to small vessels are dependent not

only on deoxyHb content, but also on vessel volume changes (Ogawa *et al* 1993). This volume dependence in the small vessels is a key issue in explaining the paradoxical phenomenon of MR signal increases with increasing deoxyHb.

True understanding of the BOLD changes requires taking into account the influence of the small vessels, i.e. capillaries. Evidence of high augmentability of the total haemoglobin content in the capillaries (Shockley and LaManna 1988) implies that MR signals can be changed by changes in capillary haemodynamics. Nevertheless, in fMRI, changes in MR signals arising from the capillaries have not been analysed because small changes were predicted (Ogawa *et al* 1993). Thus, the easily detected MR signal changes due to the large vessels have been widely used to support the canonical BOLD theory.

In this article, we describe a new theory to explain the discrepancy between the canonical BOLD theory and the relationship between fMRI signals and changes in deoxyHb content. A combined fMRI and NIRS experiment was performed using a language task as a typical example of the paradoxical phenomena of MR signals and haemoglobin changes. The validity of our theory is demonstrated by quantitative analysis of the fMRI and NIRS data.

2. Theory

2.1. Theoretical background

The first simulation of the BOLD effect derives the different relationships of the apparent transverse relaxation rate ($R_2{}^*$) for large vessels and small vessels (Ogawa *et al* 1993). $R_2{}^*$ due to large vessels is dependent only on deoxyHb content. On the other hand, $R_2{}^*$ due to small vessels depends on both deoxyHb content and blood volume in the vessels. Blood volume dependence in the small vessels can potentially explain the paradoxical correlation between MR signals and deoxyHb (Kato and Takashima 2000). $R_2{}^*$ due to small vessels is proportional to D^2/V, where D is the deoxyHb content and V is the vessel volume (Ogawa *et al* 1993). This means that even though D increases, it is possible for $R_2{}^*$ to decrease when V increases greatly.

While the influence of the small vessels on MR signals reflects a versatile combination of D and V, the predicted BOLD sensitivity per blood volume is smaller in small vessel areas than in large vessel areas (Ogawa *et al* 1993). However, MR signal changes due to the small vessels, i.e. capillaries, are not negligible because of the physiological evidence that the capillary volume is almost half the cerebral blood volume (Pawlik *et al* 1981) and is dominant in the activated area (Dunning and Wolff 1937, Borowsky and Collins 1989). We developed a new comprehensive theory that takes into account the influence of the capillaries, and to evaluate the validity of our theory with experimental data, we derived two relationships, one between oxyHb and MR signal changes and the other between deoxyHb and MR signal changes. The intravascular contribution is also included in our theory.

2.2. Relationship between extravascular MR signal and haemoglobin changes

Here, we start from the general equation for $R_2{}^*$ in each voxel:

$$R_2{}^* = c_1(1-Y)^p v = c_1 D^p v^{1-p} \tag{1}$$

where c_1 is a factor dependent on the vascularity, Y is oxygenation of the blood, v is the haemoglobin content, and p is an index ranging from 1 for large vessels to 2 for small vessels, i.e. capillaries, following the expression of Ogawa *et al* (1993) with a criterion radius of 8 μm between large and small vessels for convenience to represent qualitatively different effects. To develop our theory, we introduce a new term, apparent oxygen extraction

(AOE; μmol g^{-1} min^{-1}) defined as the deoxyHb content flowing from each region of interest (ROI) in 1 min. When activation occurs, AOE equals the activated cerebral metabolic rate of oxygen ($CMRO_2$; μmol g^{-1} min^{-1}) in an activation focus, whereas in a draining vein area, a relative increase of AOE is lower than that of the activated $CMRO_2$ because of tributary effects and contamination into the draining vein from the adjacent non-activation area. We newly define regional blood flow (rBF) (ml g^{-1} min^{-1}) as the blood flowing through each ROI in 1 min. Averaged rBF in tissue is equal to rCBF, which is the amount of arterial blood delivered to the capillary bed, whereas rBF in a draining vein area where the blood flow from tissue is concentrated is higher than rCBF. AOE and rBF have the following relationship by Fick's principle:

$$D = \frac{v \cdot \mathrm{AOE}}{H \cdot \mathrm{rBF}} \tag{2}$$

where H (μmol ml^{-1}) is the ferrous haeme concentration. In addition to Grubb's relationship between rCBF and regional cerebral blood volume (rCBV) (Grubb *et al* 1974), it is also possible to represent the relationship between rBF and v using an index α as follows:

$$v = c_2 \cdot \mathrm{rBF}^{\alpha} \tag{3}$$

where c_2 is a constant. When activation is associated with an increase in rBF, changes in haemoglobin content are larger for larger values of α. Combining the logarithmic differentiation of equations (2) and (3) to eliminate rBF gives the following equation:

$$\frac{\Delta D}{D} = \left(1 - \frac{1}{\alpha}\right)\frac{\Delta v}{v} + \frac{\Delta \mathrm{AOE}}{\mathrm{AOE}}. \tag{4}$$

This equation and the logarithmic differentiation of equation (1) to eliminate $\Delta D/D$ give the following relationship:

$$\frac{\Delta {R_2}^*}{{R_2}^*} = \left(1 - \frac{p}{\alpha}\right)\frac{\Delta v}{v} + p\frac{\Delta \mathrm{AOE}}{\mathrm{AOE}}. \tag{5}$$

In addition to Leenders's relationship between $CMRO_2$ and rCBF (Leenders *et al* 1990), the relationship between AOE and rBF can be written as

$$\frac{\Delta \mathrm{AOE}}{\mathrm{AOE}} = \beta\frac{\Delta \mathrm{rCBF}}{\mathrm{rCBF}} = \frac{\beta}{\alpha}\frac{\Delta v}{v} \tag{6}$$

where β is an index indicating the relationship between AOE and rBF. When activation is associated with an increase in rBF, changes in apparent oxygen extraction are larger for larger values of β. Substitution of equation (6) into equations (4) and (5) gives

$$\frac{\Delta D}{D} = (1 - h)\frac{\Delta v}{v} \tag{7}$$

$$\frac{\Delta {R_2}^*}{{R_2}^*} = (1 - p \cdot h)\frac{\Delta v}{v} \tag{8}$$

where the haemodynamic parameter h equals $(1 - \beta)/\alpha$. Equations (7) and (8) are combined into

$$\Delta D = \frac{(1 - h)(1 - Y)}{1 - p \cdot h}\frac{\Delta {R_2}^*}{{R_2}^*/v}. \tag{9}$$

The differential relationship between ${R_2}^*$ and extravascular MR signal intensity (S_e) is given by

$$-\Delta {R_2}^* = \frac{1}{\mathrm{TE}}\frac{\Delta S_e}{S_e} \tag{10}$$

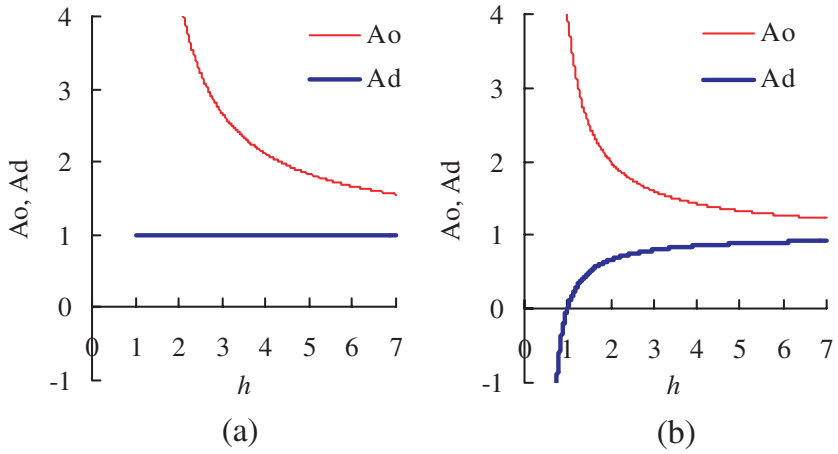

Figure 1. Different correlations between haemoglobin and MR signal changes in large vein and capillary areas. Ratios between oxyHb and MR signal (Ao), and between −deoxyHb and MR signal (Ad) as a function of the haemodynamic parameter h. (a) Large vein area ($p = 1$, $Y = 0.7$ (Kohl *et al* 2000). (b) Capillary area ($p = 2$, $Y = 0.5$ (Kohl *et al* 2000).

where TE is the echo time of a gradient echo imaging. Substituting equations (1) and (10) into equation (9) gives the following relationship between deoxyHb and extravascular MR signal changes

$$\Delta D = -\frac{Ad}{c_1 \cdot \mathrm{TE}} \frac{\Delta S_e}{S_e} \tag{11}$$

where

$$Ad = \frac{(1-h)\,(1-Y)^{1-p}}{1-p\cdot h}. \tag{12}$$

The differential relationship of the haemoglobin content is written as

$$\Delta v = \Delta O + \Delta D \tag{13}$$

where ΔO is the change in oxyHb. The following relationship is derived from equations (7) and (13)

$$\Delta D = \frac{(1-h)\,(1-Y)}{Y + h\,(1-Y)} \Delta O. \tag{14}$$

Substituting this equation into equation (11) gives the following relationship between oxyHb and extravascular MR signal changes

$$\Delta O = \frac{Ao}{c_1 \cdot \mathrm{TE}} \frac{\Delta S_e}{S_e} \tag{15}$$

where

$$Ao = \frac{h\,(1-Y) + Y}{(p\cdot h - 1)\,(1-Y)^p}. \tag{16}$$

Since it is intuitively obvious from the canonical BOLD theory that an increase in deoxyHb causes a decrease in MR signal intensity and vice versa for oxyHb, we defined the polarity of Ad and Ao so that positive values of Ad and Ao agree with this property. Ad and Ao are plotted as functions of h in figure 1. The slope of ΔD versus $\Delta S_e/S_e$ and the slope of ΔO versus $\Delta S_e/S_e$ are proportional to $-Ad$ and Ao respectively.

2.3. Influence of intravascular MR signal changes

MR signal changes (ΔS) are the sum of extravascular (ΔS_e) and intravascular (ΔS_i) contributions. The intravascular signal changes at 1.5 T are expressed as

$$\frac{\Delta S_i}{S} = V\left(2\frac{\Delta Y}{1-Y} + 0.6\frac{\Delta V}{V}\right) \tag{17}$$

where V is the blood volume fraction (Buxton *et al* 1998). Evidence that haematocrit in the capillaries increases with increasing rCBF (Johnson *et al* 1971, Mchedlishvili 1991) implies an increase in haematocrit during activation. Therefore, equation (17) should take haematocrit changes into account. The first term of equation (17) is derived from intrinsic intravascular signal changes determined by changes in the transverse relaxation rate (R_2) of the blood (Boxerman *et al* 1995). R_2 is a function of deoxygenation of the blood and haematocrit (Ht) (Thulborn *et al* 1982), written as

$$R_2 = K_1 + K_2\,(1-Y)^2\,f(Ht) \tag{18}$$

where K_1 is a static constant, K_2 is a B_0-dependent constant and $f(Ht)$ is a haematocrit factor peaking at $Ht = 50$, derived from figure 4 of the study of Thulborn *et al* (1982). Equation (17) can thus be rewritten as

$$\frac{\Delta S_i}{S} = V\left[2\frac{\Delta Y}{1-Y} - \frac{\Delta f(Ht)}{f(Ht)} + 0.6\frac{\Delta V}{V}\right]. \tag{19}$$

2.4. Capillaries

In the capillaries, the haematocrit may increase greatly during activation (Johnson *et al* 1971, Mchedlishvili 1991). Providing that the haematocrit increases from 30% (Lipowsky *et al* 1980, Hudetz 1997) to 50% and Y at rest is 0.5 (Meyer *et al* 2000, Kohl *et al* 2000), $\Delta f(Ht)/f(Ht)$ is calculated as 0.2 based on figure 4 of the study of Thulborn *et al* (1982). ΔY is assumed to be 0.08, which is half of the venous oxygenation changes (Liu *et al* 1999). For $V = 2\%$ (Pawlik *et al* 1981), $\Delta V/V = 0.2$ (Ito *et al* 2001), and $\Delta S_i/S$ is calculated to be 0.5%. The influence of the intravascular contribution in the capillaries may therefore be considered negligible compared to the observed signal changes in tissue where capillaries are rich: 1.9% (Gati *et al* 1997) and 3.4% (Yacoub and Hu 1999). In addition, it has been reported that the MR signal in an activation focus in tissue increases more than linearly (Gati *et al* 1997) and more than quadratically (Yacoub and Hu 1999) with respect to B_0. This evidence supports the hypothesis of a small contribution by the intravascular MR signal in the activation focus even at 1.5 T, because MR signal changes with a large intravascular contribution, i.e. those in large vessel areas, increase less than linearly with respect to an increase in B_0 (Gati *et al* 1997). We therefore assumed that MR signal changes in the capillary area can be represented by the extravascular contribution, even at 1.5 T.

In the case of the capillaries (in the above equations, $p = 2$), Ad varies from minus to plus dependence on h (figure 1(b)). If h is less than 1, i.e. the augmentability of the haemoglobin content is high (a high value for α) and there is a strong correlation between AOE and rBF (a high value for β), then the relationship between the changes in the MR signal and deoxyHb content becomes the reverse ($Ad < 0$) of the intuitively obvious relationship (figure 1(b)). When $h = 1$, Ad is zero. In this case, there is no change in deoxyHb when the MR signal changes, even though paramagnetism of deoxyHb is a major source of $R_2{}^*$.

The haemodynamic parameter h for the capillaries in an activated area is low. This h is calculated to be within a range of 0.8–1.6, using values for α and β derived from the literature: $\alpha = \ln(1 + \Delta\mathrm{rCBV}/\mathrm{rCBV})/\ln(1 + \Delta\mathrm{rCBF}/\mathrm{rCBF}) = 0.38$ (Grubb *et al* 1974) and

$\beta = \ln(1 + \Delta CMRO_2/CMRO_2)/\ln(1 + \Delta rCBF/rCBF) = 0.4$–$0.7$ (from a 16–30% increase in $CMRO_2$ with a 44% increase in rCBF (Kim *et al* 1999). Grubb's value (1974) for α is for the whole vasculature, including the capillaries, venules and large veins. The high flexibility (a high value for α) of the small vessels (Lee *et al* 2001) suggests that h for the capillaries is less than the values calculated using Grubb's value for α.

2.5. Large vessels

The haemodynamic parameter h may be higher in the large veins, since the lower expandability of the large veins (Lee *et al* 2001) corresponds to a low value for α. Like α, the index of AOE, or β, is also lower for the large veins. Since oxygen is extracted only in the capillaries, ΔAOE in the activated capillaries is transmitted to a large draining vein downstream from both the activated and the non-activated capillaries, whereas rBF increases in a wide area around the activation focus, an effect that has been described as a 'watering-the-garden' effect (Turner and Ordidge 2000). The increased rBF of this wide area also flows into the draining large vein. Changes in AOE are therefore diluted in the large veins by a large increase in rBF due to tributary flow from the 'watered-garden' area. Consequently, β for the large veins is smaller than that for the capillaries of an activation focus (equation (6)). The haemodynamic parameter h thus becomes higher.

The haematocrit varies less in the large vessels, and D/V is proportional to $1 - Y$:

$$\frac{\Delta Y}{1-Y} = -\frac{\Delta D}{D} + \frac{\Delta V}{V}. \tag{20}$$

Using equations (7) and (20), and $\Delta V/V = \Delta v/v$ for the large vessels, equation (17) is written as

$$\frac{\Delta S_{in}}{S} = -V\left(2 + \frac{2.6}{h-1}\right)\frac{\Delta D}{D} = -\frac{1}{n(1-Y)}\left(2 + \frac{2.6}{h-1}\right)\Delta D \tag{21}$$

where n is haemoglobin density per unit volume. Since the blood volume fraction is small, S is represented by S_e, and $\Delta S_e/S = \Delta S_e/S_e$. Using equation (11), the total signal change, which is the sum of the extra- and intra-vascular signal changes, is given by

$$\frac{\Delta S}{S} = \left[1 + \frac{Ad}{c_1 \cdot \mathrm{TE} \cdot n(1-Y)}\left(2 + \frac{2.6}{h-1}\right)\right]\frac{\Delta S_e}{S_e} \tag{22}$$

In the case of the large vessels (in equations (12) and (16), $p = 1$), which have a high value for h, Ad is equal to 1 constantly across hs (figure 1(a)). Consequently, the second term in the bracket of equation (22) is a positive value and is written as q. The total MR signal changes including the intravascular contribution are thus proportional to the extravascular signal changes. Equations (11) and (22) give

$$\Delta D = -\frac{A_d}{c_1 \cdot \mathrm{TE} \cdot (1+q)}\frac{\Delta S}{S}. \tag{23}$$

The influence of the intravascular signal changes in the large vessels changes Ad to $Ad/(1 + q)$. The same influence on Ao is derived as $Ao/(1 + q)$. Hence, the correlation between the MR signal and the haemoglobin changes is represented by the extravascular correlation (Ad and Ao) in the large vein areas, even though the MR signal includes the intravascular contribution.

A constant value of 1 for Ad indicates that the MR signal changes are always negatively proportional to changes in deoxyHb content (equation (11)). This relationship corresponds to the canonical BOLD theory that $R_2{}^*$ is quantitatively determined only by deoxyHb content. Ao is also positive, indicating a good correlation between oxyHb and the MR signal.

Positive values of both Ao and Ad reflect the increase in oxyHb and decrease in deoxyHb during activation that are intuitively obvious according to the canonical BOLD theory. This complementary change in oxyHb and deoxyHb can also be called a mirror-image change, although the theoretical result $Ao > Ad$ (figure 1(a)) implies that the amplitude of change for oxyHb is always larger than that for deoxyHb. The difference in the amplitude of change narrows when h increases, approaching a complete mirror-image change.

2.6. Paradoxical correlation of changes in oxyHb and deoxyHb with MR signal changes

Even when the influence of the intravascular contribution is included, Ao and Ad represent the correlation between MR signal and haemoglobin changes for both the capillaries and the large vessels. Since Ao is always positive in both capillary and large vein areas (figure 1), changes in oxyHb correlate consistently with MR signal changes. Moreover, Ao is always larger than Ad. This implies a better correlation between oxyHb and MR signal changes than between deoxyHb and MR signal changes, and in fact, deoxyHb changes do not necessarily correlate with MR signal changes because of the multi-polarity of Ad (figure 1(b)). In particular, when Ad is negative, the paradoxical correlation between MR signal and deoxyHb occurs. Capillary dominance is thus a key to the paradoxical correlation, whereas the large vein contribution acts separately from the paradoxical correlation.

The MR signal change in each voxel is the sum of the contributions of both large and small vessels. The different vascular contributions in grey matter, however, have been identified for microvascular and large vessel regions (Prinster *et al* 1997). The analysis by Prinster *et al* found tissue dominant activation in the visual cortex (Menon *et al* 1994) and large vein dominant activation in the motor cortex (Bandaittini *et al* 1994). It is therefore plausible to discuss activated areas in terms of differing dominance of capillaries and large veins. In our theory, a smaller value for h indicates capillary dominance and a larger value for h indicates large vein dominance. An intermediate value for h indicates comparable contributions from large and small vessels. An activation focus in tissue tends to be capillary rich (Dunning and Wolff 1937, Borowsky and Collins 1989), and the paradoxical correlation may therefore be a phenomenon specific to tissue activation focus.

3. Materials and methods

3.1. Measurement site

To observe experimentally the paradoxical difference between results obtained with fMRI and NIRS, we used a multiple-word repetition task (Kato *et al* 1998a, 1999a) as a typical example of this paradoxical phenomenon. Reproducible evidence has been reported of increases in both MR signals at 1.5–4.0 T (Kato *et al* 1998a, Frost *et al* 1999) and deoxyHb content in the activated Broca's area (Sakatani *et al* 1998, Watanabe *et al* 1998, Kato *et al* 1999a). A lesser increase or no increase at all in deoxyHb in the right inferior frontal area and the auditory area, as compared to that in Broca's area of the left inferior frontal area, has also been reproducibly observed (Watanabe *et al* 1998, Kato *et al* 1999a). We therefore chose the left prefrontal cortex and the left temporal lobe as the areas for our fMRI and NIRS measurements.

3.2. Multiple-word repetition task

A female subject (S) is given the name of an animal once and instructed to repeat it to herself silently. For example, S hears 'cat', and then repeats 'cat' to herself silently. S is then given

the names of five different animals aurally in the same way at a rate of one per 2.0 s. This task is a true single trial task with a 10 s period. S performed this task only once for both fMRI and NIRS experiments. Activation by single-word processing can be sustained for 40–60 s (Kato *et al* 1998a, 1998b, 1999a). This type of activation may cause interference with the next activation in the event-related fMRI. The task was therefore performed only once, to initiate a pure fMRI time course with a long-sustained activation.

3.3. fMRI experiments

The fMRI study was conducted with a 1.5 T system (Vision; Siemens, Erlangen, Germany) using a volume head coil. An MPRAGE (magnetization-prepared rapid gradient echo) imaging sequence was used to acquire 3D anatomical images. The imaging parameters of this 3D sequence were as follows: TR, 15 ms; TE, 7 ms; flip angle, 8°; field of view, $220 \times 220\,\mathrm{mm}^2$ in axial view; matrix size, 256×256; and slab thickness, 156 mm with 60 partitions. 128 temporal datasets of eight oblique slices (figure 2(a)) were obtained sequentially using a $T_2{}^*$-weighted EPI sequence: TR/TE, 1400/60 ms; field of view, $220 \times 220\,\mathrm{mm}^2$; flip angle, 90°; matrix size, 128×128; slice thickness, 7.0 mm; and slice gap, 0.2 mm. These multi-slice images covered the left hemisphere, including Broca's area and the auditory area (figure 2(a)). The task was started 39.2 s after the start of EPI. The initial two-thirds of the data before the task were discarded to obtain settled control data.

3.4. MRI data analysis

Maps of functional activation were generated with the STIMULATE software package (Minneapolis, MN, USA) (Strupp 1996) from EPI images acquired during the task period (10 s) and a control period (10 s) before the task. Each period contains six temporal imaging points. We used Student's *t*-test ($p < 0.05$) with a clustering over 20 pixels for the activated pixels with a signal increase of over 0.1%. Anatomical images corresponding to each EPI slice were obtained automatically from the dataset of the 3D anatomical images. Activation areas on these anatomical images were mapped by the software.

3.5. NIRS experiments

OxyHb and deoxyHb were estimated using an NIRS system with multi-channel detection (ETG-100; Hitachi Medical Co., Tokyo, Japan) (Maki *et al* 1995). Light for NIRS was directed from two laser diodes into the head through a bundle of optical fibres (1 mm in diameter). Near-infrared light with wavelengths of 780 and 840 nm was used. The distance between the photon probes was 30 mm. Depth selectivity of 15–20 mm from the probe surface (Kato *et al* 1993a) was focused on the cortices measured. The influence of blood in the extra-cranial tissue was considered to be negligible (Samur *et al* 1999). The sampling time for photon counting was 500 ms. Changes in oxyHb and deoxyHb were calculated using the differences in absorption indices at two wavelengths (780 and 840 nm). The plate ($90 \times 90\,\mathrm{mm}^2$) with a 5×5 matrix array of photon probes was placed on the left skull surface. In the NIRS experiment, the central area between two probes is the most sensitive (Kato *et al* 1993a). This was proved by the simulation of a model phantom (Okada 2000). To locate three NIRS-sensitive regions at the expected activation areas, three channel centres between the light source and the detector were carefully fitted to the left inferior frontal gyrus and the left superior temporal gyrus using 3D anatomical images with 16 fiducical markers in the area of measurement (figure 2(b)). Knowing the relative positions of the activated area and the

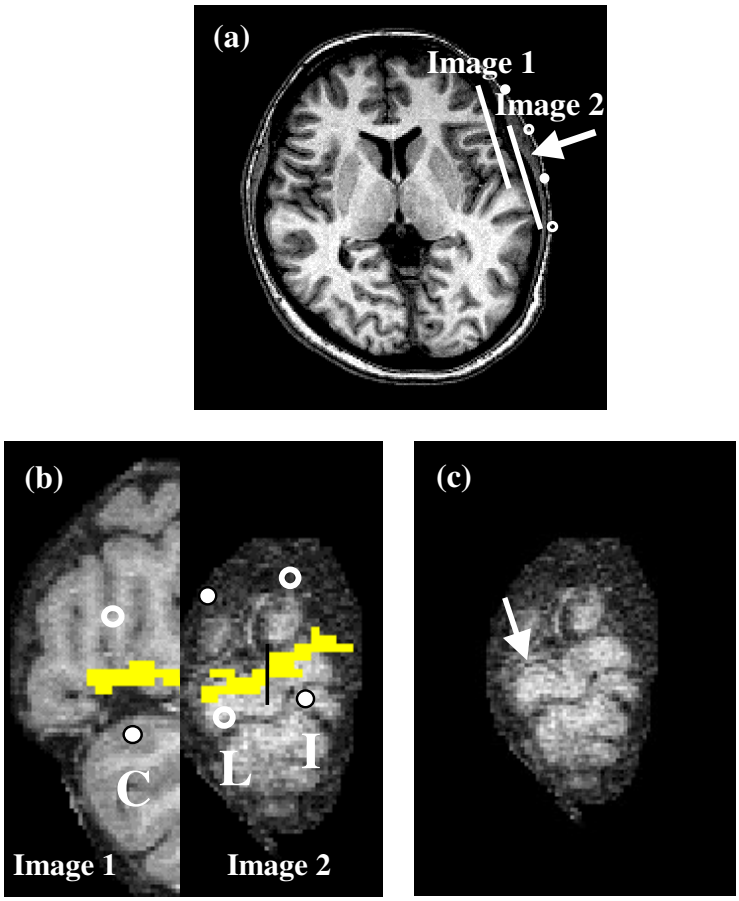

Figure 2. Location of NIRS and fMRI measurements. (a) fMRI slice positions and NIRS probe positions. Image 1 shows a slice containing Broca's area. Image 2 shows a slice containing the auditory area. The arrow indicates the viewpoint of (b). (b) Activation map and NIRS probe positions. Dots and circles represent light source and detector positions, respectively. C, capillary area: inferior frontal area (28 pixels). L, large vein area: anterior superior temporal gyrus (20 pixels). I, intermediate area: middle superior temporal gyrus (23 pixels). Activated pixels in Image 2 are divided into a large vein area and an intermediate area by the black line between the two NIRS channels. (c) Anatomical image of the auditory area. An arrow indicates a large draining vein.

fiducical markers enabled us to correctly position the NIRS probes for sensing the activated area observed by fMRI.

4. Results

The NIRS (figures 3 and 4) and fMRI (figure 5) time courses were observed in the left inferior frontal gyrus (Broca's area) and in the anterior areas in the left superior temporal gyrus (auditory cortex). Activation in Broca's area was due to haemodynamic changes in the capillaries, because the anatomical images showed no large veins in the vicinity of the activated pixels. Both oxyHb and deoxyHb in Broca's area were increased greatly by activation (figure 3(a)). However, oxyHb and deoxyHb in the auditory cortex, which included large draining veins (figure 2(c)), showed a mirror-image increase in oxyHb and decrease

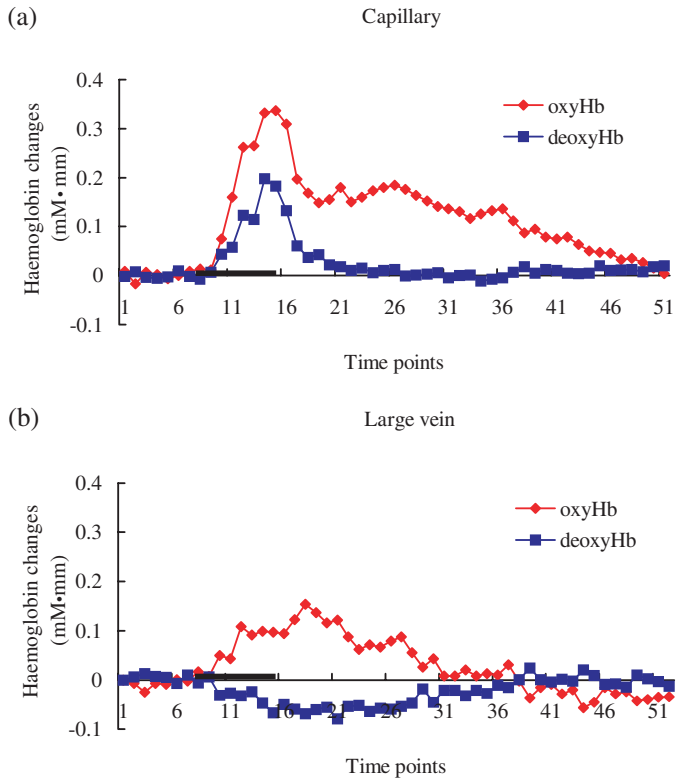

Figure 3. Time course of oxyHb and deoxyHb by NIRS in the capillary area (a) (C in figure 2(b)) and the large vein area (b) (L in figure 2(b)). Thick horizontal bars indicates the 10 s task period. To match the time points with the fMRI data, the NIRS data were plotted every 1.4 s.

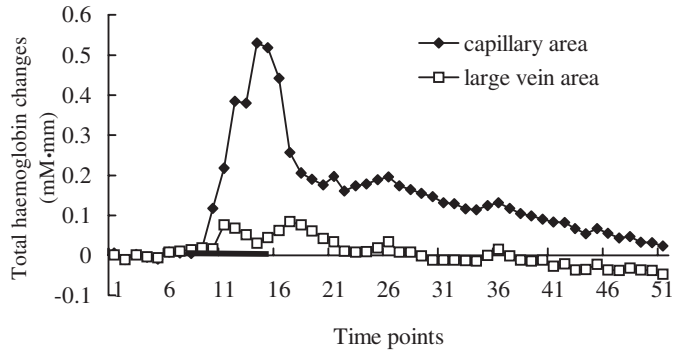

Figure 4. Time course of total haemoglobin (oxyHb + deoxyHb) in the capillary area (C in figure 2(b)) and the large vein area (L in figure 2(b)). A thick horizontal bar indicates the 10 s task period. To match the time points with the fMRI data, the NIRS data were plotted every 1.4 s.

in deoxyHb (figure 3(b)). Total haemoglobin (oxyHb + deoxyHb) increased conspicuously in Broca's area (figure 4), indicating a large increase in haemoglobin content in the active capillaries, whereas total haemoglobin in the auditory area showed only a small increase (figure 4), reflecting the stiffness of the large draining veins.

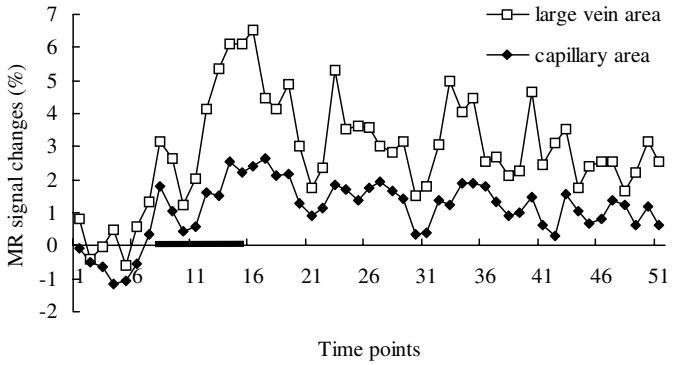

Figure 5. Time course of per cent MR signal changes in the large vein area (L in figure 2(b)) and the capillary area (C in figure 2(b)). A thick horizontal bar indicates the 10 s task period. To clearly show the signal changes associated with the task, 51 data points out of 128 were displayed.

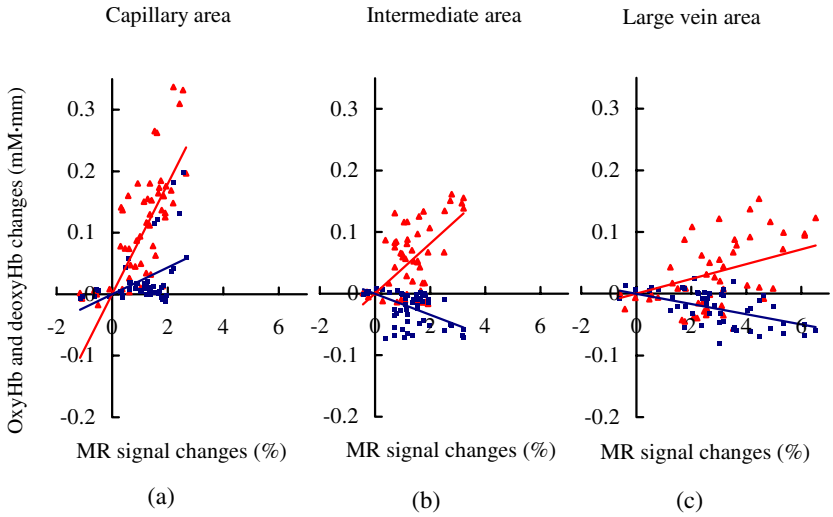

Figure 6. OxyHb (red) and deoxyHb (blue) changes versus MR signal changes in the capillary area (a) (C in figure 2(b)), the intermediate area (b) (I in figure 2(b)) and the large vein area (c) (L in figure 2(b)). The lines are linear regression lines crossing origin points determined by averaging of rest values during the 10 s before the task.

The MR signal change time courses in both Broca's area and the auditory area showed similar increases. The MR signal changes in Broca's area were smaller than those in the auditory area, which included large veins (figure 5). The smaller signal changes in Broca's area reflect the lesser influence of deoxyHb on $R_2{}^*$ in the capillaries. As for sensitivity to activated haemodynamic changes in Broca's area, measurement of the MR signal showed less sensitivity than measurement of haemoglobin changes by NIRS.

To observe the correlation between the MR signal and haemoglobin changes in Broca's area and the auditory cortices, the oxyHb and deoxyHb changes were plotted with respect to the per cent MR signal changes (figure 6). The polarity of the slope of deoxyHb versus MR signal varied with the activated area. This tendency was significant: ±95% confident ranges for the slopes were 36, 23 and 23% in the capillary, intermediate and large vein areas, respectively. The capillary-dominant Broca's area showed the paradoxical relationship of a

positive slope of deoxyHb versus the MR signal. Another paradoxical conclusion, the fact that oxyHb correlated consistently with the BOLD changes, can be drawn from the evidence that the slope of the graph of oxyHb changes versus MR signal changes was always positive. The slope of oxyHb changes versus MR signal changes was least in the large vein area and gradually increased through the intermediate area to the capillary area: ±95% confident ranges for the slopes were 15, 20 and 23% in the capillary, intermediate and large vein areas, respectively. On the other hand, the slope of deoxyHb changes versus MR signal changes negatively peaked in the intermediate area. When there are great differences in vascularity, the slopes cannot be used for precise comparison of haemodynamics in activation areas, because the slopes are a function of the unknown vascular factors c_1 (equations (11) and (15)), p (equations (12) and (16)) and q for the intravascular contribution.

To analyse the haemodynamics in the activation areas, we determined the haemodynamic parameter h by values of $\Delta D/\Delta O$ obtained from the slope of the graph of deoxyHb changes versus oxyHb changes (graph not shown). Since $\Delta D/\Delta O$ equals $-Ad/Ao$ from equations (11) and (15), $\Delta D/\Delta O$ is also the ratio of the slopes for each area in figure 6. The values obtained for $\Delta D/\Delta O$ are substituted for the theoretical values in equation (14):

$$\frac{\Delta D}{\Delta O} = \frac{1-h}{h+Y/(1-Y)}. \tag{24}$$

Since equation (24) is a differential equation, Y in the equation should have a value at each temporal point during activation. In substituting the acquired data in equation (24), we used Y values from the literature (0.5 for capillary areas, 0.7 for other areas; Kohl *et al* 2000) with plausible and sufficient deviations (±0.1) between rest and activation. The parameter *hs* thus obtained reflects the different haemodynamics of haemoglobin-content augmentability (α) and apparent oxygen extraction (β). The haemodynamic parameter h decreased gradually from 8.4 (6.5–12.1 for $Y = 0.6$–0.8) in the large vein area through 3.4 (2.8–4.5 for $Y = 0.6$–0.8) in the intermediate area to 0.6 (0.5–0.7 for $Y = 0.6$–0.4) in the capillary area. The largest value for the haemodynamic parameter h was found in the large vein area and indicates a small value for α, reflecting the stiffness of the large veins. The smallest value for h, from the capillary area, reflects high oxygen extraction and high augmentability of haemoglobin content. The intermediate value of h from the intermediate area reflects haemodynamic properties between those of the capillaries and the large veins.

Paradoxical results were observed from the MR signal changes and deoxyHb changes in Broca's area. The canonical BOLD theory does not explain an increase in deoxyHb accompanied by an increasing MR signal. On the other hand, canonical BOLD changes were observed in the large vein areas, where an increase in rCBF resulted in an increase of oxyHb and a concomitant dilution of deoxyHb during activation. The amplitude of change for oxyHb was always larger than that for deoxyHb (figure 3), in accordance with the theoretical results of $Ao > |Ad|$ (figure 1). This result agrees with the good correlation of changes in oxyHb, rather than changes in deoxyHb, with MR signal changes.

5. Discussion

5.1. A bridging theory for the paradoxical correlation

We developed a new theory to explain the paradoxical correlation between MR signals and deoxyHb changes, namely, an increase in MR signal intensity together with an increase in deoxyHb. The correlation between MR signal and deoxyHb is expressed theoretically by Ad (equation (12)). This paradoxical correlation occurs when Ad is negative for a haemodynamic

parameter of $h < 1$ in figure 1(b). This lower value for h indicates a higher value for β, reflecting a strong correlation between AOE and rBF, and may indicate higher oxygen consumption. Other studies have also demonstrated this paradoxical correlation and higher oxygen consumption in Broca's area by means other than the language task used in this study (Sakatani *et al* 1998, Watanabe *et al* 1998, Kato *et al* 1999a). Imamura *et al* (1997) found that in spite of an increase in oxyHb, deoxyHb did not change during electrical stimulation in the cat visual cortex, indicating $h = 1$. Visual stimulation has been shown to increase or slightly reduce deoxyHb in cats (Vanzetta and Grinvald 1999) and humans (Kato *et al* 1993a, 1999b), whereas oxyHb only increases, reflecting the fact that h varies around 1 in the capillaries (figure 1(b)). Focal epileptic status has been shown to cause increases in both MR signals (Warach *et al* 1996) and deoxyHb content (Adelson *et al* 1999), corresponding to negative values for h.

Capillary dominance in the activation area is a key structural cause of the paradoxical correlation. Activation in tissue alone does not follow the canonical BOLD theory. Even though the capillaries are dense in tissue (Dunning and Wolff 1937, Borowsky and Collins 1989) and their volume is large (Lipowsky *et al* 1980), the average per cent MR signal change observed in tissue was unfortunately observed to be less than the change due to large vessels (Gati *et al* 1997). Krings *et al* (1999) reported values of 2.3% for parenchymal activation and 7.3% for large superficial bridging veins in a human study at 1.5 T, and a negligible contribution of capillaries to activated signal changes in fMRI at 1.5 T has also been reported (Hoogenraad *et al* 2001). These results showing the lesser importance of the capillaries might be attributed to the fact that the investigators used a large region of interest, in which small signal changes in the activated tissue are contaminated by large signal changes due to the large veins.

We observed the paradoxical correlation between MR signal and deoxyHb in Broca's area. MR signal changes in activation in this area can be considered to be due to the capillaries. In addition to the absence of the observed large veins in the vicinity of the activated Broca's area, the small amplitude of MR signal changes in this area is suggestive of capillary-dominant haemodynamics. Maximal signal changes of 2.7% in Broca's area (figure 5) as strong activation (Sakatani *et al* 1998, Watanabe *et al* 1998, Kato *et al* 1999a) are similar to the positive MR signal changes (3.4%) observed in tissue areas with strong visual stimulation of 8 Hz flashing light at 1.5 T (Yacoub and Hu 1999). The investigators in this visual stimulation study reported signal changes of around 6% in large vein areas (Yacoub and Hu 1999). This signal change amplitude agrees with our results of maximum MR signal changes of 6.5% in the large vein area (figure 5).

The haemodynamics in the activation focus are essential to understanding the physiological changes relating to neural activation. Unfortunately, in fMRI, the important signal changes in the activation focus are small. Physiological explanations of activation based on fMRI data analysis have therefore been controversial and unclear. Even though the MR signal increase in the activation focus is small, by combining this information with NIRS data, haemodynamics during activation can be better understood. With our theory, the haemodynamic parameter h is obtained from the ratio $\Delta D/\Delta O$ measured by NIRS. $\Delta D/\Delta O$ is a function of h and Y, and is independently obtained from unknown vascularities in each activation area. If we assume a value for Y, h can be obtained. The derived value for h makes it possible to calculate detailed haemodynamic information during activation through the indices α and β, relating to haemoglobin-content augmentability and apparent oxygen extraction, respectively. These indices represent haemodynamics, because a strict coupling has been found in the relationships between rCBF and rCBV, and between rCBF and $CMRO_2$ (Leenders *et al* 1990).

The spatial selectivity of NIRS measurement is essential for deriving the haemodynamic parameter h. OxyHb and deoxyHb changes measured by NIRS are sensitive to the probe position (Kleinschmidt *et al* 1996). To separately measure activation in capillary and large vein areas by NIRS, we positioned the probes so as to correctly obtain data from each area activated in fMRI (see section 3 (figure 2(b))). Successful probe positioning enabled us to observe a large difference in total haemoglobin changes between the capillary and large vein areas (figure 4).

If haemoglobin content is always proportional to rCBV, α can be derived from data on rCBF and rCBV changes. In rat forepaw stimulation studies using the high susceptibility contrast agent MION (monocrystalline iron oxide nanocolloid), the results of Mandeville *et al* yielded values of $\alpha = 0.36$ (Mandeville *et al* 1999a) and $\alpha = 0.33$ (Mandeville *et al* 1999b) for rapid elastic capillary response. A study using an iron oxide contrast agent indicated that Grubb's value ($\alpha = 0.38$) is slightly low (Kennan *et al* 1998). MR measurements with perfluorocarbons were used to derive $\alpha = 0.75$ for small arterial vessels and $\alpha = 0.15$ for veins (Lee *et al* 2001). Since the contribution of capillary volume is absorbed into its arterial and venous constituents in MR measurements with perfluorocarbons (Duong and Kim 2000), high values for α in small arterial vessels may suggest a higher fractional volume change in the capillaries. This would indicate higher values for α than Grubb's value.

When a high susceptibility contrast agent is used for detecting rCBV changes, the rCBV data reflect plasma volume, because high-susceptibility contrast agents are freely soluble in plasma, and the measured rCBV changes therefore do not represent changes in red blood cell (RBC) content. On the other hand, optical measurements are sensitive to changes in RBC. High augmentability of RBC content in capillaries has been reported in studies using optical methods. Shockley and LaManna (1988) found that maximum RBC content during hypercapnia was 3.6 times larger than that at normoxic normocapnea in rats. From their results, we calculated values for α of 0.51 (hyercapnic), 0.57 (hypoxic) and 0.84 (hypoxic hyperventilated). When capillary volume is increased by only 12% in hypercapnia (a 6% increase in capillary diameter), the RBC content in the capillaries has been shown to be greatly increased (Villringer *et al* 1994). This evidence of high augmentability of RBC content in the capillaries reflects a higher value for α. The value for β also is higher in tissue areas where oxygen is delivered directly to the tissue. Hence, of all the areas of activation, the capillary areas have the lowest values for the haemodynamic parameter h.

The theoretical $h = 8.4$ for large vein areas gives $\alpha = 0.12$ (higher limit value within 95% confidence: $\alpha = 0.23$) with a negligible value for β in the large veins (see section 2.5). Since the large veins are harder than the small vessels (Lee *et al* 2001), α for large veins is less than the averaged value of 0.38 (Grubb *et al* 1974). The large vein value of $\alpha = 0.15$ calculated from the results of the perfluorocarbon MR study (Lee *et al* 2001) is within our theoretical range. A value for h of the intermediate area (3.4) less than that of the large vein area (8.4) reflects a higher value of α and/or β in the intermediate area than in the large vein area. When the small vessel contribution increases, average expandability of the blood vessels increases (Lee *et al* 2001). This results in a higher value for α. We interpret the higher value for β to mean that since the intermediate area is closer to the activation tissue, tributary contributions from the non-activated area in this area are less than in the large vein area (see section 2.5).

The value of β defined in equation (6) can be calculated as a value of activation focus $\beta = \ln(1 + \Delta CMRO_2/CMRO_2)/\ln(1 + \Delta rCBF/rCBF)$. Values of β derived from the literature are summarized in table 1. Imamura *et al* (1997) found that electrical stimulation in the visual cortex caused an increase in oxyHb with no change in deoxyHb, indicating $h = 1$ (figure 1(b)). While this is a paradoxical case of activation without dilution of deoxyHb during activation,

Table 1. Index β of the relation between $CMRO_2$ and rCBF.

Literature	Stimulus	Subject	Modality	β
Fox *et al* (1988)	Visual	Human	PET	0.1
Mandeville *et al* 1999a	sensory	rat	MRI	0.4
Davis *et al* (1998)	Visual	Human	MRI	0.4
Hoge *et al* (1999)	Visual	Human	MRI	0.5
Kim *et al* (1999)	Visual	Human	MRI	0.4–0.7
Imamura *et al* (1997)	Visual[a]	Cat	NIRS	0.4–0.6[b,c]
Present study	Language	Human	NIRS/MRI	0.7–0.8[b,d]

[a] Electrical stimulation in visual cortex.
[b] Using $\alpha = 0.57$ (Shockley and LaManna 1988) and $\alpha = 0.38$ (Grubb *et al* 1974).
[c] Using $h = 1$ as the haemodynamic parameter because there was no change in deoxyHb during activation.
[d] Using $h = 0.6$ as the haemodynamic parameter in Broca's area.

this derived value of β is within the range of the values for visual stimulation in the literature, excepting the value from the PET study (Fox *et al* 1988). In our study, β in the activated Broca's area was higher than that in the visual area in the literature (table 1). This suggests a large augmentation of oxygen consumption in Broca's area during activation.

5.2. Haematocrit

To understand oxygen consumption precisely, the haematocrit must be taken into account. In our theory of the extravascular signal changes, for simplicity, we assumed a constant haematocrit. Here, we suppose the relationship between an increase in haematocrit (Ht) and an increase in rBF to be $\Delta Ht/Ht = \gamma\,\Delta\mathrm{rBF}/\mathrm{rBF}$. Since Ht is proportional to the ferrous haeme concentration H in equation (2), inclusion of Ht variation in the theory alters the parameter h as $(1-\beta+\gamma)/\alpha$. Since γ should be positive in capillary areas (Johnson *et al* 1971, Mchedlishvili 1991), β derived from the obtained h in this study may actually be greater than that shown in table 1. Precise determination of oxygen extraction requires knowing the haematocrit changes as well as the haemoglobin-content augmentability.

For the large veins, in which there is lesser variation of haematocrit, the relationship between intravascular MR signal changes and haemoglobin is proportional to the relationship between the extravascular MR signal changes and haemoglobin (equation (23)). Thus, Ad and Ao derived from the theory of the extravascular MR signal changes represent the relationship of the total MR signal changes to the haemoglobin changes in the large vein area.

Hess *et al* (2000) explained the paradoxical correlation between BOLD changes and haemoglobin change based on the intravascular signal changes (Buxton *et al* 1998). Their theory cannot, however, explain our results of a large increase of deoxyhaemoglobin. In the explanation of Hess *et al*, using $Y = 0.7$ at rest, the conditions for increasing MR signal intensity with increasing deoxyHb content are calculated as $\Delta D/\Delta O < 0.2$ at 1.5 T (Buxton *et al* 1998), and $\Delta D/\Delta O < 0.1$ at 4.7 T (Hess *et al* 2000). The maximum $\Delta D/\Delta O$ in our experiment was 0.6 at 1.5 T (figure 3(a)). This value is far out of the range of the conditions for the explanation by Hess *et al*, but our theory explains the paradoxical correlation between MR signal changes and deoxyHb changes even in this case. The explanation of Hess *et al*, which focuses on the intravascular signal changes, is harder to apply at higher magnetic strengths (Buxton *et al* 1998). The capillary–extravascular contribution to the MR signal changes increases at higher magnetic field strengths. Our theory is a more accurate fit for these extravascular signal changes in capillary area.

The intravascular influence is small in the capillaries in an activation focus. In the capillaries of an activation focus, the haematocrit may increase during activation. The increase in haematocrit counteracts the increase in the intravascular MR signal because of an increase in the blood R_2 due to haematocrit changes (equation (18)). The estimated intravascular signal change (0.5%) in the capillaries (see section 2.4) is not enough to explain the maximal signal change (2.7%) in Broca's area where capillary dominance is known from the low haemodynamic parameter ($h = 0.6$). Evidence that the percentage signal change in an activation focus increases quadratically with an increase of B_0 from 1.5 to 4 T (Yacoub and Hu 1999) supports the hypothesis of a dominant extravascular contribution in capillaries even at 1.5 T.

5.3. Vasomotor regulation in microcirculation

Vasomotor regulation is an important factor in the haemodynamic response in the capillaries (Mchedlishvili 1986). Capillary-related activation has been observed in human studies using fMRI (Yacoub and Hu 1999). Although these studies showed an initial negative pattern (a so-called initial dip, duration of 3 s) in the MR signal changes, the question of whether this initial negative signal originates directly from the increased deoxyHb is still controversial (Malonek *et al* 1997). A human visual stimulation study using NIRS showed that oxyHb decreased commensurately with the initial-dip duration of 3 s, and that deoxyHb increased over 3 s incommensurately with the initial-dip duration (Kato *et al* 1999b). Similar findings have been observed in a cat study using high-resolution optical imaging (Malonek and Grinvald 1997), and a columnar initial dip was also reported by Kim *et al* (2000). Based on our theory, it is not necessary for the initial dip to always correlate with the increased deoxyHb. Even though there is no change in deoxyHb ($h = 1$) in the initial stage of strong neuronal activity, if oxyHb decreases commensurately with the initial duration of 3 s, a negative dip in the MR signal could occur. This is inferred from the phenomenon of vasoconstriction (Mchedlishvili 1991, Mchedlishvili *et al* 1967) and a lag in the increase of CBF (Malonek *et al* 1997) during cortical activation.

MR signal changes in activation have generally been discussed as a vasodilation phenomenon. However, cerebral micro-circulation is also controlled by vasoconstriction. Mchedlishvili *et al* (1967, 1991) observed the constriction of the cortical vessels (diameter 10–30 μm) and dilation of the pial arteries (diameter 45–150 μm) during cortical activation. Paradoxical evidence of MR signal changes and haemoglobin changes in vasoconstriction has also been reported recently. Sympathetic nerve activation during mental calculation vasoconstricts the blood vessels of the fingers, decreasing the blood flow (Brod *et al* 1959). In this case, Kato *et al* (2001) found that both MR signals and deoxyHb decreased. Nevertheless, the canonical BOLD theory simply predicts an increase in the MR signal with a decrease in deoxyHb. The post-negative undershoot after the offset of visual stimulation, which is accomplished by decreased oxyHb and total Hb and small changes of deoxyHb, is known from NIRS studies (Kato *et al* 1993a). This mechanism can be explained by vasoconstriction after vasodilation during visual stimulation. Similarly, visual stimulation in sleeping infants induced negative MR changes (Born *et al* 1998). Vasoconstriction may occur due to stimulation in infants, because the organization of the capillaries, with low blood vessel resistance resulting in high rCBF even at rest, is not matured enough to deliver oxygen to tissue.

Would constriction in microvessels during the initial dip period occur before vasodilation of the pial arteries for the blood supply? How can we explain the microcirculatory responses in the pial artery and micro-vessels to neural activity? Capillary dynamics in particular are not simple (Mchedlishvili 1991, Hudetz 1997). The blood oxygenation level in the capillaries

does not always parallel that in the large veins. Our new theory including the influence of dynamic changes in RBC content, i.e. dynamic haematocrit changes, makes it is possible to explain non-BOLD phenomena in the capillaries. It is important to find a model that truly reflects the physiological phenomena. When we use an inadequate model to analyse fMRI data, it is easy to misread the true physiology of activation. Indeed, fMRI of brain activation has been often criticized as an inaccurate method that is used to detect regional activation only approximately (Van Zijl *et al* 1998). Combined studies of fMRI, NIRS and microscopy could lead to a true understanding of activation physiology.

6. Conclusion

The paradoxical correlation between MR signal and deoxyHb changes is explained clearly by our new theory. This theory also answers the question of why MR-insensitive oxyHb correlates better with the MR signal than does paramagnetic deoxyHb. This study throws light on the important physiological function of the capillaries in a true understanding of signal changes in fMRI. Haemodynamics derived from fMRI data may have been previously misunderstood because the canonical BOLD theory used for their analysis does not apply to data based on the capillaries. The paradoxical correlation between MR signals and deoxyHb is proof of activated capillaries. fMRI is less sensitive to haemodymanic changes in the capillaries, whereas NIRS is sufficiently sensitive to capillary haemodynamics. A combination of NIRS and fMRI measurements could be used to reveal activation haemodynamics specific to vascularity. Our new theory bridging between fMRI and NIRS supports the idea that the combined measurements using both NIRS and fMRI may result in improved insight into brain physiology (Kato *et al* 1993b).

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