Localization of the Hand Motor Area by Arterial Spin Labeling and Blood Oxygen Level-Dependent Functional Magnetic Resonance Imaging

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Abstract: The new clinically available arterial spin labeling (ASL) perfusion imaging sequences present some advantages relatively to the commonly used blood oxygen level-dependent (BOLD) method for functional brain studies using magnetic resonance imaging (MRI). In particular, regional cerebral blood flow (CBF) changes are thought to be more directly related with neuronal activation. In this study, we aimed to investigate the accuracy of the functional localization of the hand motor area obtained by simultaneous CBF and BOLD contrasts provided by ASL functional MRI (fMRI) and compare it with a standard BOLD fMRI protocol. For this purpose, we measured the distance between the center of gravity of the activation clusters obtained with each contrast (CBF, BOLD_{ASL}, and Standard BOLD) and 11 positions defined on a well-established anatomical landmark of the hand motor area (the omega in the axial plane of the precentral gyrus). We found that CBF measurements were significantly closer to the anatomical landmark than the ones obtained using either simultaneous BOLD_{ASL} or standard BOLD contrasts. Moreover, we also observed reduced intersubject variability of the functional localization, as well as percent signal change, for CBF relative to both BOLD contrast measurements. In conclusion, our results add further evidence in support to the notion that CBF provides a more accurate localization of motor activation than BOLD contrast, indicating that ASL may be an appropriate technique for clinical fMRI studies. *Hum Brain Mapp* 34:96–108, 2013. © 2011 Wiley Periodicals, Inc.

Key words: fMRI; ASL; BOLD; hand motor area; reproducibility

INTRODUCTION

Cognitive neuroscience has obtained a powerful tool to study human brain function with the advent of functional magnetic resonance imaging (fMRI). There has been a growing interest in using fMRI techniques for clinical studies of the brain, namely the presurgical mapping of eloquent brain tissue. Imaging sequences based on the blood oxygen level-dependent (BOLD) contrast are currently the predominant method used for activation studies [Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1993]. However, the complex nature of the BOLD signal complicates the interpretation of its changes. In fact, BOLD contrast does not reflect a single physiological process, but

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rather represents the combined effects of cerebral blood flow (CBF), cerebral blood volume (CBV), and cerebral metabolic rate of oxygen (CMRO₂) [Ogawa et al., 1993]. One potential problem is that the location of BOLD signal change may not reflect exactly where the neuronal activity is changing, because its tissue specificity is often reduced by large draining veins that are not spatially confined to the area of neuronal activation [Buxton et al., 1998; Logothetis et al., 2001]. Furthermore, the signal change can vary substantially between subjects and across sessions, which are not very useful for assessing longitudinal activation changes [Aguirre et al., 1998].

Perfusion fMRI methods based on arterial spin labeling (ASL) offer a useful alternative to BOLD fMRI. The ASL methods can provide quantitative measures of perfusion, or regional CBF, without the need for the administration of exogenous contrast agents. Perfusion-weighted images are generated by inverting or saturating the magnetization of water molecules in the arterial blood supplying the imaging region and then measuring the resulting changes in tissue magnetization [Detre, 1992; Williams et al., 1992]. The difference between control (no labeling) and labeled images yields a signal that directly reflects local perfusion (see Petersen et al. [2006] for a review of ASL methods).

Several studies have compared BOLD with ASL methods for fMRI [Aguirre et al., 2002; Luh et al., 2000; Obata et al., 2004; Parkes et al., 2004; Restom et al., 2007; Tjandra et al., 2005; Wang et al., 2003a,b]. Some studies report differences between BOLD and CBF dynamics in motor areas, suggesting a hemodynamic origin for the transients of the BOLD response observed in some areas [Obata et al., 2004]. Other studies indicate that ASL provides improved sensitivity relative to BOLD for low-frequency tasks [Aguirre et al., 2002; Wang et al., 2003a]. Both within and between-subject variability have been found to be smaller when measured with perfusion fMRI when compared with BOLD fMRI [Leontiev and Buxton, 2007; Tjandra et al., 2005]. It has also been reported that perfusion changes have greater spatial specificity than BOLD changes during functional activation, suggesting that ASL may more accurately localize the region of neuronal activity [Luh et al., 2000; Tjandra et al., 2005]. In one study, the peak perfusion signal was shown to occur in voxels with a T₁ of brain parenchyma while the peak BOLD signal occurred in voxels with a T₁ characteristic of blood and cerebrospinal fluid [Luh et al., 2000]. Another study focused on the relative location of the activation areas with regard to proximal draining veins, showing a bias for the BOLD signal to be shifted toward the venous compartment relative to the ASL contrast [Tjandra et al., 2005]. It should be noted, however, that the venous contamination of BOLD signal is reduced at high and ultrahigh field [Lee et al., 1999a,b]. One limitation in some of these studies is that, for the simultaneous acquisition of CBF and BOLD contrasts, a nonoptimized echo time was used for the BOLD contrast. In fact, previous reports have shown a reduction on the order of 15% in the BOLD signal acquired simultaneously with ASL compared to conven-

tional BOLD measurements, during a finger-tapping task at 3T [Luh et al., 2000].

Since the introduction of fMRI to localize motor functions, simple and complex paradigms have been used to detect the areas responsible for motor hand control. Simple movements, like repetitive opening and closure of the hand, activated only the contralateral primary motor cortex (M1) [Yousry et al., 1995]. In contrast, more complex tasks like repetitive opposition of the thumb and each of the remaining fingers could additionally activate the ipsilateral primary motor cortex, the supplementary motor area (SMA), the premotor, and the somatosensory cortex bilaterally [Kim et al., 1993]. According to the concept of "homunculus," the cortical representation of motor hand function is known to be located in the superior aspect of the precentral gyrus [Penfield and Boldrey, 1937]. Yousry et al. [1997] evaluated the anatomical location of the motor hand area in dissected brains, computed tomography, and MR imaging and described it as having the shape of a typical hook on the sagittal plane and a structure like an inverted omega (Ω) or epsilon (ϵ) on the axial plane. Consistently, functional brain imaging studies have localized BOLD activation obtained during hand movement in the omega region of the precentral gyrus [Boroojerdi et al., 1999; Nelson and Chen, 2008; Yousry et al., 1997]. This hand knob has also been stimulated noninvasively by transcranial magnetic stimulation in order to produce hand movement, which is thought to provide a reliable method for mapping the motor cortex and, in particular, the hand motor area [Boroojerdi et al., 1999].

In the present study, we aimed to compare the performance of simultaneous CBF and BOLD ASL fMRI and standard BOLD fMRI in the functional localization of the hand motor area, both in terms of its relation to well-established anatomical landmarks as well as intersubject variability.

MATERIALS AND METHODS

Participants

Fifteen adult volunteers (6 females/9 males; mean age, 25.6; range, 22–51 years) participated in the study. All subjects were right handed, and none of them had a history of major medical, psychiatric, or neurological disorders, and written informed consent was obtained from all volunteers. During scanning, participants were instructed to perform a finger tapping motor task using their right hand, by sequential finger opposition of the thumb, and the other four digits.

Image Acquisition

Images were collected using a 3.0 T Magnetom Verio system (Siemens, Erlangen) with a 12 elements head RF coil. The fMRI sessions included two functional

experiments: (1) one using an ASL sequence to provide both a CBF contrast and a nonoptimized BOLD contrast; and (2) the other using a BOLD sequence to provide a true BOLD contrast. The order of the ASL and BOLD acquisitions was counterbalanced across subjects.

ASL Protocol

A proximal inversion with a control for off-resonance effects) Q2TIPS (QUIPSS II with thin-slice T_{I1} periodic saturation) pulsed ASL (PASL) method was used [Luh et al., 1999; Wong et al., 1997, 1998]. Two 90° RF pulses (*sinc* modulation, 25 ms duration) were applied to presaturate the imaging region, before a 180° inversion pulse (FOCI modulation) was applied to label the arterial spins over a 10-cm thick labeling region. The first inversion time $T_{I1} = 700$ ms allowed the inverted arterial spins to flow to the imaging slab. Between T_{I1} and $T_{I1s} = 1,600$ ms (T_{I1} stop time), 90° saturation pulses (*sinc* modulation, 20 ms duration) were repeatedly applied over a 20-mm thick saturation slab to define the time width of the labeling bolus. In the control measurement, the inversion pulse was executed off-resonance. Interleaved label and control images acquisition were carried out at the second inversion time $T_{I2} = 1,800$ ms using a gradient-echo (GRE) echo-planar imaging (EPI) readout module with acquisition parameters: a repetition time (TR) of 2.5 s, an echo time (TE) of 11 ms and a flip angle of 90° . The imaging region consisted of nine contiguous axial slices (no inter-slice gap) of 6 mm thickness positioned parallel to the anterior-posterior commissure (AC-PC) axis, between the vertex of the brain and the top of cerebellum, in order to include the primary motor area. The imaging slices had a field of view (FOV) of 224×224 mm² and a matrix size of 64×64 , yielding a voxel resolution of $3.5 \times 3.5 \times 6.0$ mm³. The gap between the labeling slab and the proximal slice was 18.8 mm. The slices were acquired in ascending order. The functional paradigm consisted of a blocked design with five cycles alternating 25 s of rest with 25 s of motor task. A total of 101 volumes (100 volumes plus 1 dummy scan) alternating between tag and control were acquired, resulting in a total scan duration of 4 min 12.5 s. The sequence was driven in 3D PACE mode (Siemens, Erlangen) enabling prospective motion correction.

BOLD Protocol

A standard BOLD protocol was used to acquire an optimized BOLD signal, for better comparison between BOLD and CBF measurements. In fact, the TE used in the ASL protocol (TE = 11 ms) is not optimal for the BOLD contrast, which is maximized for TE = 30 ms at 3.0 T [Ogawa et al., 1993; Tjandra et al., 2005]. Functional images were acquired using a multislice single-shot GRE-EPI sequence. The whole brain was covered with 37 contiguous axial slices of 3 mm thickness, positioned parallel to the AC-PC

axis. A total of 80 volumes were collected for each functional scan. The other imaging parameters were as follows: TR = 3.0 s, TE = 30 ms, flip angle = 90° , FOV = 256×256 mm², and a matrix size of 64×64 , yielding a voxel resolution of $4.0 \times 4.0 \times 3.0$ mm³. The slice acquisition order was ascending, interleaved. The standard BOLD acquisition followed the protocol commonly applied in clinical routine, using a blocked design with four cycles alternating 30 s of rest with 30 s of motor task. A total of 80 volumes were acquired, resulting in total scan duration of 4 min. The sequence was driven in 3D PACE mode (Siemens, Erlangen) enabling prospective motion correction.

Additional Images

Before each functional experiment, a main magnetic field (B_0) map based on a GRE sequence was acquired for the same slice prescription as the one to be used for each functional acquisition. A high-resolution whole brain structural scan was also acquired for each subject using a 3D T_1 -weighted magnetization prepared rapid acquisition gradient echo sequence. The parameters were TI = 900 ms, TR = 2,250 ms, and TE = 2.26 ms, 160 sagittal slices with 1.0 mm of thickness, FOV = 256×240 mm², and a matrix size of 256×240 , yielding an isotropic spatial resolution of 1 mm³.

Image Analysis

Image analysis was carried out with FSL (FMRIB's Software Library, <http://www.fmrib.ox.ac.uk/fsl>) and self-written MATLAB (The Mathworks, Natick, MA) and Linux shell script routines. Prospective (PACE) and retrospective motion correction algorithms (Siemens, Erlangen) were used in all functional data time-series, and subsequent analysis was performed using FEAT (FMRIB's Expert Analysis Tool) Version 5.98, part of FSL. The following prestatistics processing steps were applied to all datasets: nonbrain removal using brain extraction tool [Smith, 2002], spatial smoothing using a Gaussian kernel of full width at half maximum 5 mm, high-pass temporal filtering with a 100-ms frequency cutoff, and fieldmap-based EPI-distortion correction with FUGUE (FMRIB's Utility for Geometrically Unwarping EPIs) [Jenkinson, 2003]. An additional analysis of the BOLD data was performed to test for any effects of the different slice thicknesses between the BOLD and ASL protocols, by downsampling the BOLD data down to the ASL data's resolution along Z (from 3 to 6 mm).

Time-series statistical analysis was then performed following a general linear model (GLM) approach using FILM (FMRIB's improved linear model) with local autocorrelation correction by prewhitening [Woolrich et al., 2001] to identify task activated voxels in relation to each contrast. For the BOLD protocol, only one task-related

explanatory variable (EV1) was defined by convolving the block paradigm with a gamma function [standard deviation (SD) of 3 s and mean lag of 6 s] modeling the hemodynamic response function [Boynton et al., 1996]. For the ASL protocol, a GLM framework was also used encompassing a full perfusion signal model rather than a tag-control differencing approach, because the latter has been shown to produce biased estimates of the standard errors or suffer from a loss in efficiency [Mumford et al., 2006]. For the ASL protocol, besides the task-related EV1, one additional ASL-related explanatory variable (EV2) was defined to describe the alternation between control and label volumes when no stimulation is present. The interaction between control-label alternation (EV2) and motor task (EV1) variables was then considered by multiplying EVs 1 and 2 together to model the activation component of the perfusion-weighted signal (EV3). It is close to zero during rest and rises in amplitude during activation.

By fitting the GLM defined by the EVs to the data, the parameter estimates corresponding to each EV were determined. The contrasts of interest were then defined as EV1 versus baseline, for the *BOLD* and *BOLD_{ASL}* contrasts derived from the *BOLD* and ASL protocols, respectively; and EV3 versus baseline, for the *CBF* contrast derived from the ASL protocol. A *t* test was then applied, resulting in a *Z* (Gaussianized *t/F*) statistic map for each contrast. The *Z* maps for each subject were thresholded using a clustering procedure, whereby each cluster is determined by a voxel $Z > 2.5$ and a (corrected) cluster significance threshold $P = 0.05$ [Friston et al., 1993; Worsley et al., 1992]. We opted for a parsimonious common threshold value of $Z = 2.5$ for both protocols (following the values commonly used in the analysis of fMRI data), although their different degrees of freedom (DOF) and sensitivities preclude a direct comparison between the *Z* thresholds used. To take into account the much lower sensitivity of the *CBF* relative to the *BOLD* contrast and explore the associated activations further, an additional analysis of the ASL data was performed by obtaining *CBF* activation *Z* maps using an uncorrected voxel *P* value of 0.05 (instead of a corrected cluster *P* value of 0.05).

Spatial normalization of the subjects' data was performed after the individual statistical analysis, following the standard procedure in FSL. For *BOLD* data, registration of the functional images to the corresponding individual high-resolution *T*₁-weighted structural images and the MNI (Montreal Neurological Institute) standard brain [Collins et al., 1994] was carried out using FLIRT (FMRIB's Linear Image Registration Tool) [Jenkinson and Smith, 2001] with 7 and 12 DOF, respectively. For ASL functional data, we first registered the functional images to an equivalent EPI image but covering the whole brain (acquired during the same session from the subject), using FLIRT with 3 DOF, before further transformation to the high-resolution *T*₁-weighted structural and MNI standard brain. For each contrast, higher-level group analyses were carried out using FMRIB's Local Analysis of Mixed Effects, part of

FSL [Beckmann et al., 2003; Woolrich, 2004], which treats subjects as random effects and experimental conditions as fixed effects.

Data Analysis

Three main contrasts were considered for the evaluation of the localization, extent, and amplitude of motor activation: the ASL protocol allowed the simultaneous acquisition of the *CBF* contrast (*CBF*) and a nonoptimized *BOLD* contrast (*BOLD_{ASL}*), while the standard *BOLD* protocol provided a true, optimized *BOLD* contrast (*BOLD*). Additionally, a lower significance *CBF* contrast was obtained by using uncorrected voxel $P < 0.05$ thresholding (*CBF_{uncorr.}*), and a *Z* spatial resolution matched *BOLD* contrast was obtained by downsampling the original *BOLD* data to the ASL's slice thickness (*BOLD_{downsp.}*). The activation clusters given by each contrast (*BOLD*, *BOLD_{downsp.}*, *BOLD_{ASL}*, *CBF*, and *CBF_{uncorr.}*) for each subject were registered to the MNI standard brain, using FLIRT from FSL, in order to perform an intersubject comparison and a group analysis.

fMRI Localization of Motor Activation

The functional localization of the hand area of the contralateral primary motor cortex was studied here, for all contrasts, in terms of the center of gravity (COG) of the respective activation cluster, defined as the average location of the activated voxels within the cluster volume weighted by their respective signal change. We tested whether the COG varied with the cluster size by systematically varying the cluster *P* value between 0.05 and 0.01 and verified that the COGs obtained for each cluster were not significantly different from each other ($P > 0.05$). We therefore concluded that the COG provides a robust measure of the localization of the activation, which is largely independent of the activation cluster size. For the identification of the anatomical areas at each location, including the probabilities of activation foci belonging to specific Brodmann areas (BA) [Brodmann, 1909], the cytoarchitectonic probability maps of the Juelich Histological Atlas were used [Eickhoff et al., 2007].

Relation Between fMRI Localization and Anatomical Landmarks

Anatomical landmarks of the hand motor area of the left hemisphere were used as a reference for the localization measures obtained by each functional contrast. The hand knob structure of the precentral gyrus that is shaped like an omega in the axial plane was considered as the anatomical landmark for the localization of the hand motor area, because it has been shown to contain motor hand function [Boroojerdi et al., 1999; Yousry et al., 1997]. Nine points under the omega shaped segment of MNI standard brain were drawn by a neuroradiologist (three medial points

Figure 1.

Anatomical landmarks for the localization of the hand motor area: nine points (three medial points, M3, M2, and M1; three lateral points, L1, L2, and L3; three central points, CC, CM, and CL) over the segment of the precentral gyrus (red) and two calculated mean points (C1 and C2) (blue) (top left); four axial slices

used to localize the segment of the precentral gyrus (top right); and the same points shown in the MNI brain axial slices (bottom). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

M1, M2, and M3; three lateral points L1, L2, and L3, and three central points CM, CL, and CC), and two mean points were calculated (C1 is the mean point of the three central points, and C2 is the mean point of all nine points; Fig. 1). The Euclidean distances between the activation cluster COG's and the hand motor area landmarks were calculated for each subject and for each contrast and entered into an analysis of variance (ANOVA) using the Statistical Package for the Social Sciences, version 17.0. The rationale for this approach was to determine which of the three contrasts, *BOLD*, *BOLD_{ASL}*, or *CBF* yielded activations closest to the hand motor area. The displacement with respect to the hand motor area points and the Euclidean distance between the experiments were calculated for each subject.

Extent and Amplitude of fMRI Motor Activation

For each contrast, the extent of motor activation was measured as the number of voxels in the activation cluster. The amplitude of motor activation was measured as the mean percent BOLD signal change during the task periods relative to baseline, averaged over the activation cluster.

Intersubject Variability of Motor Activation

To assess the intersubject variability of activation areas, for each contrast, the coefficients of variation (CV) across all subjects of each spatial coordinate of the activation cluster COGs, as well as that of the cluster volume, were calculated [e.g., Magon et al., 2009; Jahng et al., 2005]. The CV is a normalized measure of dispersion of a probability distribution, which is defined as the ratio of the standard deviation σ to the mean μ expressed as a percentage [Eq. (1)].

$$CV = 100 \times \sigma / \mu \quad (1)$$

In addition, intersubject activation maps were generated for each contrast, by displaying the COGs and the local maxima locations of the activation clusters of all subjects in MNI space. The peripheral points were joined together to define a closed area illustrating the overall region occupied by the activation clusters of all subjects, for each contrast. These maps were then rendered into a 3D representation, which illustrates the overlap between activation clusters across the three different contrasts, allowing for a qualitative comparison of the average cluster location and extent as well as their intersubject variability.

Figure 2.

Activation Z maps obtained by BOLD (red), BOLD_{ASL} (green), and CBF (blue) contrasts in three subjects, using a cluster thresholding procedure with cluster $P < 0.05$ and voxel $Z > 2.5$, superimposed on the corresponding high-resolution structural images. In the bottom, the relative location of a proximal

draining vein is shown: it can be observed that the BOLD clusters are closer to this location than the CBF cluster. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS

All contrasts studied, BOLD, BOLD_{downsp.}, BOLD_{ASL}, CBF, and CBF_{uncorr.} yielded significant activation clusters in the hand motor area of the primary motor cortex of the left hemisphere, in response to the motor task, for all subjects. In some subjects, activation was also observed in

SMA and the ipsilateral primary motor cortex. Examples of the activation clusters obtained with each of the main contrasts (BOLD, BOLD_{ASL}, and CBF) are shown in Figure 2, using cluster corrected thresholding with cluster $P < 0.05$ and voxel $Z > 2.5$. Using a less demanding uncorrected thresholding procedure with voxel $P < 0.05$ (for the

Figure 3.

Activation Z maps obtained by group analysis of the BOLD (red), BOLD_{ASL} (green), and CBF (blue) contrasts, using a cluster thresholding procedure with cluster $P < 0.05$ and voxel $Z > 2.5$, superimposed on the standard MNI brain. The more inferior COG of the CBF cluster relative to the BOLD clusters is illustrated. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CBF_{uncorr.} contrast) allowed us to detect the SMA and ipsilateral M1 in 12 of 15 subjects (results not shown).

fMRI Localization of Motor Activation

The results of the group analyses for the three main contrasts are shown in Figure 3, revealing a significant activation in the hand area of the primary motor cortex of the left hemisphere, in response to the right digit-thumb opposition movement. Both BOLD modalities showed additional bilateral activation of the SMA, the premotor cortex, and the somatosensory cortex bilaterally. The volume of the BOLD activation cluster was greater than that of the BOLD_{ASL} activation cluster, and this was greater than that of the CBF activation cluster. For the CBF_{uncorr.} contrast, activation of the SMA and ipsilateral M1 was also found, but the activation volume remained smaller than that obtained with BOLD contrasts.

The COGs of the group activation clusters obtained for all contrasts are shown in Table I. The COG of the BOLD clus-

ter was found to belong to the left premotor cortex, BA6, with a probability of 35%. The COG of the BOLD_{ASL} cluster was found to belong to the left primary motor cortex, anterior BA4, with a probability of 34%, and to the left premotor cortex, BA6, with a probability of 33%. Finally, the COG of the CBF cluster was found to belong to the left primary motor cortex, posterior BA4, with a probability of 47% [Eickhoff et al., 2007]. The group cluster COGs obtained for the CBF_{uncorr.} contrast were similar to the ones obtained for the CBF contrast. The BOLD_{downsp.} contrast produced group cluster COG Z coordinates that were intermediate between those produced by the BOLD and BOLD_{ASL} contrasts, while X and Y coordinates remained similar.

The mean values of the individual COG coordinates obtained for each subject and each contrast in MNI space are also displayed in Table I. Significant spatial differences were found with ANOVA across the three main contrasts (BOLD, BOLD_{ASL}, and CBF) in MNI coordinate Z [$F(2,42) = 4.40$, $P = 0.018$], that is, in the inferior–superior direction. The post hoc multicomparison tests according to Scheffe revealed that BOLD_{ASL} COG coordinates (mean

TABLE I. Group analysis COG coordinates and group average ($\pm$ SE) of individual COG coordinates of activation clusters for all contrasts

Contrast	Group COG's coordinates (mm)			COG's coordinates (mm; mean $\pm$ SE)			CV (%)		
	X	Y	Z	X	Y	Z	X	Y	Z
CBF	-37.4	-19.1	52.4	-35.0 $\pm$ 3.1	-24.3 $\pm$ 5.3	53.2 $\pm$ 2.4	8.9	21.8	4.5
CBF _{uncorr.}	-37.2	-18.5	53.1	-34.6 $\pm$ 4.1	-22.9 $\pm$ 4.8	54.1 $\pm$ 2.7	10.2	21.0	4.9
BOLD _{ASL}	-36.0	-25.5	57.5	-31.0 $\pm$ 8.9	-30.0 $\pm$ 9.6	55.7 $\pm$ 6.2	28.7	32.0	11.1
BOLD	-38.3	-23.6	62.0	-32.3 $\pm$ 8.5	-32.8 $\pm$ 10.3	50.9 $\pm$ 4.1	26.3	31.4	8.1
BOLD _{downsp.}	-39.1	-22.4	60.6	-33.1 $\pm$ 9.1	-30.2 $\pm$ 9.8	51.3 $\pm$ 6.2	27.4	30.4	12.1

Figure 4.

Group mean Euclidean distances between the hand motor cortex (HMC) anatomical landmark points and the COGs of the activation clusters for all contrasts. Error bars denote one standard error of the group mean. *denotes significant differences between the pointed bars ($P < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

MNI coordinate Z: 55.7 ± 6.2 mm) were significantly more superficial ($P < 0.05$) than *BOLD* COG coordinates (mean MNI coordinate Z: 50.9 ± 4.1 mm), whereas *CBF* COG coordinates were not statistically different from *BOLD_{ASL}* or *BOLD* COG coordinates. There were no significant differences between contrasts in the other two dimensions, that is, in the medial-lateral [MNI coordinate X: $F(2,42) = 1.16$, $P = 0.324$] or posterior-anterior direction [MNI coordinate Y: $F(2,42) = 0.26$, $P = 0.776$]. The average COG results obtained for the *CBF* and *CBF_{uncorr.}* contrasts were not significantly different nor were the results obtained for the *BOLD* and *BOLD_{downsp.}* contrasts.

When comparing the COGs of the group activation clusters with the average COGs (in Table I), it can be observed that the group level Z coordinates obtained for the *CBF* and *BOLD_{ASL}* contrasts were close to the respective group mean Z coordinates, while a large offset was found for the *BOLD* contrast. The fact that the *BOLD_{downsp.}* contrast yielded COG Z coordinates that were intermediate between those of *BOLD_{ASL}* and standard *BOLD* contrasts suggests that the different spatial resolutions along Z between the ASL and *BOLD* protocols could at least partly explain this observation.

and *CBF*, as well as *BOLD_{downsp.}* and *CBF_{uncorr.}* activation COG are shown in Figure 4. ANOVA across the three main contrasts (*BOLD*, *BOLD_{ASL}*, and *CBF*) revealed a significant effect of the functional contrast in the distances to CL [$F(2,42) = 4.47$, $P = 0.017$], L1 [$F(2,42) = 4.37$, $P = 0.019$], C1 [$F(2,42) = 4.52$, $P = 0.017$], and C2 [$F(2,42) = 6.63$, $P = 0.009$]. The post hoc multi comparison of the mean distance values according to Scheffe revealed significant differences between the following distances: *CBF* and *BOLD_{ASL}* to CL ($P = 0.036$), *CBF* and *BOLD_{ASL}* to L1 ($P = 0.027$), *CBF* and *BOLD* to C1 ($P = 0.041$), *CBF* and *BOLD_{ASL}* to C1 ($P = 0.046$), *CBF* and *BOLD* to C2 ($P = 0.010$), and *CBF* and *BOLD_{ASL}* to C2 ($P = 0.013$). The relative location of the activation cluster COGs obtained with the three main contrasts is shown in Figure 5 (bottom). It should be noted that the *CBF_{uncorr.}* and *BOLD_{downsp.}* contrasts provided identical results to the main *CBF* and *BOLD* contrasts, respectively, with the activation COG distances to the anatomical landmarks remaining significantly different between *CBF* and *BOLD* contrasts in all cases. These observations indicate that the unmatched sensitivity and spatial resolution, respectively, between the ASL and *BOLD* protocols do not significantly affect our main result.

Relation Between fMRI Localization and Anatomical Landmarks

The mean Euclidean distance from the 11 points of the hand motor area (shown in Fig. 1) to *BOLD*, *BOLD_{ASL}*,

Extent and Amplitude of fMRI Motor Activation

The group averages of the cluster volumes and corresponding mean signal changes are shown in Table II for

Figure 5.

Intersubject activation maps in the MNI standard brain: activation cluster COGs and local maxima obtained by BOLD (red), BOLD_{ASL} (green), and CBF (blue) contrasts are displayed for all subjects. Top: 3D views of the inter-subject maps, showing reduced variability of COGs obtained with the CBF contrast rel-

ative to the two BOLD contrasts. Bottom: Axial view zoomed over the landmark positions, showing the relative locations of the COGs obtained with the different contrasts. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

all contrasts. ANOVA across the three main contrasts (*BOLD*, *BOLD_{ASL}*, and *CBF*) revealed a significant effect of contrast in the cluster volume ($P < 0.050$). Post hoc multiple comparison of mean cluster volume according to Scheffe revealed significantly fewer activated voxels for *CBF* relative to *BOLD_{ASL}* ($P = 0.004$) and for *CBF* relative to *BOLD* ($P = 0.001$). This is to be expected due to the much lower sensitivity of the ASL protocol. In fact, the

mean cluster volume increases when lowering the significance level for the *CBF_{uncorr.}* contrast. Nevertheless, it still remains smaller than both for BOLD contrasts.

Intersubject Variability of Motor Activation

The CV values are shown in Table I for the COG MNI coordinates and in Table II for the cluster volumes and

TABLE II. Group average ($\pm$ SE) of activated cluster volumes and corresponding mean signal changes for all contrasts

Contrast	Cluster volume (mm ³ ; mean $\pm$ SE)	CV (%)	Mean signal change (%; mean $\pm$ SE)	CV (%)
CBF	216.0 $\pm$ 37.6	17.4	62 $\pm$ 7	11.3
CBF _{uncorr.}	323.2 $\pm$ 56.6	17.5	65 $\pm$ 8	12.3
BOLD _{ASL}	2397.6 $\pm$ 432.0	18.0	1.14 $\pm$ 0.34	30.2
BOLD	3088.8 $\pm$ 625.6	20.3	1.49 $\pm$ 0.32	20.3
BOLD _{downsp.}	2876.1 $\pm$ 565.4	19.7	1.32 $\pm$ 0.26	19.7

mean signal changes. In all cases, the CV values for CBF activation are smaller than the CV values for both BOLD contrasts. The intersubject activation maps obtained by overlaying the activation COGs and local maxima of all subjects, for the three contrasts, are shown in Figure 5, both in 3D and axial views. In this figure, it can be seen that the CBF intersubject activation map is within both *BOLD* and *BOLD_{ASL}* intersubject activation maps. This illustrates the smaller variability of both location and extent in the case of CBF contrast relative to the BOLD contrasts.

DISCUSSION

In this study, we evaluated the localization and the intersubject variability of the hand motor area obtained by analyzing the CBF and BOLD contrasts provided by an ASL fMRI protocol and a standard BOLD fMRI protocol, during a simple thumb-digit opposition task.

Group analysis of the fMRI data obtained with each contrast showed activation of the left hand motor cortical areas in response to the right digit-thumb opposition movement, as expected. Furthermore, the BOLD contrasts (standard and ASL-derived) also showed bilateral activation of the SMA and the premotor and the somatosensory cortex bilaterally, in agreement with previous reports [Dieckhoff et al., 2010; Tjandra et al., 2005]. In fact, the higher sensitivity of the BOLD measurements resulted in systematically larger activation clusters compared to the CBF contrast. Consistently, with a recent study, we observed a superior shift in the location of the optimized BOLD activation cluster ($Z = 62.0$ mm) relative to the CBF activation cluster ($Z = 52.4$ mm) [Dieckhoff et al., 2010].

The identification of the hand motor area using well-established anatomical landmarks allowed the comparison, in the MNI standard brain, of the spatial location of the activation clusters obtained with the different fMRI contrasts. In terms of localization, we found that CBF measurements were closer to the anatomical landmarks than either the ASL-derived or standard BOLD contrasts. As far as the variability is concerned, we showed that the CV of the spatial location, as well as the percent signal change, was smaller for ASL compared to BOLD.

fMRI Localization of Motor Activation

The primary motor cortex (M1) is the cortical area essential for the execution of voluntary movements and its

hand representation is expected to be active during the thumb-digit opposition task performed by the participants in this study [Dum and Strick, 2002; Grefkes et al., 2008; Sanes and Donoghue, 2000]. In terms of standard anatomical characterization, M1 corresponds to BA4, extending from the anterior part of the paracentral lobule (medially and superiorly) to the sylvian fissure (laterally and inferiorly) along the crown and posterior surface of the precentral gyrus with a progressive tapering of its thickness. At the hand representation area, BA4 is buried within the central sulcus and rarely extends to the gyral surface [Geyer et al., 1996]. ASL-CBF data showed the highest signal increase in the location where BA4 is expected [Eickhoff et al., 2007]. However, the BOLD sequences showed maximum signal changes in a location slightly shifted anteriorly toward the premotor areas, namely area BA6.

Differences Between CBF and BOLD Contrasts

It has been shown that ASL perfusion measurements may more accurately localize brain activation relative to BOLD, due to their relatively greater sensitivity to changes in the local capillary bed of the activated neuronal population than the venule side [Lee et al., 1999a,b; Wong et al., 1997]. Indeed, BOLD fMRI studies have showed apparent activation over distant draining veins, which is not observed using perfusion contrast [Tjandra et al., 2005]. It is known that venous drainage can extend as far as few centimeters away from the capillary bed, which is therefore a potentially larger concern for BOLD, because it may lead to significant errors in the spatial localization of motor activation. Another study found that the locations of the peak signals of functional BOLD and perfusion only partially overlapped on the order of 40%, with CBF changes being more closely related with the T1 of parenchyma and those of BOLD with the T1 of blood [Luh et al., 2000]. In our study, activation detected with perfusion fMRI was confined to the hand motor area, whereas BOLD contrast also showed apparent activation over a central cortical vein, which drains the motor cortex (see Fig. 2). A previous study used the draining vein nearest to the motor hand area as a reference point and showed that the Euclidean distance for BOLD activation was smaller compared to that of CBF [Tjandra et al., 2005]. Overall, our results converge with growing evidence that perfusion activation maps are clearly better localized to brain

parenchyma and will therefore likely represent the sites of neuronal activity better than BOLD activation maps.

It should be noted that, despite their advantage in terms of localization accuracy, ASL CBF functional measurements suffer from intrinsically low signal-to-noise ratio (SNR) resulting in a much lower sensitivity than BOLD measurements. In fact, at the same significance level, the ASL CBF contrast failed to detect activation of SMAs and ipsilateral primary motor cortex, which are typically found with the BOLD contrast. Nevertheless, we found that, by lowering the significance level, CBF activation maps could also show activation in these motor areas in most subjects. In general, the sensitivity of ASL CBF analyses could be improved by performing more signal averaging (resulting in longer acquisition sequences) and, in this way, increased the SNR of the data.

Relation Between fMRI Localization and Anatomical Landmarks

Instead of the nearest draining vein, we used a well-established anatomical landmark of the hand motor area—the omega region of the precentral gyrus—as a reference [Boroojerdi et al., 1999; Nelson and Chen, 2008; Yousry et al., 1997]. We found that CBF activation was closer to the landmark points compared to BOLD contrasts. Based on a number of studies supporting the omega landmark for M1, such as Boroojerdi et al. [1999], Nelson and Chen [2008], and Yousry et al. [1997], our results therefore indicate that CBF provides more accurate localization of motor activation than BOLD signal. Furthermore, considering the original points drawn to localize the hand motor area, CBF activation was significantly closer to the central and lateral points, CL and L1, and the average points, C1 and C2 (see Fig. 4). This observation is consistent with a study that showed individual digit activated areas with predominance in the lateral region of the segment of the precentral gyrus [Kleinschmidt et al., 1997]. In fact, the central and lateral points identified in our study, CL and L1, seem to correspond to the location of the thumb, which is consistent with its strong involvement in the thumb-digit opposition task performed by the subjects. It should be pointed out that the study of Kleinschmidt et al. [1997] used a different paradigm based on self-paced isolated finger movements, together with higher spatial resolution, in order to map the fine somatotopy of the hand primary motor area.

Intersubject Variability of Motor Activation

The intersubject variability during motor activity was found to be smaller when measured with perfusion fMRI when compared with BOLD fMRI. This was reflected in the smaller variance (and CV) of the activation cluster COG's coordinates (Table I) as well as that of the associated cluster volumes and mean percent signal changes (Table II). Nevertheless, all CV values were below the

upper fiducial limit (33%) for acceptable variability in a normal distribution [Johnson and Welch, 1940]. Moreover, the group activation maps showed larger areas of BOLD and BOLD_{ASL} activations compared to CBF (see Fig. 4), further indicating the greater variability of BOLD measures compared to ASL. Overall, our results suggest that blood flow changes were more consistent across subjects than changes in BOLD contrast. The greater intersubject variability of BOLD measurements could be a consequence of their sensitivity to different physiologic parameters, including CBF, CBV, and CMRO₂, which make them susceptible to individual variations in factors such as the arterial concentration of CO₂, haematocrit. Our results extend previous observations showing that CBF provides lower intersubject variability, despite its lower sensitivity, in the location of the motor activation area [Tjandra et al., 2005]. Although intrasubject variability was not assessed in this study, it is expected that it should also be reduced by using a quantitative CBF measure rather than BOLD contrast.

Limitations of This Study

A few methodological considerations should be made here regarding the comparison of the localization of neuronal activation obtained with CBF relative to BOLD. If we consider the group mean Euclidean distance between the modalities (see Fig. 4), we observe that these values are within the intrinsic spatial resolution of collected data ($3.5 \times 3.5 \times 6.0 \text{ mm}^3$ for CBF and BOLD_{ASL} and $4.0 \times 4.0 \times 3.0 \text{ mm}^3$ for BOLD). Although the MNI standard space to which the experiments were registered has a better resolution ($2.0 \times 2.0 \times 2.0 \text{ mm}^3$), the space where functional data were collected is the one that we must consider. In fact, these differences could, at least partly, be due to registration problems between the functional and structural spaces and the MNI brain. However, the consistency of the differences between the measured distances across subjects strongly indicates that these are not the result of random errors but rather a true effect of the contrast used in each case.

Another limitation of the current study is the fact that the ASL and BOLD protocols differed in a number of acquisition parameters, precluding a more direct comparison between CBF and BOLD contrasts. In particular, the standard BOLD protocol had fewer paradigm cycles, shorter repetition time, and finer spatial resolutions, all of which contribute to a lower raw SNR. In fact, our rationale was to compare the results given by the CBF and nonoptimized BOLD contrasts obtained with the ASL protocol with the standard BOLD protocol that was indicated for a clinical fMRI study. Nevertheless, even if all acquisition parameters were fully matched between ASL and BOLD protocols, the CBF and BOLD data would still differ in terms of their sensitivity, because their SNRs are intrinsically different. Therefore, we believe that our comparisons

between CBF and BOLD contrasts are not significantly further compromised, because acquisition parameters were not fully matched, as supported by our supplementary analyses of the data. When the significance levels for the analysis of the CBF and BOLD contrasts were adjusted in order to (at least partly) take into account their different sensitivities, the activation COG distances to the anatomical landmarks remained significantly different between ASL and BOLD. Moreover, when the BOLD data were downsampled to the ASL data's resolution, so that the effective resolutions of the two acquisitions were matched along *Z*, the relative localizations of the ASL and BOLD activation clusters were maintained, which indicates that the different samplings did not significantly affect our results.

Finally, the two BOLD signals analyzed here are in fact not directly comparable, because the one collected together with the ASL sequence is not a true BOLD contrast due to nonoptimized echo time and also because of the contaminations by the flow sensitization of the sequence [Woolrich et al., 2006]. This could possibly explain the difference observed between the locations of the activations found with the BOLD and BOLD_{ASL} contrasts, and hence it should not be considered purely as a measure of the uncertainty in the localization. Nevertheless, we found no significant differences on the COG distance to the anatomical landmarks between the nonoptimized BOLD contrast obtained from the ASL protocol and the true BOLD contrast obtained from the standard BOLD protocol. Improved direct comparison between pure CBF and BOLD contrasts could be achieved by using dedicated ASL imaging sequences, such as interleaved ASL and BOLD or dual-echo acquisitions [Hoge et al., 1999; Silva et al., 1999] and separately modeling CBF and T_2^* effects in both measured signals [Woolrich et al., 2006].

CONCLUSION

In conclusion, our study provides further evidence indicating that simultaneous CBF and BOLD activation obtained by ASL fMRI provides a reliable marker for the location of motor brain activation, combining the better spatial specificity and reduced intersubject variability of CBF measures with the higher sensitivity of BOLD contrast. Such characteristics demonstrate that ASL fMRI can be used with significant advantages in clinical applications, including the presurgical mapping of eloquent brain tissue as well as longitudinal studies and clinical trials.

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