Intracortically Distributed Neurovascular Coupling Relationships within and between Human Somatosensory Cortices

The coupling of neuronal cellular activity to its blood supply is of critical importance to the physiology of the human brain and has been under discussion for more than a century. Linearity in this relationship has been demonstrated in some animal studies, but evidence is lacking in humans. In this study, we compared scalp evoked potentials and the functional magnetic resonance imaging (fMRI) blood oxygen level-dependent (BOLD) signal from healthy human volunteers with changes in the intensity of a somatosensory stimulus. By weighting the fMRI images according to the evoked potential amplitude at corresponding intensities, we tested for positive and negative covariation between these 2 data sets and the extent to which these were linear. Hemodynamic changes in primary somatosensory cortex covaried positively with neuronal activity in a predominantly linear manner, with a small quadratic contribution. Simultaneously, other cortical areas corresponding to the nonstimulated limbs were found to covary negatively and linearly in the hemispheres ipsilateral and contralateral to the stimulus. These concurrent and bilateral cortical dynamics, as well as the intraregional features of this neurovascular coupling, are both more complex than had been considered to date, with considerable implications.

Keywords: fMRI, intracortical, neurovascular coupling, SEP

Methods

Stimulation
Six healthy adults participated (4 males; mean age 24.33 years, range 22-29 years), recruited from local university members. All studies were performed under Local Ethics Committee Approval guidelines, with full informed consent obtained. Stimuli were 0.2-ms square-wave electrical pulses delivered to the median nerve at the wrist for 30-s blocks. Stimulation intensity values were chosen to span a range from just above sensory threshold to the highest level bearable for 30 s but did not exceed 30 mA or individual pain thresholds. Values were normalized to individual motor thresholds to enable comparisons between data sets and across the group. During fMRI scanning, stimuli were delivered at 100 Hz to ensure that a detectable BOLD response could be recorded (Kampe and others 2000). During SEPs recording, stimuli were delivered at 20 Hz to allow accurate identification of cortical SEP components in transient mode. Current limiting resistors were placed in the stimulating cables during fMRI as a safety precaution (Lemieux and others 1999). The null hypothesis in this experiment was that there would be no significant covariance between fMRI BOLD responses in somatosensory cortex and SEP amplitudes during changes in stimulus intensity.

Somatosensory Evoked Potentials
SEPs were recorded using Ag/AgCl 10-mm disc electrodes from contralateral parietal cortex, 3 cm posterior and 7 cm lateral to the vertex (Cz) referenced to Fz, and from the mixed nerve at the elbow of the stimulated arm. Electrode impedances were maintained at less than 8 kΩ. Over 450 averages were made of 50 ms bin width and stored for subsequent off-line analysis. Scalp potentials were amplified using a band-pass filter of 3–3000 Hz. An automatic artifact rejection system
excluded from the averages all runs containing transients exceeding ± 50 µV at any recording channel, commonly due to muscular or pulsatile artifacts. Stimulation intensity was pseudorandomized between successive recordings. The analysis was concentrated on the initial cortical component of the short-latency SEP, the N20–P25 component (termed “SEP amplitude”). The N20-P25 waveform of the human SEP is generated by a well-circumscribed population in the bank of the postcentral sulcus, that is, primary somatosensory cortex (Broughton and others 1969; Goff and others 1977; Allison and others 1980; Grimm Schreiber and others 1998). Pearson’s rank test was used for correlation analysis (e.g., between SEP amplitudes and intensity level) using the Statistical Package for the Social Sciences. Statistical significance was set at \( P < 0.05 \). Statistical significance threshold was set at \( P < 0.05 \) throughout.

**fMRI Acquisition**

Imaging was performed on a Bruker Medical S300 scanner (Bruker Medical, Ettlingen, Germany), acquiring gradient echo-planar imaging (EPI) fMRI BOLD images of 25 contiguous 5-mm oblique axial slices at 3 T. The matrix size was 128 \times 64\) (an in-plane resolution of 2 \times 4 \text{ mm})\), repetition time of 4000 ms, echo time of 27 ms, and 90° flip angle. Subjects lay supine in the scanner and wore earplugs and ear defenders for noise attenuation throughout, according to our local protocols. A functional imaging series comprised 72 sequential images of each slice during stimulus presentation in a blocked design, alternating 8 scans (32 s) on, 8 scans off, that is, a total of 4 min and 48 s. The first 8 scans were discarded to allow for \( T_{1} \) saturation effects. Stimulation intensities were unchanged during a 72-s scan sequence but were pseudorandomized between sequences. Scanning also included the once-only acquisition of a fast gradient echo \( T_{1} \)-weighted anatomical reference image of the whole brain. No neurological abnormalities were identified in any of the subjects studied.

**fMRI Preprocessing**

All image preprocessing and statistical analysis were done using statistical parametric mapping (SPM99, Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm/) on Matlab (Mathworks Inc., MA) under Linux. Each image volume was reoriented, adjusted for acquisition slice timing, and realigned to the first of each sequence. The images were further spatially normalized into a standardized stereotaxic space (Montreal Neurological Institute [MNI] space; the EPI template provided in SPM99) before being smoothed using a Gaussian filter of 4 mm full-width half maximum. Coordinates are therefore given in MNI space; areas were anatomically defined by transforming these coordinates into “Talairach space” (Talairach and Tournoux 1988).

**fMRI BOLD Analysis**

Blocks of stimuli were modeled using a boxcar function, incorporating a delay appropriate to the hemodynamic response. The size of the hemodynamic response was measured by calculating signal minus average baseline response across all scans. The modulation of hemodynamic responses by task-related activation was further characterized using both the (mean corrected) intensity and SEP amplitudes as linear regressors. This identified brain regions in which the size of task versus baseline hemodynamic response covaried linearly with these measures on a voxel-by-voxel basis (either positively or negatively).

We used SEP amplitudes as independent regressors in the fMRI analysis in order to show which voxels would best covary with SEP amplitudes. This approach does not require an a priori hypothesis regarding fMRI activation. This approach therefore eliminates the inherent assumption that the fMRI locus that correlates best with stimulus intensity will be that which correlates best with SEP amplitude. Nonlinear covariances of the fMRI with the SEP data were similarly modeled using a second-order (quadratic) derivative of SEP amplitudes (Robson 1958). Results were averaged across the group in a fixed-effects analysis to form a group-mean image, as it is widely recognized that at least 12 subjects are required for reasonable population effects to be seen in a random-effects model, due to intersubject variability (Holmes and Friston 1998). The images are shown in the standard radiological convention. All statistical maps were thresholded at \( P < 0.05 \), after correcting for multiple comparisons. Where clusters of activations were identified as significant, the coordinates of the voxel with highest \( t \)-score are given.

**Results**

**Effects of Stimulus Intensity**

**Effects of Stimulus Intensity on SEP Amplitude**

N20–P25 amplitudes of the cortical SEP correlated linearly with stimulus intensity in all subjects examined (\( P < 0.01 \) for each subject, group data \( P < 0.001 \), Fig. 1A, an example from one subject is given in Fig. 1C). Largest amplitudes were reached at 125% of motor threshold, which showed marked individual variability in absolute amplitude and intensity.

**fMRI BOLD Activity**

Irrespective of intensity, the fMRI BOLD voxel of maximal stimulus-induced activation was found in contralateral somatosensory cortex in each subject. Gradient echo fMRI BOLD voxel \( Z \)-score and percent signal change at this peak voxel increased significantly with increasing stimulus intensity (\( P < 0.05 \), \( P < 0.05 \), respectively; Fig. 1B).

**Testing for Linear Covariation with Stimulus Intensity**

**Positive Linear fMRI BOLD Covariation with Stimulus Intensity**

The fMRI BOLD areas in contralateral somatosensory cortex (peak voxel coordinates: \( Z \)-score 10.23; cluster size 626; \( P < 0.001 \)), contralateral thalamus (peak voxel coordinates: \( 16, -22, 02; Z \)-score 6.81; cluster size 30; \( P < 0.001 \)), and ipsilateral cerebellum (peak voxel coordinates: \(-20, -52, -34; Z \)-score 5.38; cluster size 17; \( P < 0.001 \)) showed significant linear covariation with stimulus intensity (Table 1; Fig. 2A).

**Negative Linear fMRI BOLD Covariation with Stimulus Intensity**

Areas that covariated negatively with increasing stimulus intensity were contralateral somatosensory cortex near the midline (peak voxel coordinates: \( 2, -26, 58; Z \)-score 4.80; cluster size 1; \( P < 0.05 \)) and ipsilateral somatosensory cortex (peak voxel coordinates: \(-40, -34, 64; Z \)-score 5.09; cluster size 5; \( P < 0.05 \); Table 1; Fig. 2B).

**Testing for Linear Covariation with SEP Amplitude**

**Positive Linear fMRI BOLD Covariation with SEP Amplitude**

Contralateral somatosensory cortex and ipsilateral cerebellum covaried significantly in a positive linear fashion with SEP amplitudes in each subject (Table 2, Fig. 3). The group analysis also showed most significant linear covariation with SEP amplitudes in contralateral somatosensory cortex (peak voxel coordinates: \(-40, -26, 64; Z \)-score 10.17; cluster size 458; \( P < 0.001 \)), contralateral thalamus (peak voxel coordinates: \( 16, -22, -02; Z \)-score 6.08; cluster size 15; \( P < 0.05 \)), and ipsilateral cerebellum (peak voxel coordinates: \(-12, -56, -32; Z \)-score 5.21; cluster size 15; \( P < 0.05 \)), across subjects (Table 3; Fig. 4A).

**Negative Linear fMRI BOLD Covariation with SEP Amplitude**

Two areas which covaried negatively with SEP amplitudes were found: the first in ipsilateral primary somatosensory cortex, in a similar location to that which covaried positively with SEP amplitudes above but in the opposite hemisphere (upper limb...
hand areas; peak voxel coordinates: –42, –32, 62; Z score 6.04; cluster size 15; P < 0.05; Table 3; Fig. 4 B); and the second in contralateral primary somatosensory cortex areas, close to the midline (leg and/or foot areas; peak voxel coordinates: 4, –44, 58; Z score 6.28; cluster size 127; P < 0.001; Table 3; Fig. 4 B).

Testing for Nonlinear Covariation with SEP Amplitude

Positive Quadratic fMRI BOLD Covariation with SEP Amplitude
Nonlinear contributions were also modeled, and a small area (peak voxel coordinates: 34, –34, 58; Z score 4.85; cluster size 2; P < 0.05; Table 3; Fig. 4 C) of fMRI BOLD activity in contralateral primary somatosensory cortex was found to covary significantly with SEP amplitudes. This cluster fell within the boundaries of the larger cluster of 458 voxels that covaried linearly with SEP amplitudes (Fig. 4 A).

Negative Quadratic fMRI BOLD Covariation with SEP Amplitude
No clusters reached significance thresholding for negative, nonlinear covariations with SEP amplitudes (results not shown).

Discussion
SEP amplitudes and fMRI BOLD responses correlated linearly with stimulus intensity in all subjects, and across subjects. Both modalities therefore exhibited the same qualitative pattern of experimental effects in primary somatosensory cortex when measured in parallel. Furthermore, the covariation of fMRI BOLD responses with SEP amplitudes (indicative of neurovascular coupling) was found to be strongly linear in this area. Short-latency SEPs are attributed mainly to synchronized extracellular currents from summated postsynaptic potentials of pyramidal cells in primary somatosensory cortex (Eccles 1951; Creutzfeldt and others 1966; Lopes da Silva and Storm van Leeuwan 1978; Nunez 1981; Lopes da Silva 1991). fMRI BOLD responses predominantly measure the CBF response from cortical vessels due to the inherent magnetic changes in hemoglobin during activation (a transient drop in the deoxy: oxy-hemoglobin ratio [Fox and Raichle 1986; Ogawa and others 1990; Kwong and others 1992; Malonek and others 1997]). Although the correlation found in these experiments does not prove causation, these findings together imply that the synaptic activity of a population of somatosensory cortical neurons play a major role in signaling the needs of the neuron to the vasculature. This is consistent with previous findings in primates (Logothetis, Pauls and others 2001) and the empirical
Intracortically Distributed Neurovascular Coupling

Note: Fixed-effects group analysis of the fMRI BOLD areas that covary linearly, (A) positively, and (B) negatively with stimulus intensity. These are contralateral somatosensory cortex, contralateral thalamus, and ipsilateral cerebellum (covary positively; A) and contralateral somatosensory cortex near the midline and ipsilateral somatosensory cortex (covary negatively; B). A fixed-effects group analysis of $n = 6$ is shown. These images are thresholded at $P < 0.05$ corrected for multiple comparisons. The corresponding data are given in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Covariation</th>
<th>Brain region</th>
<th>Coordinates $x, y, z$</th>
<th>$Z$ score</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Positive</td>
<td>Contralateral SI</td>
<td>38, −24, 62</td>
<td>10.23**</td>
<td>636**</td>
</tr>
<tr>
<td>A</td>
<td>Positive</td>
<td>Contralateral thalamus</td>
<td>50, −22, 56</td>
<td>10.16**</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Positive</td>
<td>Contralateral thalamus</td>
<td>44, −16, 54</td>
<td>10.04**</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Negative</td>
<td>Contralateral SI</td>
<td>16, −22, 2</td>
<td>6.81**</td>
<td>30**</td>
</tr>
<tr>
<td>B</td>
<td>Negative</td>
<td>Ipsilateral SI</td>
<td>2, −26, 58</td>
<td>4.80**</td>
<td>1*</td>
</tr>
<tr>
<td>B</td>
<td>Negative</td>
<td>Ipsilateral SI</td>
<td>−40, −34, 64</td>
<td>5.09*</td>
<td>5*</td>
</tr>
</tbody>
</table>

Note: Fixed-effects group analysis of $n = 6$ shown. **$P < 0.001$, *$P < 0.05$; SI = primary somatosensory cortex.

The exact metabolic nature of the neurovascular signal currently remains unknown, although there are many possibilities, including the astrocytic recycling of glutamate (the “astrocyte-neuron lactate shuttle” hypothesis) (Pellerin and others 1998), increased potassium levels causing vasodilatation (Paulson and Newman 1987), and/or increases in nitric oxide.

However, electrophysiological responses have inherent differences in signal to noise between different techniques, such that electrical signals that can be measured by extracellular electrodes are preferentially biased toward the slow oscillations of membrane potential (local field potential [LFP]) rather than action potentials or spikes. SEP measurements are too coarse to detect spiking activity, and therefore their contribution to the fMRI BOLD signals observed cannot be meaningfully analyzed using these techniques. Given evidence that human fMRI BOLD activity may be proportional to primate single-cell firing activity, this suggests that action potentials may be important in the neurovascular coupling relationship (Heeger and others 1998), increased potassium levels causing vasodilatation (Pellerin and others 1998), increased potassium levels causing vasodilatation (Pellerin and others 1998), increased potassium levels causing vasodilatation (Pellerin and others 1998).
and adenosine (Dirmagl and others 1994). All these metabolic candidates fail to demonstrate the necessary temporal and precise spatial relationship between accumulations and flow increase and have been previously discussed in detail with other possibilities (Lou and others 1987; Villringer and Dirmagl 1995; Kuschinsky 1997).

Nonlinearity

In this study, a much smaller area of fMRI BOLD activity in primary somatosensory cortex was also found to covary with SEP amplitudes in a quadratic fashion (Fig. 4C). This area fell within the larger cluster in somatosensory cortex that correlated linearly (Fig. 4A). Given the small nature of this response, any inference made must be speculative. However, a small nonlinear component to the overwhelming linear neurovascular coupling relationship (2 nonlinear/458 linearly covarying voxels, i.e., 0.44%) is consistent with most (Mathiesen and others 1998; Brinker and others 1999; Heeger and others 2000; Ogawa and others 2000; Rees and others 2000; Logothetis and others 2001) but not all recent studies (Ances and others 2000). Previous studies have found nonlinearities at the extreme ends of this relationship, such that the relationship is well approximated by a linear function over the midrange of stimuli (Hewson-Stoate and others 2005), as seen here.

There is currently a debate as to whether this nonlinearity may itself be attributable to a nonlinear neuronal response, including the contribution of transiently high neuronal-spiking activity to a hemodynamic response that is primarily synthetically driven (Bandettini and Ungereiclder 2001; Logothetis and others 2001; Birn and Bandettini 2005), or alternatively due to the inherent nonlinear relationship between metabolic demand and the BOLD signal. The BOLD response is linearly related neither to CBF nor to the cerebral metabolic rate of oxygen consumption (CMRO2) (Rees and others 1997; Hoge and others 1999; Mandeville and others 1999). Changes in cerebral oxygen consumption have been shown to increase linearly with synaptic activity but demonstrate a threshold effect, also contributing to nonlinearities (Sheth and others 2004). Alternatively, the nonlinearity may arise from slight differences in sensitivity of our acquisition techniques, as discussed later in this section.

**Negative Coupling**

Two areas of negative linear neurovascular coupling were also found in this study: in ipsilateral somatosensory cortex corresponding to the upper limb including the hand area (mirroring the area of positive linear coupling in the opposite hemisphere) and in contralateral somatosensory cortex corresponding to the sensory representation of the leg and foot (Fig. 4B). This suggests an efficient suppression of blood flow to relatively "inactive" limb cortical areas. These findings were accessible only by virtue of this type of voxel-by-voxel covariance analysis, and there are a number of possible interpretations.

We use the term "negative" BOLD to mean a reduction in BOLD activity relative to baseline which corresponds to the timing of the stimulus, also referred to as deactivation. There are 2 such types of negative BOLD signal identified in the current literature. The first is a transient initial negative dip in BOLD signal to stimulus activation characterizing the hemodynamic response function. This is thought either to be a hemodynamic steal phenomenon or to be caused by oxygen consumption in the absence of a hemodynamic response (Rother and others 2002). The other, demonstrated in this experiment, is a sustained negative BOLD response usually seen at distances of centimeters away from stimulated areas but in physiologically correlated areas, such as opposite motor (Hamzei and others 2002), sensory (Drevets and others 1995), and visual areas (Smith and others 2004). Initially thought to be a hemodynamic steal phenomenon caused by a redistribution of blood flow to adjacent areas of cortex (Harel and others 2002), it now appears much more likely to represent a neurally driven inhibitory phenomenon, when observed at sites distant to, but functionally related to, active brain areas. No areas of blood flow decreases were observed in the penumbral region of the activated area in somatosensory cortex in our study.

The fMRI BOLD signal decreases seen in ipsilateral motor cortex during unilateral hand movements are proportional to the task-related increases in contralateral M1 (in parallel with duration of movement) (Newton and others 2005). Studies have also shown that the reductions in BOLD signal in this area...
during contralateral activations are linearly related to the metabolic down-regulation, that is, CBF and cerebral metabolic rate of oxygen consumption (CMRO2) changes, suggesting an inhibitory neuronal signal underlying this negative BOLD response (Stefanovic and others 2004). Negative BOLD has also been reported during electroencephalography (EEG) spiking activity and found to occur at sites that are distant from anatomical areas related to spikes (Kobayashi and others 2005), suggesting neuronal inhibition.

Decreases in activation have also previously been attributed to higher level cortical function changes, such as the “anticipation” of expected stimuli elsewhere: CBF decreases in hand and face zones of ipsilateral somatosensory cortex (while attending to toe stimulation) have been correlated with anxiety levels during anticipation of stimuli (Drevets and others 1995). This suggests the suppression of ipsilateral responses in order to “focus on,” or “attend to,” contralateral responses where stimuli are expected in direct proportion to the anxiety level. fMRI BOLD responses in our experiment might therefore covary negatively with SEP amplitudes because they, in turn, covary with increasing stimulus intensity. However, although decreases in fMRI BOLD signal from ipsilateral somatosensory cortex do correlate with stimulus intensities, $P$ values were lower and voxel $t$-statistics less significant than the equivalent analyses with SEP amplitudes (Tables 1 and 3, respectively). Ipsilateral cortical responses may therefore be more closely (albeit negatively) related to contralateral responses rather than to the stimulus.

An alternative consideration is the changes in ongoing event-related desynchronization or synchronization at particular frequency bands (Pfurtscheller and Lopes da Silva 1999; Pfurtscheller 2001), which may represent increased cortical activation and deactivation, respectively, where “activation” represents increased resonance-like behavior of connected subnetworks. These types of changes are time locked to the event but not phase locked and therefore cannot be extracted using conventional linear methods such as averaging (as in SEP recordings), but require frequency analysis. They have been demonstrated in somatosensory and visual cortices (Neuper and Pfurtscheller 2001; Pfurtscheller and others 2001; Singh and others 2002; Moosmann and others 2003). The amplitude of negative fMRI BOLD responses to acoustic stimulation has been shown to correlate positively with measures of EEG synchronization during sleep (Czisch and others 2004), suggesting a relationship between cortical deactivation and negative BOLD signals. Further, analysis of these network synchronization changes may give a greater understanding of the underlying signal causing negative BOLD changes.

What we have shown is that the negative fMRI BOLD activity seen in contralateral somatosensory cortex correlates with neuronal activity (as indexed by the SEP) and fMRI BOLD changes in the “active” cortical area, that is, they are directly related to markers of neuronal activity elsewhere. If these findings reflect a neuronally mediated corticocortical inhibition, such that ipsilateral cortical activity is inhibited in proportion to increases in contralateral cortical activity, then it is possible that lesions of the corpus callosum might disrupt these neuronal connections.

**Experimental Confounds**

We must acknowledge some differences in the implementation of the fMRI BOLD and SEP protocols used in this experiment, although these are unlikely to create substantial experimental confounds. First, electrical stimulation of the medial nerve was applied at 100 Hz during fMRI recording and at 20 Hz during SEP recording, as in a previous study (Arthurs and others 2000). The lower frequency allows reliable identification of early components in the SEP recording, whereas the higher stimulation frequency is more efficient for determining the fMRI BOLD responses (Kampe and others 2000). SEPs at 100 Hz are inherently difficult to record due to the stimulus artifact, and the standing waveform generated is difficult to interpret at this frequency in the absence of more sophisticated analysis methodology. Short-latency SEP intensity-dependent stimulus response curves have not been found to vary significantly between 20 and 100 Hz (O. Arthurs and S. Boniface, unpublished data). However, 2 different frequencies are required to optimize each signal (fMRI and SEP), and this suggests that the different frequencies may generate subtly different responses. The use of multichannel EEG or magnetoencephalography recording may be required to accurately identify SEP component generators at high stimulation frequencies to resolve these issues.

Second, we also only modeled quadratic, second-order nonlinearities in the data and did not further investigate third order or other types of nonlinearity. However, no other types of nonlinearity reached significance in preliminary data analysis. Further, detailed modeling of this relationship may reveal more subtle nonlinearities.

Third, we used block design fMRI recording and event-related SEP recordings, although both SEPs and the fMRI BOLD response were summarized over a 30-s block, in order to eliminate and minimize any initial adaptive responses. Given the long periods over which these data sets were recorded in parallel, this is unlikely to make a significant difference. However, we acknowledge that these practical constraints and differences in techniques could account for small changes, such as the nonlinear covariations observed.

**Summary**

In conclusion, the simultaneous activation and suppression of functionally related cortical areas, as well as the intraregional features of the neurovascular coupling response, appear considerably more complex than has been considered to date and require further investigation.

**Notes**

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Address correspondence to O.J. Arthurs, Wolfson Brain Imaging Centre, University of Cambridge, Box 65, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, UK. Email: fMRI@owenarthurs.co.uk.

**References**


