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

Introduction

The coupling of the brain’s neural activity to its blood supply, termed neurovascular coupling, and its mechanisms are a fundamental feature of brain physiology that have been under discussion for more than a century and are still not fully understood. Recent studies have identified a linear relationship between measures of hemodynamic change and neuronal activity in rats (Mathiesen and others 1998; Ngai and others 1999) and primates (Mathiesen and others 1998; Heeger and others 2000; Rees and others 2000; Logothetis and others 2001) and between functional magnetic resonance imaging (fMRI) blood oxygen level-dependent (BOLD) responses in humans and primate neuronal activity (Mathiesen and others 1998; Heeger and others 2000; Rees and others 2000; Logothetis and others 2001). Some nonlinearity in this relationship has also been identified in relation to animal experiments (Ances and others 2000; Jones and others 2004; Hewson-Stoate and others 2005). The relationship is less well characterized in humans, but modern neuroimaging methods combined with more traditional electrophysiological techniques now allow for a definition of the neurovascular relationship in normal human subjects.

A further physiological question relates to whether the brain increases blood flow to functionally active areas at the expense of other nonfunctioning areas. Neuroimaging has thrown some light on this: some studies have identified decreases in blood flow responses in functionally related (but not adjacent) cortical areas in both sensory (Drevets and others 1995; Peyron and others 1999) and motor areas (Allison and others 2000) during activation. However, whether these findings are related to decreased neuronal activity causing reductions in hemodynamic change (i.e., an underlying negative neurovascular coupling mechanism), or are a purely vascular phenomenon, remains unresolved.

The experiments presented here investigate the direction and linearity of the neurovascular coupling relationship in normal human subjects. We compared changes in cerebral blood flow (CBF) (using blocked design fMRI BOLD) and scalp electrophysiology (using somatosensory evoked potentials [SEPs]) in parallel experiments with changes in the intensity of a median nerve electrical stimulus. By weighting the fMRI images according to the evoked potential amplitudes at corresponding intensities, we sought to test the hypothesis that these 2 data sets covaried with each other, either positively or negatively, in a linear or nonlinear manner. We thus identified whether these relationships might vary within the somatosensory cortex itself or between hemispheres.

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 1997). 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 (C3) 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
Results

Effects of Stimulus Intensity on SEP Amplitude

Effects of Stimulus Intensity

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: 38,–24, 62; 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 covaried 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. 4B); 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. 4B).

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. 4C) 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. 4A).

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 Leeuwen 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
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by Arthur and others (2002; Heeger and others 2003; Rees and others 2000). The hemodynamic response may therefore be related to the underlying electrical activity, including both LFP and spiking activity. The importance of this possibility and several other factors that may affect the lack of a perfect relation between BOLD and SEPs have been discussed elsewhere at length (Arthurs and Boniface 2000; Heeger and Rees 2002; Rees and others 2002; Logothetis and Pfeuffer 2004). Our results suggest that the cortical activity measured using the early component of the SEP makes a strong linear contribution to the vascular changes that dominate the human BOLD response.

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

### 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>
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<tbody>
<tr>
<td>2A</td>
<td>Positive</td>
<td>Contralateral SI</td>
<td>38, 50, 62</td>
<td>10.23**</td>
<td>636**</td>
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<tr>
<td></td>
<td>Negative</td>
<td>Contralateral thalamus</td>
<td>16, 44, 54</td>
<td>10.84**</td>
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<tr>
<td>2B</td>
<td>Positive</td>
<td>Contralateral SI</td>
<td>2, 50, 58</td>
<td>4.80**</td>
<td>1**</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>Ipsilateral SI</td>
<td>−40, 34, 64</td>
<td>5.09</td>
<td>5**</td>
</tr>
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</table>

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

### Table 2

<table>
<thead>
<tr>
<th>Figure</th>
<th>Subject</th>
<th>Brain region</th>
<th>Coordinates x, y, z</th>
<th>Z score</th>
<th>Cluster size</th>
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<tr>
<td>3A</td>
<td>1</td>
<td>Contralateral SI</td>
<td>50, 22, 52</td>
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<td>466**</td>
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<tr>
<td>3B</td>
<td>2</td>
<td>Ipsilateral Cerebellum</td>
<td>−16, −62, −30</td>
<td>6.34**</td>
<td>27**</td>
</tr>
<tr>
<td>3C</td>
<td>3</td>
<td>Contralateral SI</td>
<td>40, 22, 62</td>
<td>9.22**</td>
<td>105**</td>
</tr>
<tr>
<td>3D</td>
<td>4</td>
<td>Ipsilateral Cerebellum</td>
<td>−20, 50, −38</td>
<td>5.75**</td>
<td>10**</td>
</tr>
<tr>
<td>3F</td>
<td>5</td>
<td>Contralateral SI</td>
<td>44, 28, 50</td>
<td>8.14**</td>
<td>113**</td>
</tr>
<tr>
<td>3F</td>
<td>5</td>
<td>Ipsilateral Cerebellum</td>
<td>−24, 62, −30</td>
<td>7.49**</td>
<td>102**</td>
</tr>
<tr>
<td>3F</td>
<td>6</td>
<td>Contralateral SI</td>
<td>46, 36, 58</td>
<td>5.85**</td>
<td>29**</td>
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</table>

Note: Fixed-effects group analysis of n = 6 shown. **P < 0.001, *P < 0.05; SI = primary somatosensory cortex.
These images are thresholded at somatosensory cortex. Note: Fixed-effects group analysis of
— Negative, nonlinear
4
positively and nonlinearly with SEP N20-P25 amplitudes (Fig. 4). These are contralateral somatosensory cortex and contralateral thalamus (covary positively and linearly, A), contralateral somatosensory cortex near the midline and ipsilateral somatosensory cortex (covary negatively and linearly, B), and contralateral somatosensory cortex (covary positively and nonlinearly, C). 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 3.

Figure 4. The fMRI BOLD areas that covary positively and linearly with SEP N20-P25 amplitudes (A), negatively and linearly with SEP N20-P25 amplitudes (B), and positively and nonlinearly with SEP N20-P25 amplitudes (C). These are contralateral somatosensory cortex and contralateral thalamus (covary positively and linearly, A), contralateral somatosensory cortex near the midline and ipsilateral somatosensory cortex (covary negatively and linearly, B), and contralateral somatosensory cortex (covary positively and nonlinearly, C). 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 3.

Table 3

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<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>
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<tr>
<td>A</td>
<td>Positive, linear</td>
<td>Contralateral SI</td>
<td>40, -26, 64</td>
<td>10.17**</td>
<td>458**</td>
</tr>
<tr>
<td></td>
<td>Positive, linear</td>
<td>Contralateral thalamus</td>
<td>50, -20, 56</td>
<td>10.14**</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Positive, nonlinear</td>
<td>Contralateral SI</td>
<td>-34, -34, 58</td>
<td>4.95*</td>
<td>2*</td>
</tr>
<tr>
<td></td>
<td>Positive, nonlinear</td>
<td>Contralateral SI</td>
<td>-4, -44, 58</td>
<td>6.28**</td>
<td>127**</td>
</tr>
<tr>
<td></td>
<td>Negative, linear</td>
<td>Contralateral SI</td>
<td>-42, -32, 62</td>
<td>6.04**</td>
<td>15*</td>
</tr>
<tr>
<td>C</td>
<td>Negative, nonlinear</td>
<td>Ipsilateral SI</td>
<td>-26, -22, -2</td>
<td>6.08**</td>
<td>15*</td>
</tr>
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</table>

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

and adenosine (Dirnagl 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 Dirnagl 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 corresponded 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 ERP 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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**References**


