Negative BOLD signal changes in ipsilateral primary somatosensory cortex are associated with perfusion decreases and behavioral evidence for functional inhibition

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A B S T R A C T

We used functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) to study the negative blood oxygenation level dependent (BOLD) signal and its underlying blood flow changes in healthy human subjects. This was combined with psychophysiological measurements to test that the negative BOLD signal is associated with functional inhibition. Electrical stimulation of the median nerve at 7 Hz evoked robust negative BOLD signals in the primary somatosensory cortex (SI) ipsilateral to stimulation, and positive BOLD signals in contralateral SI. The negative BOLD signal in ipsilateral SI was accompanied by commensurate decreases in relative regional cerebral blood flow (rCBF). Conjunction analysis of the fMRI and PET data revealed a region in the ipsilateral postcentral gyrus showing overlap of negative BOLD signals and relative rCBF decreases. The current perception threshold (CPT) at the ipsilateral finger during concomitant stimulation of the contralateral median nerve increased significantly, suggesting augmented functional inhibition. Since the CPT in the ipsilateral hallux did not significantly change in response to median nerve stimulation, it is more likely that the CPT-increase for the finger is due to functional inhibition (Kastrup et al., 2008) than to changes in selective attention. In conclusion, our data provide evidence that stimulus-induced reductions in relative rCBF may underlie the negative BOLD signal, which in turn may reflect increments in functional inhibition.

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Introduction

Although functional magnetic resonance imaging (fMRI) is widely used to map brain functions, the mechanisms behind the positive and in particular the negative blood-oxygenation-level-dependent (BOLD) signals are incompletely understood (Lauritzen, 2005; Logothetis, 2008). In this study, we combined fMRI and positron emission tomography (PET) to explore changes in relative regional cerebral blood flow (rCBF) underlying the negative BOLD signal in the somatosensory system. Previous work suggested that the negative BOLD signal indicates functional inhibition (Kastrup et al., 2008). As this experiment did not control for the possible confound of changes in selective attention, we used an optimized psychophysiological paradigm to address this issue directly.

The stimulus-evoked negative BOLD signal may be explained by either a larger decrease in rCBF compared to cerebral metabolic rate of oxygen (CMRO2) (Nair, 2005), larger fractional increase in CMRO2 compared to rCBF (Vafaee and Gjedde, 2004), comparable decreases in rCBF and CMRO2 (Shmuel et al., 2002; Stefanovic et al., 2004), or a slow rise time in rCBF in face of rapid increments in nerve cell activity (Nair, 2005). Unilateral mechanical (Hlushchuk and Hari, 2006) or electrical stimulation (Kastrup et al., 2008; Klingsner et al., 2010) has been shown to induce negative BOLD signals in the ipsilateral primary somatosensory cortex (SI) in humans. Animal studies that focus on ipsilateral changes to thalamocortical stimulation report ipsilateral negative BOLD signals (Boorman et al., 2010; Devor et al., 2008) that are associated with vasoconstriction and decreases in rCBF (Devor et al., 2008). These results indicate activation of energy-consuming interneurons which may release vasoconstrictor molecules (Cauli and Hamel, 2008).
2010) or reduce recurrent excitation (Logothetis, 2008) or induce a combination of both.

In humans, a combined arterial spin labeling (ASL) and fMRI study reported decreases in rCBF and BOLD signal in ipsilateral primary motor cortex (MI) in response to a unilateral motor task (Stefanovic et al., 2004). The authors concluded that the negative BOLD signal reflected functional inhibition, but this was not proven unequivocally as no psychophysiological measurements of inhibition were included. Furthermore, many ASL techniques have a tendency to underestimate rCBF changes (Chen et al., 2008). Therefore, in this study we compared perfusion measurements using high-resolution PET and BOLD signal measurements and analyzed both modalities in one statistical framework. This was complemented with a behavioral study to test that the negative BOLD signal in SI ipsilateral to median nerve stimulation is accompanied by congruent decreases in rCBF and that the increases in CPT are more likely related to functional inhibition (Kastrup et al., 2008) than alterations of attention.

**Material and methods**

**Subjects**

Twelve right-handed healthy volunteers (7 male, 5 female; age: 28 ± 7 years) were included in the fMRI and PET investigations, which were conducted on different days in randomized order. One volunteer did not complete both scans and his results were therefore excluded from further analysis. Nine of the volunteers participated in the behavioral study that was carried out after the brain imaging sessions. The local ethics committee (De Videnskabetiske Komiteer for Region Hovedstaden) had approved all experiments and all subjects gave written informed consent prior to the study.

**Electrical stimulation**

We placed bipolar electrodes over the right wrist to stimulate the median nerve. The monophasic square wave pulses (duration 200 μs; frequency 7 Hz) were delivered by a constant current neurostimulator (DantecKeypoint, Denmark). The individual motor threshold was determined by increasing the current intensity until the right thumb showed a visible contraction. The stimulation intensity was set to 25% below the motor threshold. Although the final stimulation intensity used in the fMRI study (6.8 mA ± 2.2 mA) was considerably lower than in the PET study (8.4 mA ± 4.4 mA), this difference did not reach significance ($p = 0.2$, two-sided Wilcoxon signed-rank test). The difference between the means of the sensory thresholds was mainly due to an outlier whose stimulation intensity values differed by 10 mA between the sessions. The data were nevertheless included in the study as we obtained virtually identical results in the analysis of the functional imaging data when including the data compared to exclusion. Further reasons for the variability in the sensory thresholds between the sessions might encompass differing setups (e.g. scanner environment, fluctuations in the state of attention of the subjects, etc.). None of the subjects showed any muscle twitching during the experiment nor did they report that stimulation was painful at this intensity.

**fMRI data acquisition and analysis**

The fMRI block design started with a 39 s rest period and consisted of eight 60 s stimulation blocks alternating with seven 39 s rest periods, for a total duration of 792 s (Fig. 1). Each of the 60 s stimulation blocks consisted of seven epochs that lasted for 6 s with an interstimulus-interval of 3 s. fMRI data acquisition was performed with a 3.0 T Philips InteraAchieva scanner (Philips Medical Systems, Best, The Netherlands) equipped with an eight-element phased-array receive head coil. A gradient echo EPI sequence (32 slices of 4 mm thickness; slice gap 0.1 mm; field of view 230×230 mm; in-plane acquired resolution 2.9×2.9 mm; repetition time 3.0 s; echo time 35 ms; flip angle 90°; no-SENSE) was used to record 280 volumes during each of the two identical functional runs. Anatomical images were acquired with a T1-weighted 3D turbo field echo sequence (170 sagittal slices; 1 mm thickness; in-plane resolution 1×1 mm; repetition time 9.9 s; echo time 4.6 ms; flip angle 8°).

We pre-processed and analyzed the fMRI data with SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm). To avoid T1 equilibration effects, the first three volumes of each run were discarded automatically by the scanner. The remaining 277 volumes were realigned to the first image using a six-parameter rigid-body transformation to correct for motion artifacts (Friston et al., 1996). The images were spatially normalized to the Montreal Neurological Institute (MNI) space (Evans et al., 1993), interpolated to 2×2×2 mm and smoothed using an 8 mm (full-width-half-maximum) isotropic, three-dimensional Gaussian filter.

Low frequency confounds were removed by a high-pass filter at 1/200 Hz and each experimental condition was modeled as a boxcar function convolved with the canonical hemodynamic response function. Statistical parametric maps for positive and negative T-contrasts were

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**Fig. 1.** Stimulation paradigm used in the fMRI and PET study. A) On/off stimulation paradigm applied in fMRI. Grey blocks represent 60 s stimulation periods interleaved with 39 s periods of rest. All eight stimulation blocks and respective rest blocks amount to 277 volumes in our setup. B) A single 60 s on/off stimulation epoch consisted of 7 blocks, represented by dark grey striped blocks with a duration of 6 s each and a 3 s inter-stimulus interval. The same 60 s paradigm was used in PET. Stimulation frequency was 7 Hz, indicated by black stripes. Stimulation intensity was below motor-threshold.
calculated using the general linear model implemented in SPM8. In order to obtain group activation patterns, a random-effects analysis was performed. For the whole brain analysis, a significance level of \( p < 0.05 \), corrected for multiple comparisons (family-wise error) was employed. For activations within the somatosensory system (i.e., contra- and ipsilateral SI and SII) we used an uncorrected \( p \)-value of \( < 0.001 \) because of a strong anatomical a priori hypothesis. The anatomy toolbox (Eickhoff et al., 2005) was used for defining independent ROIs of SI and SII, used as brain masks. The SI and SII ROIs comprised Brodmann areas (BA) 1, 2, and 3a and 3b, and areas within the parietal operculum (OP) 1, 3 and 4 respectively. As OP 2 is most likely not part of SII but represents the human equivalent of the macaque vestibular area (parieto-insular vestibular cortex) (Eickhoff et al., 2006), this area was not included in the SII brain mask. Computation of percent signal change of the fMRI data was done with rfxplot (Gläscher, 2009).

PET data acquisition and analysis

Electrical stimulation of the right median nerve lasted for 60 s and consisted of alternating 6 s periods of 7 Hz stimulation (7 repetitions) and 3 s rest periods as applied in the fMRI stimulation paradigm. We chose our stimulation paradigm to maximize signal intensity in both imaging modalities. Minimal stimulation time in PET was 60 s, but optimal length of one block in fMRI is 18 to 20 s (Skudlarski et al., 1999). As the time course of the ipsilateral negative BOLD signal is time dependent and returns to baseline under ongoing stimulation (Hlushchuk and Hari, 2006; Kastrup et al., 2008), using a paradigm of 60 s of sustained stimulation would have had a detrimental effect on the signal intensity of the negative BOLD signal. We therefore opted for an ON/OFF paradigm to keep habituation of BOLD response and sensitization to electrical stimulation at a minimum (Mobascher et al., 2010).

Head movement was minimized by using a foam cushioned headrest with a strap over the subject’s forehead during scanning. Prior to the emission scans, a 7 min transmission scan was acquired with a rotating \(^{137}\)Cs single-photon point source for attenuation correction. Changes in relative rCBF were measured using a high-resolution research tomograph (HRRT) PET camera (Siemens Medical Solutions, Knoxville, TN) that generates 207 transaxial slices of 1.21 mm thickness with a 312 mm transaxial and 252 mm axial field-of-view. Prior to each scan, a bolus of 400 MBq of \([^{15}\text{O}]-\)labeled water was injected in the brachial vein of the non-stimulated arm using an automatic water injection system (AWIS 1997, Scansys, Denmark). A single static 90 s frame was acquired during and after bolus arrival in the brain with a 10 min delay between successive scans. No arterial blood sampling was performed. PET images were reconstructed with correction for scatter and attenuation. We acquired 6 scans during median nerve stimulation and 6 at rest in semi-random order. The TXTV segmentation method, included in the HRRT Users software release 1.1, was used for reconstruction of the transmission scans. Emission scans were reconstructed using 3D-OSEM PSF algorithm (10 iterations, 16 subsets) and filtered with a 5 mm full-width at half maximum (FWHM) Gaussian filter. PET images were realigned, spatially normalized to the standard template of the Montreal Neurological Institute (Evans et al., 1993) and smoothed with an isotropic Gaussian kernel with a FWHM of 8 mm. Statistical parametric maps for positive and negative T-contrasts were smoothed with an isotropic Gaussian kernel with a FWHM of 8 mm.

Calculation of activation task onset in PET

The onset of the electrical stimulation paradigm had to be precisely timed to fit the temporal window of \([^{15}\text{O}]-\)water activation scans with respect to tracer-arrival in the brain. The time point of the input functions peak was calculated with an in-house-written Matlab program and estimated from whole brain counts. The program plotted the true counts (decay and offset corrected) that were obtained from the first emission scan (rest-scan) over a timeline. Individual subject input function configurations were generated to optimize stimulation onsets. Because the ipsilateral negative BOLD signal in SI peaks approximately 6 to 9 s after stimulation onset, stimulation had to be started 6 to 9 s prior to the bolus peak. For every individual scan we also subtracted the time from scanner-start to injection-start (14.6 ± 1.9 s).

Behavioral experiments

In the behavioral experiments, we tested the hypothesis that the ipsilateral negative BOLD signal reflects genuine functional inhibition, as reflected by an increase in CPT in the finger conteralateral to the negative BOLD signal in SI (Kastrup et al., 2008; Klingner et al., 2010), that cannot be attributed to changes in selective attention. We thus introduced left hallux stimulation as a control condition (Fig. 2C). This was done in order to control for stimulus-induced alteration of selective attention as a possible confound. We chose the toes because they are represented in the posterior part of the paracentral lobule on the medial surface of the brain (Eickhoff et al., 2008; Snell, 2009), an area that is not comprised by the ipsilateral negative BOLD signal in our experiment. If the increase in CPT is caused by a shift in attention towards stimulation of the contralateral arm, we expect an increased threshold both when testing the finger and the foot. In contrast, a selective effect for the hand area would be indicative for neuronal inhibition.

For stimulation of the left index finger and hallux, two stainless steel ring electrodes (Digitimer; cathode proximal; anode–cathode distance approximately 1 cm) were used. The sensory threshold was assessed by slowly increasing (maximum 20 s) the intensity of the constant current stimulation of the finger or hallux until the subject reported detecting the stimulus. The paradigm consisted of alternating stimulation and rest epochs. We randomly stimulated the right median nerve, or the left index finger/hallux or left index finger/hallux and right median nerve concurrently. All combinations were repeated 4 times with interstimulus intervals between 4 and 20 s. The overall length of the paradigm was 9 min. Mean intensities for left finger and hallux stimulation with and without concurrent right median nerve stimulation were calculated for each subject and analyzed with a two-sided Wilcoxon signed-rank test. Psychophysiological data are presented as the mean ± standard error of the mean (SEM).

Results

fMRI

Electrical stimulation of the right median nerve induced statistically significant BOLD signal increases within contralateral SI and SII (Fig. 3A). Within SI, the activation was attributed to BA 1 and BA 3b and within SII to OP 1 (Fig. 2A). Table 1 gives an overview of the activated areas together with their stereotactic coordinates. Fig. 3A shows the negative BOLD signal in ipsilateral SI (BA 1). Please note the extension of the ipsilateral negative BOLD signal into the motor cortex. Fig. 3C shows that the signal intensity of the most significant voxels within the predefined ROIs of SI and SII in contralateral SI (\( x = -44, y = -22, z = 60 \)) and SII (\( x = -42, y = -22, z = 16 \)) increased by 0.6 ± 0.1% and 0.8 ± 0.1% respectively and decreased by 0.5 ± 0.1% in ipsilateral SI (\( x = 46, y = -26, z = 62 \)).

PET

Fig. 3B shows significant relative rCBF increases within SI, allocated to contralateral BA 1, and contralateral (OP 1 including OP 4) and
ipsilateral (OP 4) SII. Significant decreases in relative rCBF occurred in subdivisions of ipsilateral SI, assigned to BA 1 and BA 3b. Further relative rCBF increases are listed in Table 2. As illustrated in Fig. 3D, the percentage rCBF signal change increased in the most significant voxels within the predefined ROIs of SI and SII by 3.0±0.5% in contralateral SI (x=−44, y=−28, z=64) and by 5.4±0.7% in contralateral SII (x=−42, y=−20, z=16). In ipsilateral SI (x=44, y=−24, z=58), relative rCBF decreased by 2.9±0.9%.

The most significant voxels in the fMRI and PET analysis differ spatially, but considering the different imaging modalities and their

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**Fig. 2.** Behavioral paradigm used to measure current perception threshold (CPT) of A) the left index finger and C) the left hallux. B) CPT of the finger increased significantly (p<0.005) during concurrent stimulation of the median nerve. D) CPT measured in the left hallux did not change during concomitant median nerve stimulation. Grey blocks and triangles represent stimulation periods. For the finger and hallux, stimulation intensity [mA] was slowly increased until detection threshold was reached. Stimulation of the right median nerve lasted for 20 s, whereas rest periods varied between 4 and 20 s.

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**Fig. 3.** Areas showing increases (red) and decreases (blue) in BOLD signals or relative rCBF during unilateral median nerve stimulation (thresholded at p<0.005, uncorrected, for display purposes). A) BOLD signal increases to unilateral median nerve stimulation occurred in the contralateral primary (SI) and secondary somatosensory cortex (SII) and the primary motor cortex (MI). BOLD signal decreased in ipsilateral SI and MI. B) Relative rCBF increased (red) in contralateral SI and bilaterally in SII and decreased (blue) in ipsilateral SI. Signal changes are projected onto coronal (first row) and transverse slices of the SPM T1 template. Sectional planes are y=−20 and z=±54. Mean percentage (±SEM) of C) BOLD signal and D) relative rCBF changes are extracted from the most significant voxels in contralateral SI, SII and ipsilateral SI.
Table 2
Conjunction analysis of fMRI and PET data

<table>
<thead>
<tr>
<th>Localisation</th>
<th>Peak level</th>
<th>BOLD signal</th>
<th>Peak voxel MNI coordinates</th>
<th>T-Statistic</th>
<th>p-Value</th>
<th>Cluster level</th>
<th>Cluster size in voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Contralateral hemisphere</td>
<td>Postcentral gyrus (BA 1, BA 3b)</td>
<td>Increase</td>
<td>−44</td>
<td>−22</td>
<td>60</td>
<td>16.2</td>
<td>p&lt;0.001 uncorrected.</td>
</tr>
<tr>
<td></td>
<td>Rolandic operculum (OP 1)</td>
<td>Increase</td>
<td>−42</td>
<td>−22</td>
<td>16</td>
<td>14.58</td>
<td>p&lt;0.001 uncorrected.</td>
</tr>
<tr>
<td></td>
<td>Precentral gyrus (BA 6)</td>
<td>Increase</td>
<td>−36</td>
<td>−12</td>
<td>56</td>
<td>16.37</td>
<td>p&lt;0.05 FWE</td>
</tr>
<tr>
<td>Ipsilateral hemisphere</td>
<td>Postcentral gyrus (BA 1)</td>
<td>Decrease</td>
<td>46</td>
<td>−26</td>
<td>62</td>
<td>5.03</td>
<td>p&lt;0.001 uncorrected.</td>
</tr>
</tbody>
</table>

Respective spatial resolutions and preprocessing steps (e.g. alignment procedures), distances between the most significant voxels (7.2 mm for contralateral SI, 4.9 mm for ipsilateral SI and 2 mm for contralateral SII) are still relatively small.

Conjunction analysis of fMRI and PET data

We performed a conjunction analysis ("Minimum Statistic compared to the Global Null") (Friston et al., 2005; Price and Friston, 1997) of the PET and fMRI data on a voxel-basis to identify areas with significant signal changes in both imaging modalities (Fig. 4.). As expected, common signal increases occurred in a cluster of 298 voxels within contralateral SI that was mainly allocated to BA 1 based on the cytoarchitectonic maps of the anatomy toolbox (peak voxel at x = −48, y = −14, z = 52) and a cluster of 687 voxels within SII (OP 1, peak voxel at x = −42, y = −20, z = 16). Common signal decreases occurred in a cluster of 203 voxels within ipsilateral SI that was mainly attributed to BA 1 (peak voxel at x = −44, y = −23, z = 58).

Correlation analysis between fMRI and PET data

We performed an independent whole brain voxel-by-voxel correlation analysis (Casanova et al., 2007) of the BOLD signal and relative rCBF changes. To explore the correlations within somatosensory areas, we used the brain masks of SI and SII based on the cytoarchitectonic maps derived from the anatomy toolbox and observed significant correlations (p<0.01, uncorrected) in contralateral (BA 3a and 3b, peak voxel at x = −26, y = −20, z = 44) and ipsilateral (BA 2, peak voxel at x = −44, y = −32, z = 44) SI and bilaterally in SII (OP 1, OP 4, peak voxel at x = −64, y = −18, z = 12 and OP 3, peak voxel at x = −44, y = −16, z = 22 respectively). To investigate whether the common signal changes of the rCBF changes and the BOLD signal (i.e. conjunction analysis) also showed significant correlations, we masked the correlation analysis with the conjunction analysis, which revealed a cluster of 5 voxels within the conjunction area in ipsilateral SI, attributed to BA 2 and spreading into BA 1. Fig. 5 shows the respective values of the individual subjects extracted from the peak voxel at x = 48, y = −32, z = 54.

The fact that the changes in BOLD signal and rCBF within the conjunction area were significantly correlated at p<0.01, uncorrected, in ipsilateral SI but not within the conjunction area of contralateral SI was unexpected and thus remains to be addressed by future research. At a more liberal threshold of p<0.05, uncorrected, however, we observed correlations between the two imaging modalities within the conjunction area of contralateral SI as well (BA 1, most significant voxel at x = −50, y = −20, z = 56).

Behavioral experiments

Mean detection thresholds measured at the left index finger increased significantly from 2.5 mA ± 0.3 to 3.0 mA ± 0.4 when tested without and with simultaneous stimulation of the right median nerve (two-sided Wilcoxon signed-rank test: p<0.005, Fig. 2B). There was no significant change in CPT for stimulation of the left halleux with and without concurrent median nerve stimulation; mean thresholds were 6.6 mA ± 1.2 and 6.9 mA ± 1.0, respectively (two-sided Wilcoxon signed-rank test: p = 0.25; Fig. 2D).

Discussion

Our results demonstrate that the ipsilateral negative BOLD signal in SI is associated with a decrease in relative rCBF. We furthermore show that although changes in directed attention cannot be entirely excluded, the most parsimonious explanation for the ipsilateral negative BOLD signal is functional inhibition as indicated by an increased CPT of the finger.

The physiological meaning of the negative BOLD signal is still controversial and both purely vascular phenomena such as vascular steal and neurovascular mechanisms related to synaptic inhibition have been put forward as explanatory mechanisms. According to the “vascular steal” hypothesis, blood is redirected to neurally active regions and drained from nearby areas, thus reducing the BOLD signal without changes in neuronal activity in regions of the negative BOLD

Table 1
Main activation clusters of the fMRI BOLD signal increases and decreases with corresponding statistics.

<table>
<thead>
<tr>
<th>Localization</th>
<th>Peak level</th>
<th>BOLD signal</th>
<th>Peak voxel MNI coordinates</th>
<th>T-Statistic</th>
<th>p-Value</th>
<th>Cluster level</th>
<th>Cluster size in voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral hemisphere</td>
<td>Postcentral gyrus (BA 1, BA 3b)</td>
<td>Increase</td>
<td>−44</td>
<td>−22</td>
<td>60</td>
<td>16.2</td>
<td>p&lt;0.001 uncorrected.</td>
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<tr>
<td>Rolandic operculum (OP 1)</td>
<td>Increase</td>
<td>−42</td>
<td>−22</td>
<td>16</td>
<td>14.58</td>
<td>p&lt;0.001 uncorrected.</td>
<td>477</td>
</tr>
<tr>
<td>Precentral gyrus (BA 6)</td>
<td>Increase</td>
<td>−36</td>
<td>−12</td>
<td>56</td>
<td>16.37</td>
<td>p&lt;0.05 FWE</td>
<td>55</td>
</tr>
<tr>
<td>Ipsilateral hemisphere</td>
<td>Postcentral gyrus (BA 1)</td>
<td>Decrease</td>
<td>46</td>
<td>−26</td>
<td>62</td>
<td>5.03</td>
<td>p&lt;0.001 uncorrected.</td>
</tr>
</tbody>
</table>

Table 2
Main activation clusters of the relative rCBF increases and decreases with corresponding statistics.

<table>
<thead>
<tr>
<th>Localization</th>
<th>Peak level</th>
<th>rCBF</th>
<th>Peak voxel MNI Coordinates</th>
<th>T-Statistic</th>
<th>p-value</th>
<th>Cluster level</th>
<th>Cluster size in voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral hemisphere</td>
<td>Postcentral gyrus (BA 1)</td>
<td>Increase</td>
<td>−44</td>
<td>−28</td>
<td>64</td>
<td>6.35</td>
<td>p&lt;0.001 uncorrected.</td>
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<tr>
<td>Rolandic operculum (OP 1, OP 4)</td>
<td>Increase</td>
<td>−42</td>
<td>−20</td>
<td>16</td>
<td>8.42</td>
<td>p&lt;0.001 uncorrected.</td>
<td>777</td>
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<tr>
<td>Anterior cingulate cortex</td>
<td>Increase</td>
<td>−2</td>
<td>12</td>
<td>30</td>
<td>5.93</td>
<td>p&lt;0.05 FWE</td>
<td>46</td>
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<tr>
<td>Thalamus</td>
<td>Increase</td>
<td>−16</td>
<td>−20</td>
<td>6</td>
<td>5.83</td>
<td>p&lt;0.05 FWE</td>
<td>54</td>
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<tr>
<td>Precentral gyrus (BA 6)</td>
<td>Increase</td>
<td>−34</td>
<td>−18</td>
<td>72</td>
<td>5.59</td>
<td>p&lt;0.05 FWE</td>
<td>382</td>
</tr>
<tr>
<td>Insula</td>
<td>Increase</td>
<td>−36</td>
<td>4</td>
<td>8</td>
<td>5.17</td>
<td>p&lt;0.05 FWE</td>
<td>25</td>
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<tr>
<td>Ipsilateral hemisphere</td>
<td>Postcentral gyrus (BA 1, BA 3b)</td>
<td>Decrease</td>
<td>44</td>
<td>−24</td>
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<td>3.75</td>
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<tr>
<td>Rolandic operculum (OP 4)</td>
<td>Increase</td>
<td>54</td>
<td>−4</td>
<td>10</td>
<td>6.89</td>
<td>p&lt;0.001 uncorrected.</td>
<td>623</td>
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<tr>
<td>Insula</td>
<td>Increase</td>
<td>40</td>
<td>−14</td>
<td>4</td>
<td>6.27</td>
<td>p&lt;0.05 FWE</td>
<td>77</td>
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<tr>
<td>Middle cingulate cortex</td>
<td>Increase</td>
<td>10</td>
<td>8</td>
<td>36</td>
<td>5.52</td>
<td>p&lt;0.05 FWE</td>
<td>16</td>
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</table>
or BA 1, which gave rise to augmented receptive fields in the opposite homologous hemisphere indicating disinhibition (Clarey et al., 1996), and in humans following unihemispheric transcranial magnetic stimulation (TMS) over the parietal cortex (Blankenburg et al., 2008), the occipital cortex (Bocci et al., 2011) or MI (Avanzino et al., 2007; Daskalakis et al., 2002; Gerloff et al., 1998). These findings provide further evidence for the theory that activation of transcallosal fibers induces net synaptic inhibition in the ipsilateral hemisphere. Transcallosal inhibition is believed to increase efficiency (Adam and Güntürkün, 2009; Hellige, 1990) and to serve as an “inhibitory barrier” to prevent maladaptive interhemispheric cross talk (Kinsbourne, 1982).

Although almost all callosal fibers are excitatory (Conti and Manzoni, 1994; van der Knaap and van der Ham, 2011), they can exert an inhibitory effect by targeting gamma-aminobutyric acid (GABA)ergic interneurons (Bloom and Hynd, 2005; van der Knaap and van der Ham, 2011). The ipsilateral negative BOLD signal is therefore most likely mediated by transcallosal activation of inhibitory GABAergic interneurons that do contain vasoactive substances with both dilatory and constrictory properties (Cauli and Hamel, 2010; Enager et al., 2009). Therefore, it is likely that excitation of specific classes of cortical inhibitory interneurons induced the localized reduction in relative rCBF in this study. This notion is consistent with results from recent studies reporting a negative correlation between elevated GABA levels and the amplitude of the BOLD response (Donahue et al., 2010; Muthukumaraswamy et al., 2011). In comparison, reductions in GABA levels correlated positively with the degree of motor learning and fMRI signal strength in left MI (Stagg et al., 2011). Finally, GABA concentrations in the anterior cingulate cortex correlated positively with the intensity of the negative BOLD signal in the same area (Northoff et al., 2007).

Interneuronal activity is energy demanding since several classes of interneurons have a two to threefold higher discharge rate than pyramidal neurons (Markram et al., 2004), and the duration of inhibitory synaptic currents is two times longer than that of excitatory currents (Galarreta and Hestrin, 1997; Gupta et al., 2000). Therefore a positive BOLD signal may be expected, and animal reports indeed indicate that they both rise in rCBF and by inference positive BOLD signals may be produced by stimulation of inhibitory interneurons (Enager et al., 2009; Mathiesen et al., 1998). The activity of inhibitory interneurons could also account for the increased 2-deoxyglucose uptake measured in rat brain areas with an ipsilateral negative BOLD signal (Devor et al., 2008). However, interneurons also possess a number of energy saving mechanisms. Their resting membrane potential is lower than that of pyramidal cells. Therefore, the electrochemical gradient along which chloride moves is smaller and thus less energy is required to pump chloride ions back. The number of inhibitory interneurons is much lower than that of excitatory pyramidal cells; only 15% to 20% of the cortical neurons are GABAergic interneurons (Buzsáki et al., 2007; DeFelipe, 1993; Hendry et al., 1987; Markram et al., 2004; Somogyi et al., 1998). A negative BOLD signal induced by activity of interneurons may be explained by reduced recurrent activity of downstream excitatory neurons (Logothetis, 2008) but also by vascular effects. Sympathetic inhibition may diminish or abolish the release of vasodilators like neuronal nitric oxide synthase and phospholipase A2 by reducing the opening probability of voltage-sensitive calcium channels (Caesar et al., 2003; Lauritzen, 2005). Finally, interneurons can directly influence micro-veessels through the release of vasoconstrictors such as neuropeptide Y (Cauli et al., 2004).

We investigated the negative BOLD signal employing PET and fMRI systematically in the same subjects. In addition, our study benefits from using an HRRT PET scanner that has a spatial resolution of up to 1.4 mm (Olesen et al., 2009), a nearly isotropic spatial resolution in a 20 cm diameter volume and a higher sensitivity than conventional PET scanners (de Jong et al., 2007). Comparing fMRI and PET data is challenging due to differences in methodology and their sensitivity to variations in physiology. Several factors can account for the observed differences in the activation maps of the fMRI and PET data.
While the PET signal mainly reflects tissue perfusion at the capillary level (Dimitrakopoulou-Staats et al., 1998), the fMRI signal at lower field strength predominantly originates from the draining veins (Kinahan and Noll, 1999). This can influence both amplitude and location of the measured signal (Boxerman et al., 1995; Hlustík et al., 1998). It has been shown that H$_2^{15}$O PET has a higher signal contrast than BOLD-fMRI (Ramsey et al., 1996). FMRI is additionally vulnerable to susceptibility and pulsation artifacts. Finally, differences in the statistical processing of PET and fMRI data (e.g. global normalization is used for PET data only) may also contribute to differences in activation patterns. It is therefore not surprising that studies investigating the spatial overlap of FMRI and PET measurements reported an average mismatch of 10 to 20 mm (Bookheimer et al., 1997; Clark et al., 1996; Fox et al., 1999, 2001; Hasnain et al., 1998; Xiong et al., 2000). We used a conjunction analysis of the FMRI and PET results, thereby combining both data sets in one statistical framework. The novelty of our findings is that they provide strong evidence that the ipsilateral negative BOLD signal is unlikely to be caused by attentional mechanisms. Finally, our data extend earlier findings that the association of the negative BOLD signal with decreases in rCBF is not limited to MI (Stefanovic et al., 2004), but also occurs in SI. The only comparable study examining the ipsilateral negative BOLD signal and changes in CBF in the motor cortex performed a ROI based correlation analysis of the two datasets acquired by FMRI and ASL (Stefanovic et al., 2004). We chose PET instead of arterial spin labeling (ASL), because there is a risk of underestimating CBF when using ASL. Indeed, studies have shown that CBF values measured by ASL can be up to 30% lower than those measured by PET (Chen et al., 2008; Ye et al., 2000). Furthermore, within-subject CBF variations were higher for ASL (standard deviation: 20 ml/100 g/min in grey matter and 15 ml/100 g/min in white matter) than for PET measurements (4–5 ml/100 g/min) (Carroll et al., 2002). Consequently, H$_2^{15}$O PET is still considered the gold standard for measuring CBF-changes.

We also found positive and negative BOLD signal changes in MI. It is known that tactile stimulation of the hand or foot may activate MI (Polonara et al., 1999), a finding that has been interpreted in terms of anticipation of action (Fabri et al., 2011). Transcranial recordings of evoked responses in man after median nerve stimulation also documented interhemispheric connections between SI–MI–SI (Golding et al., 1970; Rager et al., 2011; Reis et al., 2007). The additional behavioral results suggest that increases in CPT were not caused by refocused attention towards the opposite median nerve. The results hence confirm the functional inhibitory impact of the ipsilateral negative BOLD signal as increased CPTs indicate functional impairments of sensory processing that signify inhibition. Previous studies investigating negative ipsilateral BOLD signals in the somatosensory cortex did not include a control for attention as a confounding variable. Studies of the somatosensory and pain system reported raised detection thresholds for tactile stimulation during auditory distraction (Gescheider and Niblette, 1967) and increased reaction times in a thermal discrimination task during attention–deflecting visual cues (Bushnell et al., 1985; Gescheider and Niblette, 1967) or divided attention towards tactile stimuli (Post and Chapman, 1991). Therefore, we introduced the toe as a control condition as its cortical representation is not encompassed by the ipsilateral negative BOLD in SI and it has a similar degree of lateralization as the finger. Any purported CPT changes in the hallux during concomitant opposite median nerve stimulation would thus be attributable to changes in directional attention. Since CPTs of the contralateral hallux did not change, the most likely explanation for the increased CPT of the contralateral finger is that it is caused by functional inhibition rather than by relocation of attention towards the opposite arm. Therefore, we take the increased CPT as evidence for functional inhibition in the region with negative BOLD signal and reduced relative rCBF. We did not find a significant correlation between CPT and the negative BOLD signal as reported by others (Kastrup et al., 2008; Klinger et al., 2010), nor did we find a correlation between the behavioral data and rCBF changes. Differences in the setups might explain this discrepancy. In contrast to Kastrup and Klingner and their respective coworkers, who measured CPTs within a time interval of 30 s (Kastrup et al., 2008; Klinger et al., 2010), we used a maximal time-window of 20 s. Therefore our current-slope as a function of time was steeper which might have introduced inaccuracies. We also used pure somatosensory stimulation of 7 Hz at an intensity that was set 25% below the motor threshold, whereas the aforementioned studies applied a 40 Hz stimulation at motor threshold (Kastrup et al., 2008) or at 10% below motor threshold (Klinger et al., 2010). This results in higher stimulation intensities, which in turn are known to be positively correlated with the strength of the negative BOLD response (Klinger et al., 2010). One could speculate that our weaker BOLD and possibly also weaker rCBF response might be another reason for our lack of correlation between CPT and the BOLD signal or CPT and the rCBF change. One last explanation could be inter-session or intra-subject effects as the behavioral measurements and the PET and fMRI scans were performed on different days and both the BOLD signal and rCBF are known to have a day-to-day variability (Leontiev and Buxton, 2007).

In conclusion, our data show that the negative BOLD signal in ipsilateral SI triggered by unilateral median nerve stimulation is associated with congruent decreases in relative rCBF. Although influences of attention could also be attributed to increased CPTs in the finger during concomitant contralateral median nerve stimulation, our psychophysiological findings further support functional inhibition as the main perpetrator of the increases in CPT and the negative BOLD signal and decreases in rCBF.

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