

Transcranial cortex stimulation and fMRI: Electrophysiological correlates of dual-pulse BOLD signal modulation

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Are the local hemodynamic changes in BOLD-fMRI correlated to increased or decreased neuronal activity or both? We combined transcranial electrical cortex stimulation (TES) with simultaneous fMRI and electromyographic (EMG) recording to study the influence of inhibitory and excitatory neuronal activity on the concomitant BOLD signal change. Unilateral or bilateral TES was applied with a postero-anterior orientation. This activates pyramidal cells transsynaptically and allows for the induction of cortical inhibition and excitation of the pyramidal cell, respectively. In this project interhemispheric inhibition (IHI) served as an in vivo model to investigate electrophysiologically well defined inhibitory and excitatory effects.

Methodology: Included event-related fMRI, which triggered TES; online recording of the EMG response monitored the inhibitory and excitatory influences on discharging corticospinal neurons.

Results: Revealed that a single suprathreshold stimulus induced a positive BOLD response both in the ipsilateral as well as in the contralateral primary motor cortex (M1). The contralateral co-activation of the homotopic M1 should be a functional correlate of transcallosal connections. If a contralateral conditioning stimulus preceded the test stimulus by 10 ms (IHI), the subsequent ipsilateral BOLD signal was significantly reduced. We find that cortical inhibitory processes are accompanied by attenuation of the local neurovascular signal.

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Introduction

It is a matter of controversy whether local hemodynamic changes detected in functional imaging are correlated to increased or decreased neuronal activity. For example, studies on the rat cerebellar cortex suggest that it is not possible to relate neurally evoked blood flow changes to an increased firing rate of principle output neurons (Mathiesen et al., 1998; Lauritzen, 2001). In contrast, they postulate that blood flow changes are coupled to the

afferent input function, meaning all aspects of presynaptic and postsynaptic processing (Lauritzen and Gold, 2003). Monosynaptic excitation as well as mixed disynaptic inhibition/excitation is shown to correlate with a *relative increase* in the local hemodynamic response. However, it is unclear in which manner these results for the rat cerebellar cortex can be transferred to the human cerebral cortex.

So far, functional imaging studies in humans addressed the influence of inhibition and excitation on the hemodynamic response by using, e.g., visual (Shmuel et al., 2002, 2003) or motor tasks. In the latter case, results of recent fMRI and PET studies using unimanual motor tasks demonstrated both an increased and a decreased BOLD signal in ipsilateral primary motor cortex (M1) (e.g. Kawashima et al., 1998; Cramer et al., 1999; Allison et al., 2000; Nirikko et al., 2001; Hamzei et al., 2002; Kobayashi et al., 2003; Stefanovic et al., 2005; Newton et al., 2005).

These contradictory studies analyzed the BOLD response based on *voluntary movement*. In contrast, we utilize transcranial electrical cortex stimulation (TES) to directly induce inhibitory and excitatory neuronal activity in the motor cortex, i.e., independent of voluntary movement. We combined TES with simultaneous fMRI in an event-related design. The aim was to examine BOLD signal changes related to electrophysiologically well defined inhibitory and excitatory neuronal processes of single-pulse stimulation and interhemispheric inhibition (IHI). In humans, IHI has been induced by transcranial cortex stimulation with a conditioning testpulse. Here, as first described by Ferbert et al. (1992), a conditioning transcranial magnetic stimulus (TMS) to one motor cortex reduces the EMG activity produced by a second pulse to the contralateral motor cortex within an interstimulus interval of 6 to 50 ms (Ferberbert et al., 1992; Meyer et al., 1995, 1998b; Di Lazzaro et al., 1999; Hanajima et al., 2001; Chen et al., 2003). This phenomenon is likely to occur on a cortical level (Ferberbert et al., 1992; Di Lazzaro et al., 1999; Hanajima et al., 2001). It is mediated through excitatory commissural neurons, whose propagation speed is about 10–15 ms (Meyer et al., 1998a,b), which excite local inhibitory interneurons in the contralateral homologous motor cortex (Chen et al., 2003). Studies in the cat revealed that stimulation of one motor cortex produces a mixture of a point-to-point excitation of pyramidal neurons in the homologous area of the contralateral hemisphere,

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surrounded by a broad area that exerts inhibitory influences on pyramidal neurons (Asanuma and Okuda, 1962).

The simultaneous combination of TES with fMRI has several technical advantages (Brandt et al., 1996, 2001) as compared to the combination of TMS and fMRI. The gold electrodes do not cause significant artifacts in the MRI images. The electrode position can be marked exactly in the MRI images by using vitamin E capsules attached to the back of the electrode. The electrode cables – if oriented inline with the magnetic field of the scanner – do not cause significant artifacts. Additionally, a further major advantage of using TES in fMRI is that one can utilize multiple electrode sets and stimulate several regions of the brain during one experimental session (here both hemispheres) and/or control condition. A potential disadvantage as compared to TMS is the unpleasant nociceptive cutaneous sensation at the site of stimulation, which can be accommodated for with an adequate control condition.

TES was applied with a postero-anterior electrode orientation (TES p-a), which was shown to activate pyramidal cells transynaptically leading to comparable cortical stimulation effects as found for TMS p-a (stimulus onset latency, short intracortical inhibition and facilitation) (Brocke et al., 2005). First, we investigated if IHI can be induced by TES p-a in the same way as known for TMS p-a.

Second, single-pulse and bilateral TES p-a were combined with simultaneous fMRI in an event-related design. We could thus compare each time-point in the BOLD signal (e.g. during single or bilateral stimulation) with the electrophysiological correlate defined by the EMG response. This new experimental setup allowed us to induce well defined inhibitory and excitatory neuronal activity during fMRI and to study its influence on the concomitant BOLD signal changes.

Materials and methods

Subjects

Six right-handed healthy volunteers (male, 27, 28, 29, 30, 32 and 32 years of age) were subject to all exclusion criteria for fMRI and TES. The local ethics committee approved the protocol, and subjects gave their written informed consent. To our knowledge there have been no reports about seizures induced by TES in healthy subjects with the intensity and frequency used in the present study. The experimenters were neurologists familiar with the recognition and treatment of seizures.

Magnetic resonance imaging

All fMRI experiments were conducted using a 3 T Signa LX scanner (General Electric Company, USA). Functional images were collected with BOLD-contrast using a T2*-weighted single-shot EPI sequence (TR=3s, TE=30 ms; FA=70°, 16 slices, 64×64 matrix, FOV 220, voxel size=3.4×3.4×3 mm). Anatomical high quality three-dimensional data sets for each subject were recorded using a T1-weighted sagittal FLASH sequence (TR/TE=8/3 ms, FA=20°, voxel size=0.5×0.5×1 mm).

Transcranial electrical cortex stimulation

TES of the right and left motor hand area was performed using two Digitimer D 185 cortex stimulators (Welwyn, Great Britain, 50 μ s time constant) with non-ferromagnetic cables and gold

electrodes (diameter of 1 cm). The electrodes were attached to the scalp over the motor cortex with an electrode paste and Colodium glue to prevent dislocation.

Both cortex stimulators were triggered separately by an external trigger software (Spike 2 version 4.12, Cambridge Electronic Design, Cambridge, UK) using a CED 1401 power laboratory interface (Cambridge Electronic Design, Cambridge, UK) located outside the magnet room.

The exact functional definition of individual optimal scalp position and stimulus intensity was performed outside the scanner: First, the position was predefined using focal TMS. Second, the optimal site for TES was determined as the location, at which stimuli of suprathreshold intensity consistently produced the largest muscle responses in the first dorsal interosseus muscle. Resting motor threshold (RMT) was defined as the intensity needed to evoke a muscle response in relaxed muscle of >50 μ V in 5 of 10 consecutive trials. Suprathreshold stimulus intensity was adjusted as to evoke a muscle response amplitude of about 1 mV in the relaxed muscle and ranged between 24% and 32% of maximum stimulator output.

TES was performed in a postero-anterior arrangement (TES p-a) perpendicular to the central sulcus of each hemisphere. For each hemisphere the anode was fixed over the motor hand area, 5 cm lateral (right or left) to the intersection line from the vertex to the external auditory meatus. The cathode was placed 5 cm anterior of the anode on a line parallel to the midline.

Electromyographic recordings

To quantify the stimulation effect of single- and paired-pulse TES surface EMG responses during scanning were recorded bilaterally from the first dorsal interosseus muscle (FDI) in a belly tendon montage. In order to minimize the interaction between EMG cables and the magnetic fields, non-ferromagnetic cables in a twisted-pair orientation and gold cup electrodes (diameter of 1 cm) were used. With a sampling rate of 5 kHz, responses were continuously recorded, amplified and band-pass filtered (20 Hz–4 kHz) by CED 1902 amplifiers (Cambridge Electronic Design, Cambridge, UK). Data were collected through a CED 1401 power laboratory interface outside the magnet room and stored on a personal computer using Spike 2. As the T2*-weighted single-shot echo planar imaging (EPI) sequence induced a gradient artifact in the recorded EMG activity lasting approximately 85 ms, only the last 100 ms of the EMG signal between each slice (interslice time 187.5 ms) was analyzed (see Fig. 1).

Experimental setup

In the MRI scanner, the subject's head was positioned in the coil on a vacuum pillow to prevent electrode-related pressure points and head movements. The TES and EMG cables were run axial to the coil and connected to the cortex stimulators and the EMG amplifier at the far end of the scanner room. By carefully placing all cables axial to the coil without cable loops: (a) current induction should be prevented and (b) artifacts in the EMG-signal as well as the fMRI data should be minimized.

Both the cortex stimulators and the EMG amplifier were connected by BNC cables via low-pass filters (2.5 MHz cutoff frequency; minicircuits, NY, USA) through a $\lambda/2$ -tube to the 1401-interface outside the scanner.

Six subjects were stimulated with TES p-a over the primary motor cortex during continuous bilateral recording of EMG during

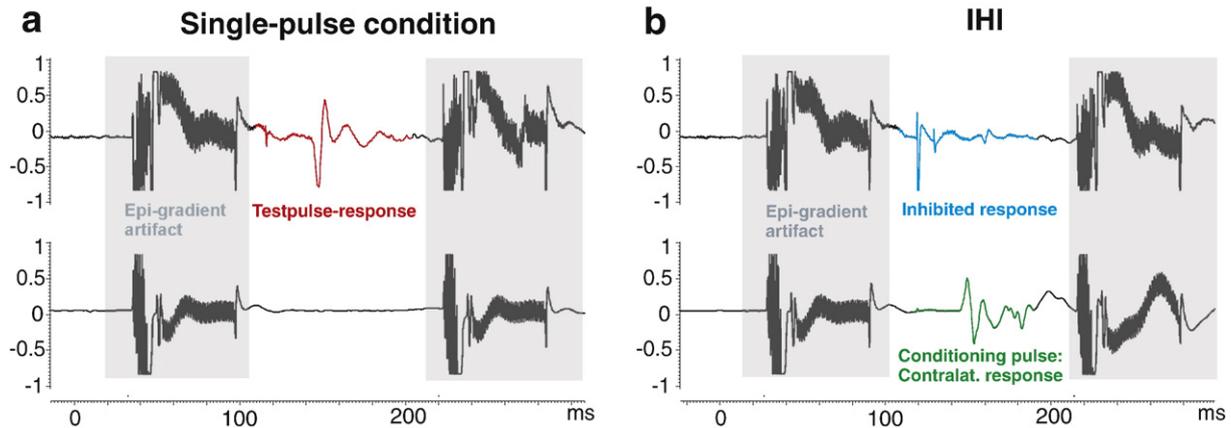


Fig. 1. EMG data and fMRI. Representative EMG data recorded continuously during fMRI: Epi-gradient artifact and induced responses. (a) Single-pulse stimulation. Upper graph: The electromyographic response in the target muscle (FDI contralateral to iM1) during single-pulse stimulation of iM1 is colored red. Furthermore, single-pulse TES induced a stimulus artifact in the EMG data preceding the motor response. Lower graph: No muscle response could be detected ipsilateral to the single-pulse stimulation. The typical epi-gradient artifacts in the EMG data are marked with gray. (b) IHI condition. Upper graph: The response amplitude in the FDI contralateral to iM1 (colored blue) is significantly inhibited during bilateral stimulation (IHI). Furthermore, bilateral TES induced two stimulus artifacts in the EMG data preceding the motor response. Lower graph: In contrast to the single-pulse condition, a muscle response (colored in green) could be detected in the FDI contralateral to cM1, resulting from the additional conditioning pulse on cM1.

simultaneous fMRI data recording. TES was performed using a conditioning test design described previously (Ferbort et al., 1992). In short, for each run the suprathreshold test stimulus was applied over the hand area of right primary motor cortex (ipsilateral M1=iM1) with the target muscle at rest in order to evoke EMG responses in the contralateral first dorsal interosseous muscle (FDI) of about 1 mV. In half of the trials, an additional suprathreshold conditioning stimulus was applied over the hand area of the contralateral primary motor cortex (cM1). The complete conditioning test design consisted of the following conditions: (a) one single suprathreshold test-stimulus (single-pulse condition), (b) one contralateral suprathreshold conditioning stimulus followed by the ipsilateral test stimulus with a 10 ms interstimulus interval (IHI condition).

Triggered by an external signal, single- and paired-pulse TES was always applied after the slice related EPI gradient EMG artifact (see Fig. 1). CED 1401 power laboratory interface displayed all outgoing signals with respect to signals detected from the scanner at each volume. The two conditions (single-pulse and IHI) were presented in a randomized event-related design, consisting of 5 scanning sessions of 70 volumes each corresponding to 210 s duration. Each session contained 8 single and 8 paired-pulse conditions with an interstimulus interval of 12.1875 s (corresponding to 3 volumes + interslice time), resulting in 2×40 trials for each subject. Sessions were separated by 5 min to avoid carryover effects. Subjects were instructed to keep their eyes closed and to relax their hands during the experiments.

Data analysis

For each subject the peak-to-peak amplitudes of TES-induced muscle responses were measured and averaged separately for both conditions (single-pulse and IHI, $N=40$ per subject). For further comparison the averaged response amplitudes of conditioned TES (IHI) were normalized to the mean size of the unconditioned (single-pulse) response amplitude in the FDI contralateral to the testpulse on iM1. For each subject the significance of mean am-

plitude reduction by IHI (as compared to single-pulse) was tested separately using paired *t*-test ($p < 0.05$, $N=40$).

The analysis of the fMRI data was performed using BrainVoyager 2000 (Brain Innovation, Maastricht, The Netherlands). The data from each subject were analyzed separately as a series of case studies. The fMRI data were preprocessed for movement and data warping, smoothed in space (3-mm isotropic Gaussian kernel) and time (Hrf-Convolution). For each subject, the structural and functional data were transformed into standardized space (Talairach and Tournoux, 1988) and functional volumes were brought into coregister with the high-resolution structural data sets to generate volume–time courses.

Data were analyzed in two ways. First, multiple-regression analyses on a single-subject basis were computed after z normalization across sessions. As each session contained two stimulation conditions (8 single-pulse condition and 8 IHI condition) in a randomized order, multiple-regression models were fitted to compute statistical maps across sessions for the effect of both conditions. Therefore each condition was modeled as a stick function and convolved with a canonical hemodynamic response function. The episodes between both stimulation conditions (single-pulse and IHI) were defined as “baseline” (no stimulation). To test whether significant increases occurred when stimuli were applied, voxels activated by the contrast “single pulse” versus “baseline” at $p < 10^{-5}$ (uncorrected) or better were marked. In a separate analysis, resulting in a second statistical map, regional effects defined by the contrast “IHI” versus the same “baseline condition” were marked. Fig. 2 depicts a representative example of the resulting statistical maps.

Second, based on the statistical map “single-pulse” versus “baseline”, two regions of interest (ROIs) were defined in conjunction of functional activation, known anatomical landmarks (“hand knob” Yousry et al., 1997) and a spheres with a 8 mm radius over the primary ipsilateral and contralateral motor cortex (iM1 and cM1).

We addressed the level of hemodynamic response in iM1 and cM1 to the different stimulation conditions on a single-subject

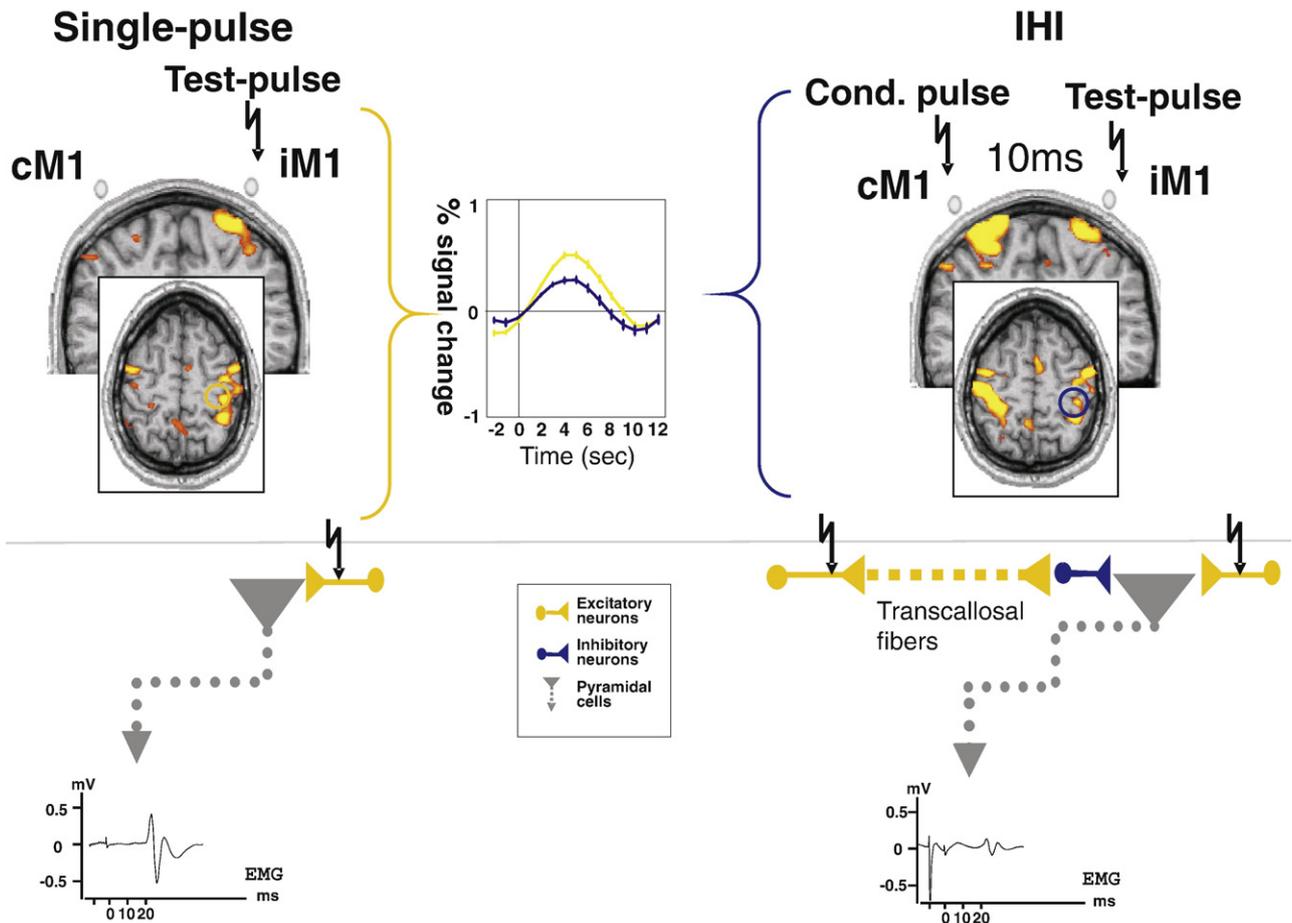


Fig. 2. Single-pulse TES of iM1 versus IHI. Averaged hemodynamic response curves during testpulse and IHI condition in one subject and individual activation maps for single-pulse and IHI stimulation projected onto the same subject's three-dimensional data set in a coronar and axial cut. The position of the stimulation electrode (anode) is marked with a vitamin E capsule, which is visible in the MR images. (a) Testpulse. A unilateral suprathreshold testpulse (110% resting motor threshold, RMT) was applied on iM1. As shown in the schematic model below, the BOLD response in iM1 (marked with a yellow circle) during ipsilateral single-pulse stimulation is likely to result from a transmission of excitation via horizontal interneurons, which are excited by TES p-a. A corticospinal transmission of excitation results in an EMG response, which could be recorded in the contralateral target muscle. (b) IHI. A suprathreshold conditioning pulse (110% RMT) was applied on cM1 10 ms prior to a second suprathreshold testpulse on iM1. The blue curve in the center graph represents the averaged hemodynamic response curve in iM1 during IHI. The blue circle in panel b indicates iM1. The schematic model below illustrates the postulated electrophysiological mechanisms underlying interhemispheric inhibition. Note the reduced EMG amplitude, resulting from a transcallosally transmitted inhibition as schematically illustrated above. Center graph: Averaged hemodynamic response curves from ROI analysis of iM1 during unilateral stimulation of iM1 (yellow curve, yellow circle in panel a) and IHI (blue curve, blue circle). x axis: time in seconds, y axis: percent signal change. Corresponding to the obvious difference between the two curves IHI induced a significantly smaller peak response as a single-pulse and iM1 (paired *t*-test of peak responses, $p < 0.05$, $N = 40$).

basis. For this purpose, the average event-related peak activity during single-pulse or IHI condition was calculated individually in each of the 6 subjects for both ROIs. The 2 s before the event was used to compute the baseline. Two seconds before and twelve seconds after the event were included in the depicted response plot (see Figs. 2–6). Furthermore, to test whether the peak responses of the resulting hemodynamic response curves differ significantly between the two conditions ($N = 40$ within each subject), *t*-test was performed for both ROIs on a within subject-basis (paired *t*-tests, $N = 40$, $p < 0.05$, see Table 2).

Fixed-effects analysis

In a separate step a fixed-effects group analysis was performed to depict hemodynamic effects related to each condition. A mul-

ti-ple-regression model was fitted to compute statistical maps across *z*-transformed sessions and subject data. Both conditions (single-pulse and IHI) were contrasted against the baseline condition separately leading to two activation maps (single-pulse-baseline and IHI-baseline) depicting fixed-effects for both conditions (see Fig. 4a).

In an additional analysis we identified condition related circumscribed effects by contrasting single-pulse versus "IHI". The predictor "single-pulse" was set to 1 and "IHI" set to -1, resulting in the activation map also shown in Fig. 4a.

Somatosensory control condition

In order to control for nociceptive somatosensory co-activation an additional control condition was performed. Stimulation was

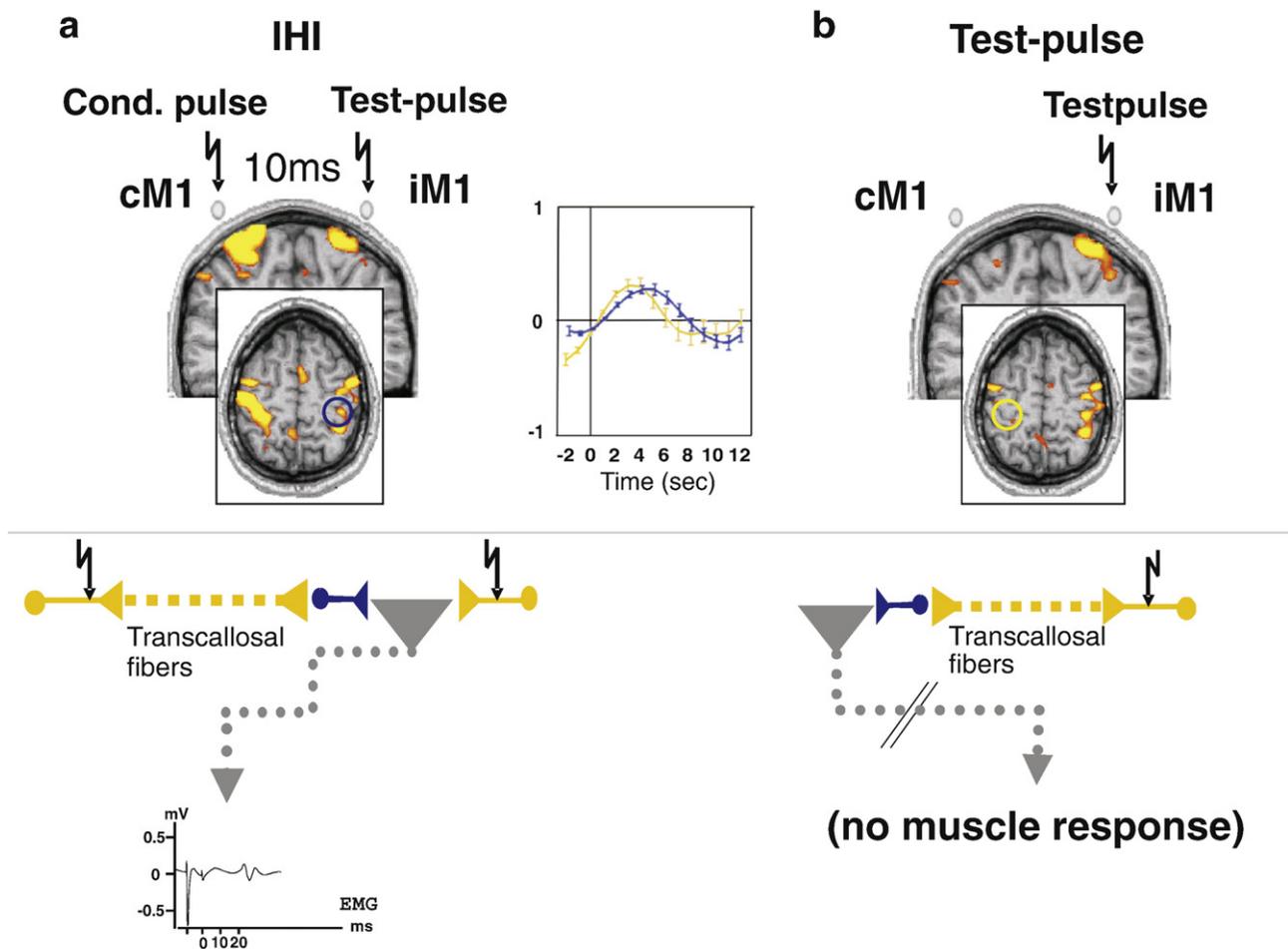


Fig. 3. Ipsilateral versus contralateral M1. Averaged hemodynamic response curves during IHI and testpulse condition in one subject and individual activation maps for IHI and single-pulse stimulation projected onto the same subject's three-dimensional data set in a coronar and axial cut. (a) IHI. A suprathreshold conditioning pulse (110% RMT) was applied on cM1 10 ms prior to a second testpulse on iM1. The blue curve in the center graph represents the averaged hemodynamic response curve in iM1 during IHI. The blue circle indicates the relevant ROI. The schematic model below illustrates the postulated electrophysiological mechanisms underlying interhemispheric inhibition. (b) Testpulse. A unilateral suprathreshold testpulse was applied on iM1. Individual activation maps and averaged hemodynamic response curves in contralateral M1. The schematic model below illustrates that the BOLD response in cM1 during single-pulse stimulation of iM1 is likely to result from transcallosally transmitted inhibitory and excitatory neuronal activity originating in iM1. Despite the fact that no response could be detected in the muscle contralateral to cM1, the net BOLD response was positive (yellow curve in the center graph). Center graph: Averaged hemodynamic response curves from ROI analysis of iM1 during IHI (blue) and cM1 during unilateral stimulation of iM1 (yellow). *x* axis: time in seconds, *y* axis: percent signal change. The obvious similarity of the two curves (blue and yellow) is in line with the statistical comparison of the averaged peak responses in iM1 during IHI and cM1 during single-pulse stimulation of iM1, revealing no significant differences (paired *t*-test, $p > 0.5$, $N = 40$).

performed with the same stimulus intensities and the same anodal position as during cortex stimulation. In contrast, the cathode was fixed on the scalp only 1 cm anterior of the anode. Stimulation with this inter-electrode distance should mimic the discomfort induced by TES on the scalp without inducing a motor response. Additionally, continuous EMG recording was used to ensure that no motor effect was induced by the electrical pulses. Forty trials of single electrical pulses were applied always after the EPI gradient EMG artifact in an event-related design identical to the previous examination.

Control for unspecific interhemispheric effects of bilateral TES

In order to control for unspecific interhemispheric effects of the bilateral TES on iM1 an additional control experiment was

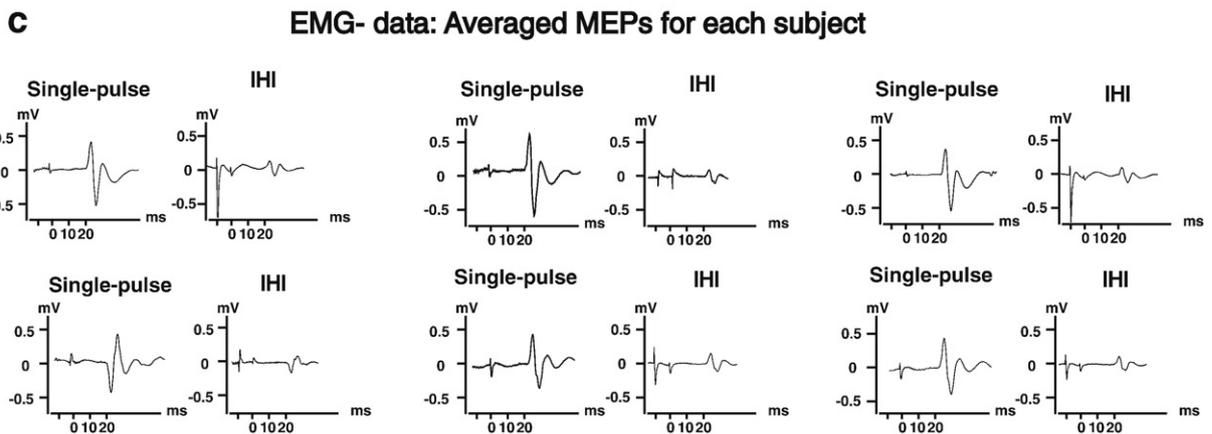
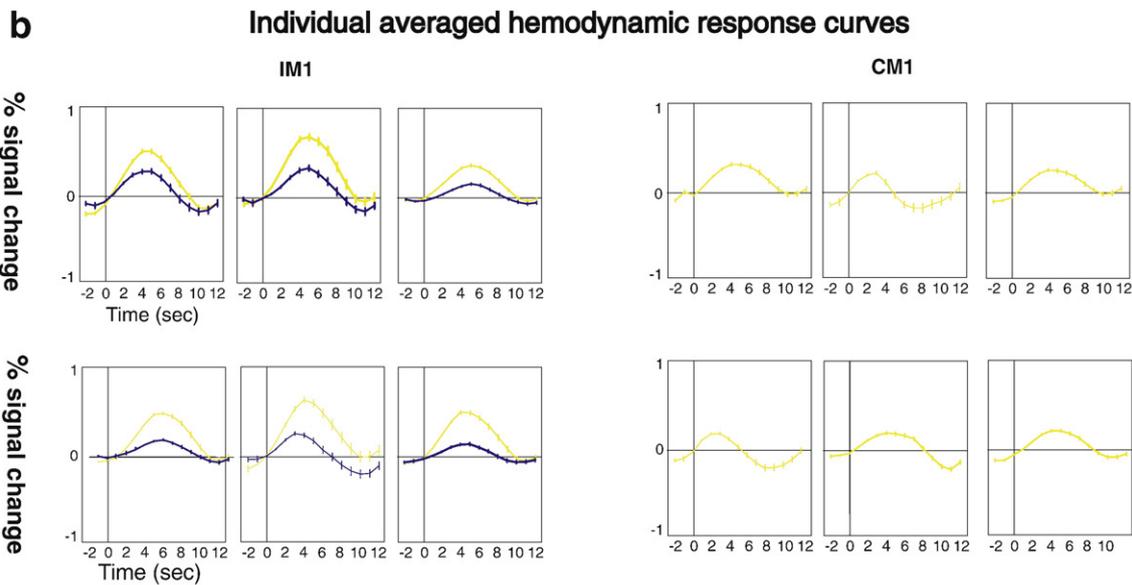
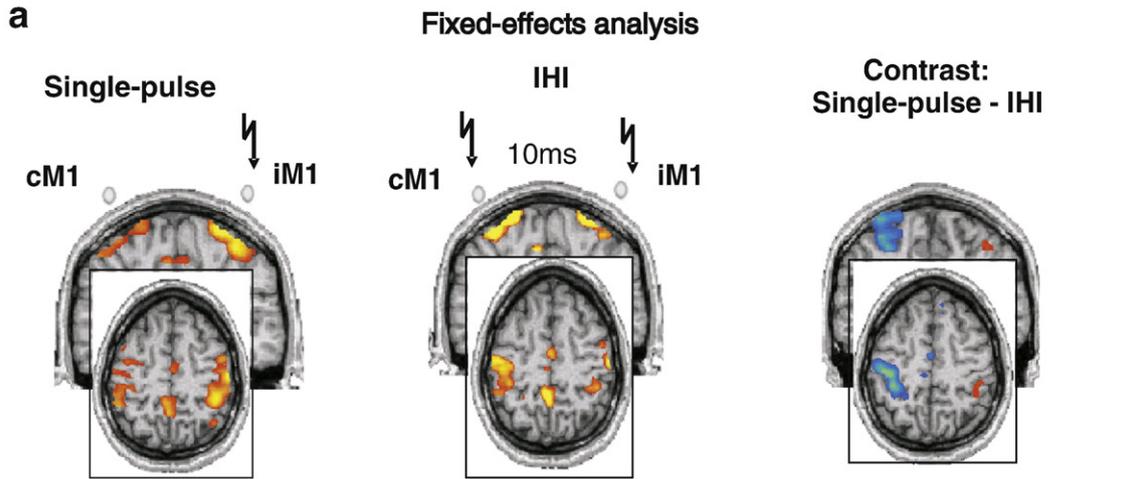
performed in one subject: the same experimental setup and data analysis as in the main experiments were used (single-pulse vs. paired-pulse TES p-a, 40 trials each condition), but the inter-stimulus interval (ISI) was set to 5 ms instead of 10 ms. According to previous studies (e.g. Ferbert et al., 1992) this ISI is too short to induce IHI. Nevertheless unspecific interhemispheric effects due to bilateral TES should be comparable to the experiments with an ISI of 10 ms.

Control condition: MEP size and corresponding BOLD response in iM1

In one subject an additional control experiment addressed the influence of induced motor responses on the BOLD response in iM1: again, a suprathreshold testpulse (22% of maximal

stimulator output) was applied on iM1 in the single-pulse condition. In the paired-pulse condition a contralateral suprathreshold conditioning pulse was applied on cM1 10 ms prior to the testpulse.

For the single-pulse condition two groups of results were separated by the induced MEP sizes: events with (1) small motor evoked potentials (MEP) of about 0.4 mV and (2) larger MEP of about 0.8 mV. The multiple-regression analysis and the comparison



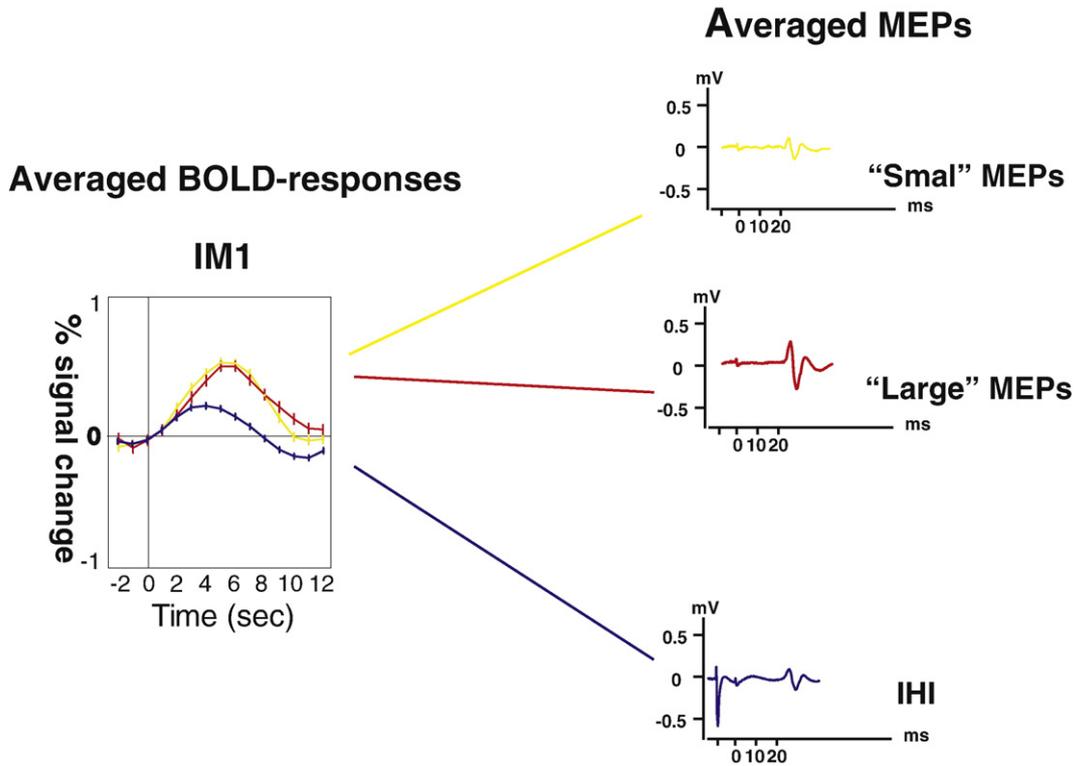


Fig. 5. Control condition: “Small” versus “large” MEPs. Left: Averaged hemodynamic response curves from iM1 during: (a) single-pulse stimulation inducing small MEPs, (b) single-pulse stimulation inducing large MEPs and (c) MEPs induced by IHI. Right: Averaged (a) “small” MEPs induced by single-pulse TES, (b) “large” MEP induced by single-pulse TES and (c) MEPs induced by IHI.

of peak BOLD responses in iM1 (see above) were performed for three conditions (“small MEP”, “large MEP” and “IHI”).

Results

Electrophysiological results: IHI versus single-pulse stimulation

The stimulation effects of the ipsilateral testpulse and contralateral conditioning pulse on both primary motor cortices could be monitored by continuous EMG recordings from the right and left FDI. Motor responses to externally triggered unilateral and bilateral TES could always be detected in the interval between EPI gradient EMG artifacts (see Fig. 1).

Resting motor threshold (RMT) ranged from 22% to 29% (i.e., 220 to 290 V) of maximum stimulator output for both motor hand

areas. The intensities of the suprathreshold ipsilateral and contralateral stimuli were adjusted to induce similar motor responses of about 1 mV in the FDI contralateral to the stimulated dominant hemisphere and ranged from 24% to 32% (i.e. 240 to 320 V, 110% of RMT). In six subjects, suprathreshold ipsilateral single-pulse stimulation induced mean response amplitudes between 0.79 ± 0.31 mV and 1.46 ± 0.36 mV (see Table 1); suprathreshold contralateral stimulation induced amplitudes between 1 ± 0.28 mV and 1.42 ± 0.35 mV.

The comparison of the single-pulse and the IHI condition revealed significant differences within all six subjects: a contralateral conditioning pulse over cM1 that was given 10 ms before the ipsilateral testpulse over iM1 (IHI condition) induced a significant attenuation of motor response amplitudes to the testpulse in each subject (19.3–30% of unconditioned motor response amplitude, see

Fig. 4. Outline of statistical results for all subjects. (a) Fixed-effects analysis. The multiple-regression analysis on a multi-subject basis (6 subjects) revealed the fixed-effects related to both conditions (single-pulse and IHI). Both (single-pulse and IHI) were contrasted against the baseline condition separately leading to two activation maps (single-pulse-baseline and IHI-baseline) and were projected onto one subject’s three-dimensional data set in a coronar and axial cut. The resulting statistical map for “single-pulse” is shown on the left and for “IHI” is in the center. As revealed on a single-subject basis as well, local and distant stimulation effects of single-pulse and paired-pulse stimulation were identified in ipsilateral and contralateral M1, bilateral M2, SMA and S1. The activation map on the right depicts the results from the contrast analysis “single-pulse” versus “IHI”. This contrast revealed an exclusive activation during single-pulse in the primary motor cortex ipsilateral to the stimulus (marked in red). In contradistinction, an exclusive activation during IHI was found in a widespread region of the contralateral primary motor cortex (marked in blue). (b) Individual averaged hemodynamic response curves. Left: Averaged hemodynamic response curves from individual ROI analysis of iM1 during unilateral stimulation of iM1 (yellow curve) and during IHI (blue curve) for each subject. *x* axis: time in seconds, *y* axis: percent signal change. Corresponding to the obvious difference between the two curves, IHI induced a significantly smaller peak response in iM1 as compared to single-pulse stimulation (paired *t*-test of peak responses, $p < 0.05$, $N = 40$) in all subjects. Right: Averaged hemodynamic response curves from individual ROI analysis of cM1 during contralateral single-pulse stimulation of iM1. (c) EMG data: Averaged MEPs for each subject. Each curve is an average of the MEPs induced by single-pulse or IHI ($N = 40$ per condition) in each of the six subjects. Corresponding to the obvious MEP attenuation during IHI the significance of mean amplitude reduction by IHI (as compared to single-pulse) was tested separately for each subject by using paired *t*-test ($p < 0.05$, $N = 40$).

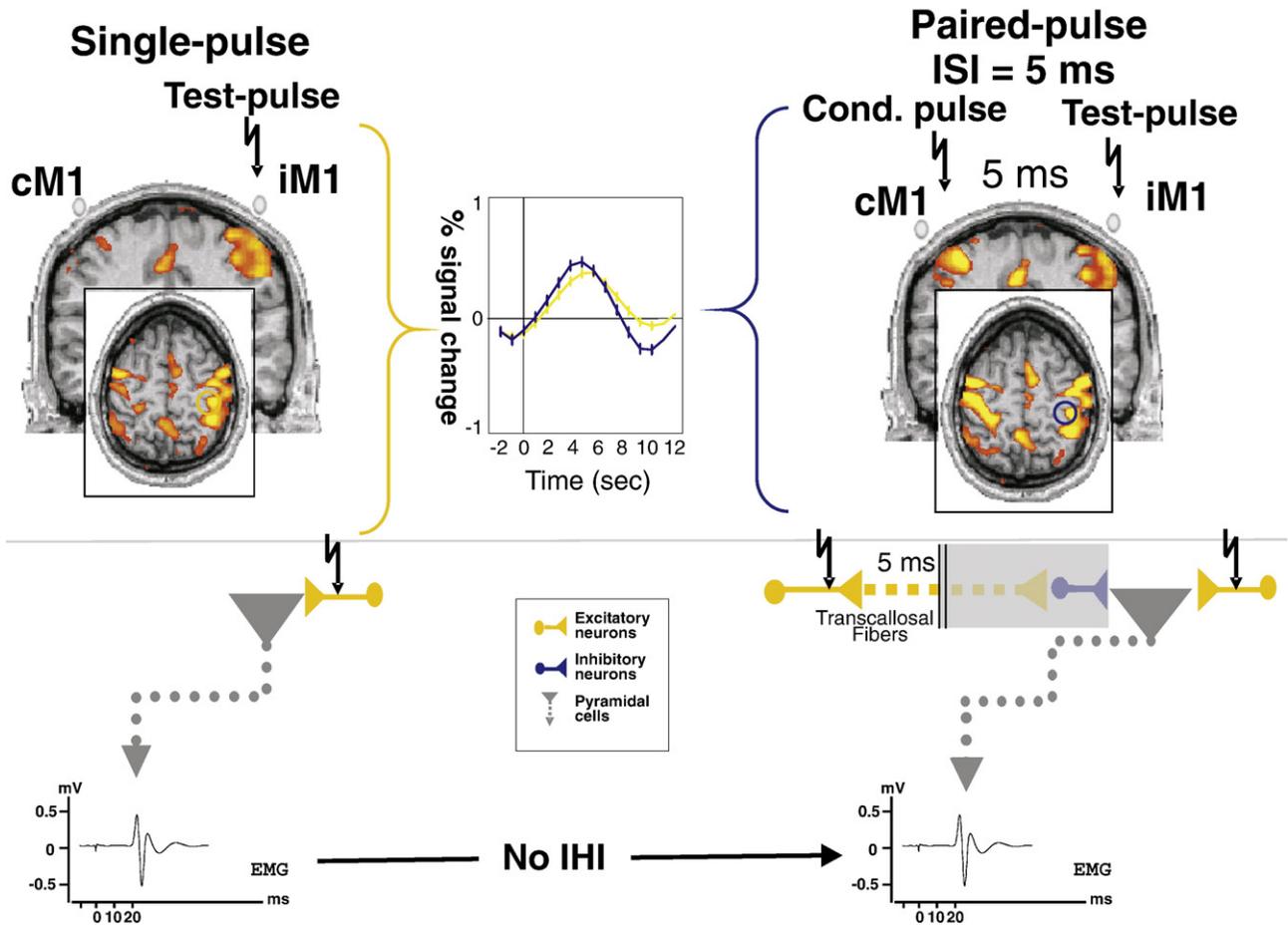


Fig. 6. Control condition: Single-pulse versus paired-pulse, ISI=5 ms. Single-pulse TES above iM1 and bilateral TES was applied in the same experimental setup as in the main experiments, but with a reduced interstimulus interval (ISI) of 5 ms. The resulting statistical maps for both conditions (single-pulse and paired-pulse TES) were projected onto the same subject's three-dimensional data set in a coronar and axial cut. MEP analysis revealed no significant difference between paired-pulse TES (MEPs of 0.7 ± 0.28 mV) and single-pulse stimulation (0.8 ± 0.2 mV) as revealed by paired *t*-test ($p > 0.5$, $N = 40$). Consequently, the comparison of peak BOLD responses in iM1 for single-pulse ($0.65 \pm 0.05\%$ signal change) and paired-pulse TES ($0.63 \pm 0.04\%$ signal change) revealed no significant difference (paired *t*-tests, $p > 0.5$, $N = 40$). The averaged hemodynamic response curves derived from iM1 for both conditions are shown in the center graph yellow: single-pulse; blue: paired-pulse. The yellow and blue circles in the statistical maps indicate the relevant ROIs.

Table 1; paired *t*-tests, $p < 0.05$) across all sessions. Fig. 1 shows a representative example of the EMG responses for both the testpulse stimulation and the conditioned TES p-a as detected between the

Table 1
Testpulse vs. IHI: EMG results: amplitudes and significance

Subject	Testpulse (mV)	IHI (mV)	IHI: % testpulse	<i>p</i> -value
1	0.96 ± 0.36	0.36 ± 0.1	22.9	<0.05
2	1.46 ± 0.36	0.43 ± 0.04	29.4	<0.05
3	1.22 ± 0.37	0.32 ± 0.03	26.2	<0.05
4	1.4 ± 0.32	0.27 ± 0.06	19.3	<0.05
5	0.79 ± 0.31	0.23 ± 0.05	29.1	<0.05
6	1.1 ± 0.21	0.33 ± 0.07	30	<0.05

Note. Peak-to-peak amplitudes and standard deviation of the electromyographic responses induced by both conditions. Electrophysiological results for all subjects. The comparison of the averaged peak-to-peak response amplitudes for both conditions revealed a significant amplitude reduction (paired *t*-test, $p < 0.05$, uncorrected) by IHI to 19.3–29.4% of unconditioned test-pulse response.

epi-gradient artifacts. For the average peak-to-peak response amplitudes for both conditions and all six subjects see Table 1, and for the averaged MEPs for both conditions and all subjects see Fig. 4c.

Electrophysiological results for control conditions

Reafference control

In one subject single-pulse suprathreshold electrical stimuli on iM1 (22% of maximal stimulator output) induced motor responses with higher variations of the induced motor responses (0.6 ± 0.4 mV) in the contralateral FDI in an additional experimental run. During IHI the additional contralateral conditioning stimulus led to motor response amplitudes of 0.28 ± 0.1 mV in the FDI contralateral to iM1. This experimental run was analyzed separately as control condition and excluded from the other analysis in the main experiments.

Control for unspecific interhemispheric effects

After reducing the ISI between conditioning pulse and testpulse to 5 ms the EMG data analysis revealed no significant difference

between the motor responses to single-pulse (0.8 ± 0.2 mV) and paired-pulse (0.7 ± 0.28 mV) TES induced in the contralateral FDI (paired *t*-tests, $p > 0.5$, $N = 40$).

fMRI results of main experiments

Statistical maps

A representative example is given in Fig. 2 showing individual activation maps for single-pulse and IHI stimulation projected onto the same subject's three-dimensional data set in a coronal and axial cut.

Local increases in BOLD signal induced by single and paired-pulse TES *p*-a were detected in the region underneath the testpulse electrode (see vitamin E capsule, Fig. 2) extending from the surface to the depth of the central sulcus in all subjects. This area corresponds to the hand area of primary motor cortex as identified by anatomical landmarks (White et al., 1997; Yousry et al., 1997) and by Talairach coordinates (Talairach and Tournoux, 1988) (mean $-28/-28/53$) of maximal peak response. Several further distant co-activations were observed during both unilateral and bilateral TES. They were identified anatomically and by Talairach coordinates: a co-activation was always found in the contralateral hand area (cM1), which was interestingly homotopic to iM1 (mean $30/-28/53$, Fig. 2). In all subjects, additional ipsilateral and contralateral co-activations were observed in the dorsal premotor cortex (M2, mean: right: $25/-8/53$; left: $-25/-7/52$), the supplementary motor cortex (SMA, mean $-1.3/-8/56.5$) and bilateral in the primary somatosensory cortex (S1, mean: right: $-50/-32/46$; left: $48/-27/46$).

Fixed-effects analysis

The multiple-regression analysis revealed fixed-effects related to both conditions. Fig. 4a depicts the resulting statistical maps separately for each condition (single-pulse-baseline and IHI-baseline). As demonstrated on a single-subject basis local and distant stimulation effects of single-pulse and paired-pulse stimulation were identified in ipsilateral and contralateral M1 (iM1, $-28/-28/53$ and cM1, $31/-27/53$), bilateral M2 (right: $24/-8/53$; left: $-24/-7/52$), SMA ($-1.3/-8/56.5$) and S1 (right: $-50/-30/47$; left: $49/-27/46$).

The activation map, shown in Fig. 4a on the right, depicts further results from the contrast “single-pulse-baseline” versus “IHI-baseline” (predictor “single-pulse” set to 1 and “IHI” set to -1). This contrast revealed an exclusive activation during single-pulse in the primary motor cortex ipsilateral to the stimulus (marked in red, $-28/-28/53$). This area corresponds well with the primary motor hand area as revealed by its anatomical landmarks as well as in relation to Talairach coordinates. In contradistinction, an exclusive activation during IHI was found in a region of the contralateral primary motor cortex (marked in blue, $31/-27/53$).

Hemodynamic response in M1: IHI versus single pulse

In the main part of our analysis we addressed the level of hemodynamic response in the bilateral M1 for different conditions: on a within subject basis event-related activity was analyzed separately for each of the 6 subjects in both ROIs (iM1 and cM1). iM1 and cM1 had been defined both anatomically and by statistical maps of single-pulse TES BOLD response. Fig. 2 gives a representative example of the averaged hemodynamic response curves in iM1 and cM1 after either single-pulse or paired-pulse TES in one subject

(subject 1). The averaged hemodynamic response curves in iM1 and cM1 for each subject are depicted in Fig. 4b. Table 2 shows the mean peak responses in iM1 for both conditions and all subjects.

The apparent difference between the single-pulse and the IHI condition in the level of BOLD response in iM1 (see Figs. 2 and 4) could be confirmed. While *single-pulse TES* induced peak responses between 0.3 ± 0.03 and $0.71 \pm 0.04\%$ signal change in iM1, *IHI* led to significantly lower ($p < 0.05$; paired *t*-test, $N = 40$) peak responses in the same ROI (0.15 ± 0.07 to $0.35 \pm 0.03\%$) in all six subjects.

BOLD response in cM1

The level of BOLD response in cM1 (contralateral to homotopic hand area) during single-pulse TES was also compared to the BOLD response in iM1. Here, in all subjects the peak response in cM1 (between 0.2 ± 0.01 and $0.4 \pm 0.02\%$ signal change) was significantly weaker ($p < 0.05$; paired *t*-test, $N = 40$) as compared to iM1. Fig. 3 shows the averaged peak BOLD response in cM1 during unilateral stimulation of iM1 for one subject, and Fig. 4b contains the responses separately for all subjects. On the other hand, an additional contralateral conditioning pulse induced a significantly higher peak response (0.3 ± 0.04 to $0.75 \pm 0.05\%$ signal change) in cM1 as compared to the single-pulse condition.

cM1 versus iM1

The contralateral co-activation in cM1 during single-pulse stimulation of iM1 was compared with the BOLD response in iM1 during paired-pulse stimulation (i.e. modified by transcallosal transmitted inhibition). For all subjects the statistical analysis revealed that the average hemodynamic peak response in iM1 during IHI (0.15% to 0.35% signal change) was comparable to the peak response in cM1 during single-pulse stimulation of iM1 (peak between 0.2% and 0.4% , $p > 0.5$; paired *t*-test, uncorrected). Fig. 3 gives a representative example.

Somatosensory control condition

Control stimulation with 1 cm inter-electrode distance did not directly activate motor cortex but induced nociceptive cutaneous somatosensory effects. Here we found co-activation only in the posterior insular cortex (right: $50/-15$; left: $-47/-1/11$), right thalamus ($11/-19/11$), putamen ($20/-1/12$) and anterior cingulum (right $2/22/37$; left: $-4/21/39$). These structures are typically activated by unpleasant or painful stimuli (e.g. Treede et al., 1999).

Table 2
BOLD response in iM1: testpulse vs. IHI: % signal change and significance

Subject	Testpulse	IHI	<i>p</i> -value
1	0.7 (0.05)	0.25 (0.03)	<0.05
2	0.3 (0.03)	0.15 (0.02)	<0.05
3	0.55 (0.04)	0.24 (0.02)	<0.05
4	0.5 (0.07)	0.15 (0.07)	<0.05
5	0.71 (0.04)	0.35 (0.03)	<0.05
6	0.5 (0.03)	0.25 (0.03)	<0.05

Note. Mean peak responses and standard error mean (SEM) of averaged hemodynamic response curves for all subjects and both conditions. The comparison revealed a significant attenuation (paired *t*-test, $p < 0.05$, uncorrected) of the peak BOLD response during IHI as compared to the single-pulse stimulation (testpulse).

Control for unspecific interhemispheric effects—ISI = 5 ms

The comparison of peak BOLD responses in iM1 for single-pulse ($0.65 \pm 0.05\%$ signal change) and paired-pulse TES ($0.63 \pm 0.04\%$ signal change) revealed no significant difference (paired *t*-tests, $p > 0.5$, $N = 40$) with the interstimulus interval set to 5 ms. Fig. 6 depicts the resulting statistical maps as well as the averaged hemodynamic response curves for both conditions.

Reafference control

In an additional control experiment, two groups of responses to single-pulse stimulation of iM1 were separated for further fMRI data analysis: events with (1) small MEP (15 events 0.3 ± 0.1) and (2) large MEP (15 events 0.8 ± 0.2). The comparison of averaged event-related peak activities in iM1 revealed no significant difference for “small MEP” and “large MEP”. In contrast, IHI led to a significantly reduced peak response in iM1 (paired *t*-test, $p < 0.05$), while the mean MEP response during IHI was not significantly different to those of the small MEP condition (see Fig. 5 for an illustration).

Discussion

The present study demonstrated that single- and paired-pulse TES p-a can successfully be combined with simultaneous fMRI and EMG. In a first step, we could show that IHI can also be induced by bilateral TES p-a as has previously been shown for TMS p-a.

Second, this new experimental setup allowed us to induce well defined inhibitory and excitatory neuronal activity during fMRI and to study its influence on the induced BOLD signal changes. Besides a specific pattern of local and distant co-activations of known sensorimotor areas, the BOLD analysis revealed several new results. The major finding was that IHI induced a net reduction of the BOLD response in iM1.

Furthermore, the BOLD response in cM1 (a) might reflect a functional transcallosal connection between homotopic areas of ipsilateral and contralateral M1 and (b) served as a control for potential reafference effects.

Electrophysiological results

Bilateral TES p-a and IHI

We can now demonstrate that TES p-a with the classical conditioning testpulse design may induce interhemispheric inhibition (IHI) as has previously been shown for TMS p-a (e.g. Ferbert et al., 1992; for a review see Chen et al., 2003). For all subjects the EMG analysis revealed that a conditioning suprathreshold electrical stimulus on cM1 significantly reduces the EMG activity produced by a second electrical stimulus to iM1 with an interstimulus interval of 10 ms (see Fig. 2). These electrophysiological results are comparable with paired-pulse TMS studies analyzing IHI: a conditioning transcranial stimulus to one motor cortex reduces the EMG activity produced by a second pulse to the contralateral motor cortex within an interstimulus interval of 6 to 50 ms (e.g. Ferbert et al., 1992; Meyer et al., 1995, 1998b; Di Lazzaro et al., 1999; Hanajima et al., 2001; Chen et al., 2003). This phenomenon is likely to occur on a cortical level (Ferbort et al., 1992; Di Lazzaro et al., 1999; Hanajima et al., 2001), mediated by excitatory commissural neurons, whose propagation speed is about 10–15 ms (Meyer et al., 1998a,b), and, which act on local inhibitory interneurons in the

contralateral motor cortex (Chen et al., 2003). Altogether, the novel finding that TES p-a may induce IHI in the same way as known for TMS p-a is in line with previous results (Brocke et al., 2005), suggesting that TES p-a activates pyramidal cells preferentially transsynaptically and leads to comparable stimulation effects on a cortical level (stimulus onset latency, sICI and sICF) (Brocke et al., 2005).

fMRI results

Specific pattern of sensorimotor areas

In all subjects the analysis of statistical maps for the single-pulse- as well as the IHI condition revealed a similar pattern of local and distant BOLD responses, which can be attributed to a network of functionally associated sensorimotor areas: more specifically, the single-pulse- and the IHI condition induced BOLD responses in ipsilateral and contralateral M1, bilateral premotor areas (M2), supplementary motor cortex (SMA) and the primary somatosensory cortex (S1). These results are compatible with previous reports of combined transcranial cortex stimulation (TES or TMS) and simultaneous functional imaging (fMRI or PET): transcranial cortex stimulation always induced a pattern of local (e.g. FEF, V3a or M1) and distant activations in functionally and anatomically connected areas (e.g. Paus et al., 1997; Brandt et al., 2001; Strafella and Paus, 2001; Bestmann et al., 2004), demonstrating for example specific visuomotor or sensorimotor networks.

IHI as model for inhibition and excitation

Whether neuronal inhibition is reflected in an increased or decreased BOLD response was analyzed by using IHI as a model for cortical inhibitory and excitatory mechanisms in combination with simultaneous fMRI. Previous work studying inhibition and excitation during functional imaging often used different types of unilateral voluntary motor tasks leading to contradictory results (e.g. Kawashima et al., 1998; Cramer et al., 1999; Allison et al., 2000; Nirkkko et al., 2001; Hamzei et al., 2002; Kobayashi et al., 2003; Stefanovic et al., 2005; Newton et al., 2005). Those paradigms were based on the postulation that the inhibitory effect of ipsilateral finger movement is probably mediated transcallosally in a similar way as known from TMS studies (conditioning pulse on contralateral M1) (Ferbort et al., 1992; Wassermann et al., 1994; Chiappa et al., 1995). Furthermore, it was shown that the type of unimanual motor task strongly influences the excitability of the ipsilateral motor cortex (Liepert et al., 2001). Nevertheless, during voluntary motor tasks other forms of inhibition, for example, between contralateral M2 and ipsilateral M1, may contribute to the inhibitory effects. Those interactions were analyzed for example by Mochizuki et al. (2004). They suggested a commissural connection between ipsilateral premotor cortex and contralateral M1, which might play a role in bimanual coordination during voluntary movement.

In contrast to previous fMRI studies using voluntary motor tasks, in the present study IHI was studied by using transcranial cortex stimulation in the classic and electrophysiologically well examined paired-pulse paradigm (e.g. Ferbert et al., 1992). We combined unilateral and bilateral TES with fMRI to differentiate the BOLD response coupled to a single stimulus over iM1 from the response in the same area, which was transcallosally modified by an additional contralateral conditioning pulse (IHI). The functional effect of each stimulus could be electrophysiologically quantified by continuous EMG recording.

IHI: net BOLD reduction in iM1

BOLD analysis revealed that bilateral stimulation (IHI) induced a positive but significantly lower peak response in iM1 as compared to ipsilateral single-pulse stimulation. Thus, the additional cM1 pulse led to a net attenuation of BOLD response in iM1.

The presumable electrophysiological mechanisms underlying the stimulation effects of both conditions are illustrated in Fig. 2:

1. Single-pulse TES p-a is likely to induce an excitation of pyramidal cells transsynaptically via horizontal interneurons on iM1 as known for TMS p-a (Brocke et al., 2005).
2. IHI is probably mediated through excitatory commissural neurons, which act on local inhibitory interneurons in iM1 (Chen et al., 2003). Thus, the net reduction of BOLD signal in iM1 during IHI is found even though we must suppose additional inhibitory and excitatory neuronal activity in iM1 as compared to single-pulse stimulation (Fig. 2).

This net BOLD reduction is somewhat surprising. Studies in the rat cerebellar cortex suggest that the hemodynamic response is influenced by presynaptic and postsynaptic activity as well as synaptic signal processing, but not the spike rate (e.g. Lauritzen and Gold, 2003). Consequently, a mixed disynaptic inhibition/excitation should correlate with an increase in the local hemodynamic response. Yet, we find a net decrease. How can we explain this discrepancy? The fact that inhibitory synapses are less numerous and strategically better located than excitatory synapses (DeFelipe and Farinas, 1992; Beaulieu and Colonnier, 1985; Koos and Tepper, 1999) may indicate that inhibition is more efficient and, therefore, less energy consuming than excitation (Waldvogel et al., 2000).

However, recent work suggests that the hemodynamic response is preferentially driven by neurotransmitter-related signal processing and not by the local energy needs. Furthermore, the BOLD signal is supposed to be coupled preferentially to postsynaptic events (e.g. Attwell and Iadecola, 2002).

Thus, in the present study during IHI the testpulse may act on pre-inhibited pyramidal cells, which would result in net reduced postsynaptic activity. This could implicate that the effect of the reduction of postsynaptic activity during IHI is stronger than that of the additional inhibitory and excitatory presynaptic input leading to a reduced net BOLD increase in iM1.

Nevertheless, these inferences about the physiological underpinnings of the observed BOLD effects are derived indirectly from models of the hemodynamic response function, neurovascular coupling and the physiological underpinning of single- and paired-pulse transcranial cortex stimulation. The present experimental setup does not allow direct inferences of neural activity as also the EMG-signal must be understood as filtered by a neuromuscular impulse function. However, we can conclude that interhemispheric inhibition induced by bilateral TES is accompanied by a net reduction of the BOLD signal in iM1. Thus, more generally speaking, cortical inhibitory processes are accompanied by attenuation of the local neurovascular signal.

Co-activation in homotopic cM1

During single-pulse stimulation above iM1, no stimulus was applied on *contralateral M1*. Consequently, no motor response was

induced in the target muscle of contralateral M1 as revealed by EMG (see Fig. 3). Nevertheless, single-pulse TES induced a small but positive BOLD response in cM1, which was *homotopic* to the activated region of iM1. This is likely to reflect the transcallosal connection between homotopic areas of M1.

Influence of reafference effects

In general, one could argue that BOLD reduction in iM1 during IHI as compared to single-pulse stimulation on iM1 could be influenced by reafference differences corresponding to different MEP amplitudes in the contralateral target muscle. To control for reafference effects, two control conditions were analyzed:

First, single-pulse stimulation of iM1 induced a positive BOLD response also in cM1 via transcallosally transmitted excitatory and inhibitory neuronal activity, i.e. no MEP was induced in the contralateral target muscle. This dissociation of positive BOLD response and muscle response in cM1 depicts the independency of IHI and reafference. In analogy, this offers insight into ipsilateral motor cortex (iM1) activity: the comparison of the averaged peak responses in iM1 during IHI with those in cM1 during single-pulse stimulation revealed no significant difference between iM1 and cM1 BOLD activity but significant difference in the induced motor responses in the respective target muscles. A motor response could always be detected contralateral to iM1 in the IHI condition; no motor response could be detected contralateral to cM1 during single-pulse stimulation of iM1.

Thus, we argue that the differences during paired-pulse stimulation (IHI) as compared to the single-pulse condition result from transcallosal transmitted excitatory and inhibitory neuronal activity and not from differences in reafference due to subsequent motor responses (MEP size).

This theoretical comparison offers indirect evidence for the mechanisms underlying the BOLD responses in iM1. A second control condition comparing MEP size and the BOLD response in iM1 supported the previous results:

The comparison of the peak BOLD responses in iM1 corresponding to small (0.3 ± 0.1 mV) or large (0.8 ± 0.2 mV) contralateral motor responses revealed no significant difference between the two groups. In contradistinction, the BOLD response in iM1 was significantly reduced during the IHI condition and was accompanied by comparable MEP amplitudes (0.28 ± 1 mV) as in the “small” MEP group. Thus, the BOLD responses for the IHI condition and the small MEP group were relatively different and the muscle responses relatively equal. This again suggests a dissociation between induced MEP size and corresponding BOLD responses also in iM1.

This interpretation is supported by an additional parametric regression analysis of the relationship between each specific MEP size and the corresponding peak BOLD response for all subjects. Both conditions were analyzed separately, resulting in $N=40$ events per condition (single pulse, IHI) and per subject. Analyses revealed no parametric dependency between the EMG amplitudes and the peak BOLD response amplitudes for the single-pulse condition (R ranged from $R=0.059$ to 0.138 , R^2 ranged from 0.0034 to 0.019) and for IHI (R ranged from $R=0.086$ to 0.146 , R^2 ranged from 0.0074 to 0.021).

Altogether, we conclude that the BOLD response difference between the single-pulse and IHI condition in iM1 does not preferentially result from differences in reafference (e.g. somatosensory feedback projections from the target muscle) but additional inhibitory stimulation effects of IHI.

Control for unspecific effects of bilateral stimulation

According to previous studies (e.g. Ferbert et al., 1992) the ISI of 5 ms between bilateral suprathreshold stimulation of M1 is too short to induce IHI. Consequently, no IHI could be detected in the resulting motor responses with an ISI of 5 ms. Furthermore, the analysis of fMRI data demonstrated that the change to ISI=5 ms led to a missing BOLD reduction. As all other stimulation parameters are kept equal to the main experiment, the missing BOLD reduction is likely to result from the missing IHI. These results support the postulation that the detected net BOLD reduction in iM1 during IHI in the main experiments results from transcallosally transmitted inhibition of the pyramidal neurons and not from unspecific interhemispheric effects of bilateral stimulation on the BOLD response.

Group analysis

As shown in Fig. 5, the same pattern of activation as revealed by intraindividual GLM analysis could also be reproduced by a fixed-effects analysis for all 6 subjects and both conditions (single-pulse, IHI). Furthermore, the contrast analysis (single-pulse versus IHI) for this group revealed exclusive activity for single-pulse TES in iM1, supporting the difference in BOLD response in iM1 between single-pulse and IHI. On the other hand, the same contrast analysis revealed exclusive activity for IHI in cM1, supporting again the results of intraindividual ROI analysis discussed above. Altogether the group analysis additionally demonstrates the consistency of the fMRI results across all subjects.

Conclusion

- (1) IHI can also be induced by TES p-a in a comparable way as known for TMS p-a. This cortical stimulation effect supports the hypothesis of previous studies that TES p-a preferentially activates the corticospinal neurons indirectly to induce *I*-waves, leading to comparable effects as TMS p-a.
- (2) It could be demonstrated that the influence of cortical inhibitory and excitatory stimulation effects on the neurovascular response in the human brain can be analyzed non-invasively by combining single- and paired-pulse TES p-a with simultaneous fMRI.
- (3) We suggest that the BOLD response in cM1 during single-pulse stimulation of iM1 may reflect a transcallosal connection between homotopic areas of ipsilateral and contralateral M1.
- (4) IHI is accompanied by a net reduction of the BOLD signal in iM1. Thus, more generally speaking, cortical inhibitory processes are accompanied by attenuation of the local neurovascular signal.

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