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Enhancement of long-range EEG coherence by synchronous bifocal transcranial magnetic stimulation

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Abstract

Interregional coupling of distant brain regions can be measured by electroencephalographic (EEG) coherence reflecting the spatial–temporal correlation between two oscillatory signals. It has been suggested that this coherence in activity is a signature of functional integration of multimodal neuronal networks. Repetitive transcranial magnetic stimulation (rTMS) is a well-established technique for non-invasive cortical stimulation. Its modulating effects outlast the train of stimulation and affect behavior. In the present study, we tested the hypothesis that cortico-cortical coherence between distant brain areas can be selectively enhanced by synchronous bifocal rTMS. Cortico-cortical coherence was assessed in 16 healthy human subjects before and after three trains of synchronous high-frequency (10 Hz) rTMS to the left primary motor cortex and the visual cortex at the occipital pole simultaneously. Stimulation of the left M1 alone served as the control condition. Coherence and spectral power were measured between these areas on the stimulated and the homologue contralateral side. Synchronous bifocal rTMS induced an increase of interregional coupling in the alpha and lower beta band on the stimulated side without effects on spectral power. These data indicate that synchronous bifocal rTMS is a feasible technique for selective modulation of interregional EEG coherence. Furthermore, they raise the hypothesis that interventional enhancement of long-range coherence may effectively modulate interregional integration with behavioral consequences.

Introduction

There is converging evidence that synchronization of neuronal activity represents a pivotal mechanism for intramodal (von der Malsburg & Schneider, 1986; Gray *et al*., 1989) and transmodal (von Stein *et al*., 1999) 'binding' of object features. Furthermore, this mechanism seems to be critically involved in interregional integration subserving effective behavior in animal models and in humans (Classen *et al*., 1998; Hummel & Gerloff, 2005; Kay, 2005; Sauseng *et al*., 2007). Disturbed neuronal synchronization has been related to disorders such as schizophrenia, epilepsy, autism, Alzheimer's disease and Parkinson's disease (Uhlhaas & Singer, 2006).

In humans, oscillatory activity and interregional coupling can non-invasively be measured by electroencephalographic (EEG) spectral analysis. In order to address the question of interaction, i.e. information transfer between distant cortical areas, the cross-correlation between the power spectra of different EEG channels can be calculated (Rappelsberger *et al*., 1994; Classen *et al*., 1998). This parameter is suited to quantify the correlation of activity between electrodes and thus provides information about interregional synchronization (Nunez, 2000).

At the behavioral level, it has been demonstrated that multimodal integration tasks such as visuomotor tracking are associated with enhanced interregional synchronization of the cortical areas involved (Classen *et al*., 1998). Moreover, the level of coherence was shown to be positively correlated with behavioral success in a visuotactile task (Hummel & Gerloff, 2005). In turn, the enhancement of coherence by non-invasive brain stimulation may represent an option to further the efficacy of functionally relevant cortico-cortical integration.

Repetitive transcranial magnetic stimulation (rTMS) is a well-established technique for non-invasive cortical stimulation. Its modulating effects outlast the train of stimulation (Chen *et al*., 1997), are not limited to the stimulated area (Lee *et al*., 2003; Plewnia *et al*., 2003) and affect behavior (Kobayashi *et al*., 2004). It has been demonstrated that coherence can be differentially modulated by applying rTMS to the motor and premotor cortex with frequencies of 10 Hz (Jing & Takigawa, 2000), 5 Hz (Serrien *et al*., 2002; Oliviero *et al*., 2003; Fuggetta *et al*., 2008) and 1 Hz (Chen *et al*., 2003; Strens *et al*., 2002). Exemplifying the efficacy of non-invasive associative, i.e. bifocal, stimulation, Stefan *et al*. (2000) combined peripheral electrical stimulation to the median nerve and rTMS to the primary motor cortex. Most likely, based on the Hebbian rule, the converging transsynaptic activation of cortical output neurons by rTMS and somatosensory afferent information by median nerve stimulation led to a lasting, reversible and topographically specific increase of neuronal activity (Wolters *et al*., 2003).

In the light of these previous data, we set out to test the hypothesis that trains of synchronous bifocal high-frequency rTMS applied with

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two stimulation coils to M1 and the visual cortex at the occipital pole (V1) can induce a topographically selective enhancement of interregional long-range coherence. To our knowledge, this is the first study to apply bifocal rTMS in order to modify cortico-cortical coherence.

Methods

Subjects

Sixteen right-handed healthy volunteers (seven male, nine female; mean age 26.9 ± 2.5 years) were included in the study and gave written informed consent to the experimental procedure approved by the University of Tübingen local ethics committee in accordance with the Declaration of Helsinki. None of the subjects had a history of physical, mental or neurological illness.

EEG recording

Continuous EEG was recorded from 28 (silver–silver chloride) surface electrodes, mounted in a cap (Easy Cap, Herrsching-Breitbrunn, Germany). EEG signals were sampled at 1000 Hz and band-pass filtered in the range 0.5–100 Hz (NeuroScan, Herndon, VA, USA). Impedances were kept below 5 kΩ. Linked earlobe electrodes served as reference. In order to address the problem of common reference (Fein *et al*., 1988), bipolar recordings were used for further analysis. Signals common to activation and rest conditions were minimized using a subtractive approach (see below). The following regions of interest (ROIs) were defined for further analysis: FC3-CP3/FC4-CP4 (left/right sensorimotor cortex), O1-Oz/O2-Oz (left/right visual cortex).

Offline analysis

Epoching and artifact rejection was performed offline. EEG data were analysed with respect to coherence (Coh) and power (Pow). For both analysis techniques, each sequence period was segmented into non-overlapping epochs of 1024 ms (allowing a frequency resolution of ∼1.0 Hz). All sweeps were inspected visually for artifacts, which were removed manually. Spectral power and coherence were calculated using Matlab Release14 (The MathWorks, Natick, MA, USA) in three frequency bands: alpha (8–12.9 Hz), and lower (13–20.9 Hz) and higher (21–29.9 Hz) beta. These frequency ranges have proved to be valuable in recent studies on coherence during motor and visuomotor activity (Classen *et al*., 1998; Gerloff *et al*., 1998; Manganotti *et al*., 1998). The Pow values were also calculated for each frequency bin (width 1 Hz). Coh was calculated according to the following equation:

$$\mathrm{Coh}_{xy}(f) = P_{xy}(f)2/[P_{xx}(f)P_{yy}(f)] \quad (1)$$

In order to separate the influence of stimulation from background coherence levels (stimulation-related coherence: SRCoh), transformed coherence in the baseline state ($\mathrm{Coh}_{xy[\mathrm{base}]}$) was subtracted from transformed coherence after stimulation ($\mathrm{Coh}_{xy[\mathrm{stim}]}$). To achieve constant variance for statistical analysis, coherence estimates were normalized by the inverse hyperbolic tangent (Halliday *et al*., 1995).

$$\mathrm{SRCoh}_{xy} = \mathrm{a\,tanh}(\mathrm{Coh}_{xy[\mathrm{stim}]}) - \mathrm{a\,tanh}(\mathrm{Coh}_{xy[\mathrm{base}]}). \quad (2)$$

SRCoh magnitude increments were expressed as positive values, and decrements were expressed as negative values. Frequency bins were averaged according to the concept of pooled coherence as described by (Amjad *et al*., 1997).

To extract the effects specific for bifocal stimulation, the difference ($\mathrm{SR}_{\Delta}\mathrm{Coh}$) between SRCoh after bifocal stimulation ($\mathrm{SRCoh}_{\mathrm{bif}}$) and SRCoh after monofocal stimulation ($\mathrm{SRCoh}_{\mathrm{mon}}$) was calculated:

$$\mathrm{SR}_{\Delta}\mathrm{Coh} = \mathrm{SRCoh}_{\mathrm{bif}} - \mathrm{SRCoh}_{\mathrm{mon}}. \quad (3)$$

For power analysis, EEG signals were filtered, epoched, visually inspected for artifacts and computed as described for SRCoh. Power was pooled into the same frequency bands as was done for coherence estimates.

To analyse the time course of oscillations after each stimulation, and to obtain a sufficient window length for coherence analysis, a sliding window technique (four windows, window length 60 s, window distance 30 s, thus covering a range of 0–150 s after stimulation) was applied (Fig. 1B). For each window (w1–w4), coherence and power were calculated. For further analysis of effect duration (10, 20 and 30 min after the last stimulation), coherence and power were computed for each of these blocks (180 s).

Experimental procedure

Magnetic stimulation was performed with figure-of-eight-shaped magnetic coils (diameter of each wing: 70 mm) connected to magnetic stimulators (Magstim, Whitland, Dyfed, UK) at the position of the lowest motor threshold (MT) for stimulation of the right M abductor

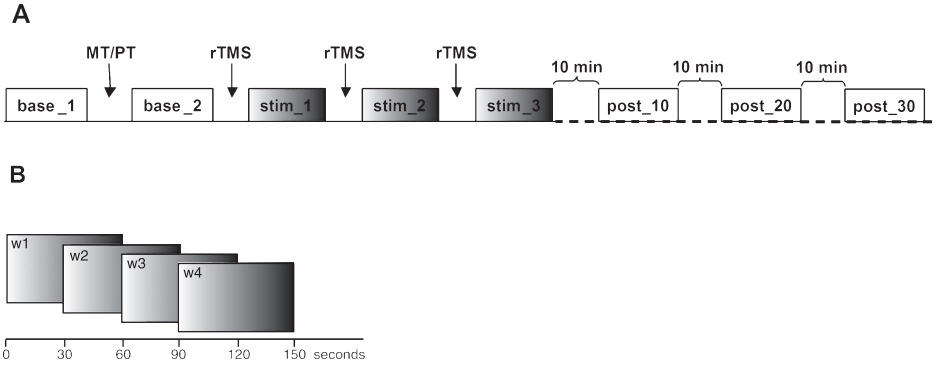

FIG. 1. (A) Experimental design. Each block comprised 180 s of EEG registration. In the blocks immediately after rTMS (stim_1–stim_3, shaded in grey) the last 30 s of EEG had to be discarded because of coil-positioning artifacts. (B) Short-term effects immediately after stimulation (stim_1–stim_3) were analysed adopting a sliding window technique with four windows (w1–w4, window length 60 s, window distance 30 s, 0–150 s).

pollicis brevis (APB) and at the occipital pole (V1). The position of the lowest MT (optimal position) was determined by placing the coil with its center touching the skull, at an angle of 45° to the midline and moving it in 1-cm steps across the scalp above the left motor cortex. Stimulation intensity was varied in steps of 2% of the maximum intensity output. MT was defined as the lowest stimulus intensity evoking a motor evoked potential (MEP) amplitude > 50 μV in five of ten consecutive trials in the relaxed APB. MEPs were recorded by surface electrodes in a belly tendon montage, amplified (CED 1902, CED, Cambridge, UK), analog/digitally converted (CED micro 1401, CED). To determine the phosphene threshold (PT), the coil was placed tangentially over the occipital pole (Kammer, 1999). The PT was defined as the minimal intensitiy required to induce phosphenes in five of ten consecutive trials starting with 60% of the maximum stimulator output, which was increased by 5% steps until phosphenes were reported. Stimulation intensity was then reduced by 2% until subthreshold intensity. Subjects who did not perceive phosphenes at intensities > 84% of stimulator output were stimulated with 100% stimulator output. If PT was below 84% stimulator output, the intensity was set to 120% of PT.

Subjects were seated comfortably in a chair and instructed to fixate a cross displayed on a monitor. First, EEG at rest was recorded for 3 min. After determination of MT and PT as described above, a second baseline EEG of 3 min was recorded. Then the amplifier was blocked and three trains of rTMS were applied simultaneously over the left M1 and V1. Each of the trains consisted of 25 pulses (10 Hz, 120% MT/PT). The frequency of 10 Hz was chosen to activate coherence particularly in this frequency range, as coherence of alpha and low beta oscillations has been shown to have a special functional significance in information processing in large-scale networks underlying visuo-motor (Classen *et al*., 1998) and visuo-tactile (Hummel & Gerloff, 2005) integration. One second after the last TMS pulse of each rTMS train, the amplifier was deblocked automatically and EEG at rest was recorded for 180 s. The last 30 s of EEG data had to be discarded because of artifacts due to the coil positioning procedure for the subsequent train. For the evaluation of effect duration, EEG at rest was recorded for 3, 10, 20 and 30 min after the last train of stimulation (Fig. 1A).

To test the specificity of the effects of bifocal stimulation, monofocal rTMS to the left M1 was performed following the procedure described above (Fig. 1A). This control experiment was performed with the same subjects following a randomized cross-over design.

Statistical analysis

First, the effects of bifocal TMS on spectral coherence ($SR_{\Delta}Coh$) and power ($SR_{\Delta}Pow$) immediately after stimulation (short-term effects) were assessed by repeated-measures analysis of variance (rmANOVA) with $WINDOW_{1-4}$, $SIDE_{left,\ right}$, and $REPETITION_{stim\_1,stim\_2,stim\_3}$ (first, second and third stimulation train) as within-subject factors. Greenhouse–Geisser correction for non-sphericity was applied and corrected degrees of freedom and *P*-values were given, when appropriate. Scheffé tests were used for *post-hoc* comparisons. Second, the effect duration of bifocal TMS on $SR_{\Delta}Coh$ and $SR_{\Delta}Pow$ was analysed by a two-way rmANOVA with the factors $SIDE_{left,\ right}$, and BLOCKbase, w4, 10 min, 20 min, 30 min. Of note, coherence estimation of window w4 (90–150 s after each stimulation) is based on three EEG periods (stim_1, stim_2, stim_3) of 60 s each (a total of 180 s). Coherence estimates of 10, 20 and 30 min after stimulation are calculated using each 180-s window.

Results

Short-term effects: alpha (8–12.9 Hz) coherence and power

The three-way rmANOVA on the four 60-s windows of $SR_{\Delta}Coh_{\alpha}$ (w1–w4) after stimulation (Fig. 2A) revealed an interaction of the factors WINDOW and SIDE ($F_{2.1,31.1} = 3.386$, $P < 0.05$). The main effects WINDOW ($F_{1.7,25.7} = 0.386$, $p = 0.65$), SIDE ($F_{1,15} = 1.390$, $P = 0.26$) and REPETITION ($F_{2,30} = 0.116$, $P = 0.89$) did not reach significance. There were no other significant interactions. As the main effect REPETITION did not reach significance, the three periods (stim_1–stim_3) were collapsed. *Post-hoc* analysis (Scheffé tests) indicated that on the stimulated (left) side, after 90 s (w4), bifocal rTMS exerted an increase of $SR_{\Delta}Coh_{\alpha}$ ($P < 0.05$) compared with w1 (0–60 s) and w2 (30–90 s). After 90 s (w4), the comparison of the stimulated

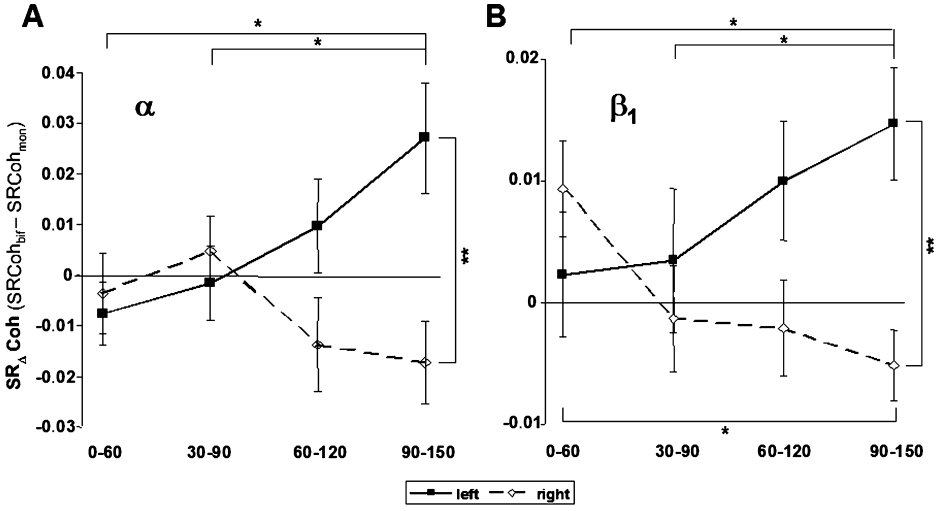

FIG. 2. (A) Time course of the difference between $SRCoh_{\alpha}$ after bifocal and $SRCoh_{\alpha}$ after monofocal stimulation ($SR_{\Delta}Coh_{\alpha}$) on the stimulated (left) and non-stimulated side (*$P < 0.05$, **$P < 0.01$; Scheffé-test). Note that in the alpha band the difference in stimulation-related coherence ($SR_{\Delta}Coh$) increases 60 s after the end of rTMS. The positive $SR_{\Delta}Coh$ on the stimulated (left) side indicates an enhancement of SRCoh after bifocal rTMS whereas the negative $SR_{\Delta}Coh$ on the non-stimulated (right) side represents a decrease of SRCoh. (B) Time course of the difference between $SRCoh_{\beta 1}$ after bifocal and $SRCoh_{\beta 1}$ after monofocal stimulation ($SR_{\Delta}Coh_{\beta 1}$) on the stimulated (left) and non-stimulated side (*$P < 0.05$, **$P < 0.01$; Scheffé-test). Error bars represent the standard error of the mean (SEM).

TABLE 1. Stimulation-related power (SRPow) after bifocal and monofocal rTMS in the alpha , lower beta and higher beta bands

	Time after rTMS						
	0–60 s	30–90 s	60–120 s	90–150 s	10 min	20 min	30 min
(a) Alpha band (8–12.9 Hz)							
Bifocal							
FC3-CP3	12.9 ± 64.8	−16.3 ± 199.3	−69.3 ± 177.4	−2.8 ± 100.9	−33.6 ± 316.6	−33.3 ± 255.7	−77.16 ± 272.6
FC4-CP4	−30.0 ± 95.0	32.7 ± 151.7	−85.9 ± 254.6	−3.3 ± 71.1	−40.6 ± 229.7	−4.8 ± 265.8	−39.6 ± 120.7
O1-Oz	−8.7 ± 59.5	−11.4 ± 68.5	0.2 ± 33.1	3.0 ± 23.6	2.8 ± 42.0	14.8 ± 57.6	−23.2 ± 71.9
O2-Oz	−12.5 ± 77.4	−10.2 ± 20.2	27.2 ± 41.3	8.9 ± 46.2	−0.8 ± 34.2	2.3 ± 70.4	−13.0 ± 51.9
Monofocal							
FC3-CP3	−9.4 ± 121.3	−21.9 ± 122.8	−29.9 ± 95.4	24.0 ± 88.9	−15.9 ± 248.3	−11.2 ± 127.2	−48.3 ± 155.7
FC4-CP4	3.9 ± 109.8	6.8 ± 121.0	−29.5 ± 185.9	12.8 ± 129.5	−13.8 ± 268.9	34.7 ± 98.4	37.1 ± 132.9
O1-Oz	10.7 ± 11.4	1.3 ± 50.0	−3.6 ± 27.2	17.2 ± 43.1	−8.6 ± 29.5	6.8 ± 31.0	11.1 ± 52.9
O2-Oz	8.8 ± 22.1	−24.5 ± 110.9	4.4 ± 23.2	21.2 ± 58.7	−31.5 ± 88.0	−12.5 ± 27.9	20.4 ± 62.0
(b) Lower beta band (13–20.9 Hz)							
Bifocal							
FC3-CP3	3.3 ± 36.0	−4.5 ± 53.8	1.3 ± 32.9	−10.2 ± 25.8	−7.7 ± 53.2	−14.1 ± 40.2	−14.2 ± 45.8
FC4-CP4	2.6 ± 28.3	−2.6 ± 39.5	2.6 ± 39.0	−2.3 ± 31.5	−7.2 ± 52.1	−3.3 ± 25.6	−9.8 ± 33.9
O1-Oz	0.2 ± 20.4	−2.8 ± 20.4	3.6 ± 9.8	−5.0 ± 12.4	−17.4 ± 82.9	2.2 ± 17.8	−12.7 ± 44.2
O2-Oz	−2.8 ± 44.5	15.1 ± 31.4	2.3 ± 22.7	−7.9 ± 44.0	−27.3 ± 98.4	−5.7 ± 27.6	−7.5 ± 31.5
Monofocal							
FC3-CP3	−15.4 ± 35.8	2.4 ± 28.5	0.0 ± 24.9	−6.7 ± 38.1	6.76 ± 44.2	−5.6 ± 44.7	−0.2 ± 42.3
FC4-CP4	−1.1 ± 40.9	11.0 ± 36.6	12.5 ± 30.6	−6.2 ± 26.8	18.6 ± 29.4	7.5 ± 47.6	8.2 ± 48.6
O1-Oz	−2.4 ± 15.7	1.0 ± 7.2	3.8 ± 11.9	0.4 ± 8.4	−5.4 ± 12.5	−1.4 ± 10.1	−3.4 ± 18.1
O2-Oz	−2.7 ± 16.3	7.0 ± 12.8	11.9 ± 23.7	2.5 ± 13.6	4.1 ± 22.4	5.37 ± 14.5	2.7 ± 18.1
(c) Upper beta band (21–29.9 Hz)							
Bifocal							
FC3-CP3	−7.3 ± 18.8	−2.4 ± 18.4	8.6 ± 20.2	−0.1 ± 10.8	−3.5 ± 12.7	−11.3 ± 17.2	−6.5 ± 13.8
FC4-CP4	4.3 ± 19.5	4.4 ± 15.2	1.0 ± 14.4	3.1 ± 16.5	−1.5 ± 16.2	0.7 ± 40.0	−2.0 ± 19.5
O1-Oz	14.4 ± 42.9	2.2 ± 17.9	−5.2 ± 21.8	−3.3 ± 18.6	−0.3 ± 29.1	−4.2 ± 17.2	−4.3 ± 15.2
O2-Oz	5.6 ± 21.3	7.0 ± 15.5	1.7 ± 21.5	2.5 ± 17.8	3.9 ± 41.0	0.2 ± 16.5	−3.6 ± 8.2
Monofocal							
FC3-CP3	−9.2 ± 17.5	−1.2 ± 17.8	5.7 ± 19.7	3.1 ± 18.3	−3.0 ± 14.9	−0.3 ± 13.0	2.4 ± 18.6
FC4-CP4	8.2 ± 14.1	12.0 ± 16.2	12.0 ± 16.9	4.4 ± 16.0	9.3 ± 27.8	5.0 ± 15.7	4.8 ± 21.2
O1-Oz	−0.4 ± 5.2	2.6 ± 4.9	1.8 ± 5.5	3.3 ± 6.5	2.1 ± 6.3	1.1 ± 7.5	7.5 ± 29.2
O2-Oz	0.8 ± 8.5	1.9 ± 7.2	1.5 ± 4.3	2.9 ± 6.6	0.4 ± 18.2	0.8 ± 3.8	5.8 ± 29.5

Data are presented as means ± SD.

(left) and the non-stimulated (right) side indicated a difference in $SR_{\Delta}Coh_{\alpha}$ between the left and the right side ($P < 0.01$). rTMS of either form did not exert any significant effects on $SR_{\Delta}Pow_{\alpha}$ (Table 1a).

Short-term effects: lower beta (13–20.9 Hz) coherence and power

The three-way rmANOVA on windowed $SR_{\Delta}Coh_{\beta 1}$ after stimulation (Fig. 2B) showed a significant effect of SIDE ($F_{1,15} = 4.677$, $P = 0.047$) and interaction of the factors WINDOW and SIDE ($F_{3,45} = 5.453$, $P = 0.003$). The main effects WINDOW ($F_{2.2,33.1} = 0.328$, $P = 0.74$) and REPETITION ($F_{2,30} = 0.252$, $P = 0.78$) did not reach significance. There were no other significant interactions. *Post-hoc* tests showed an enhanced $SR_{\Delta}Coh_{\beta 1}$ in w4 on the stimulated (left) side compared with the non-stimulated (right) side ($P < 0.01$) and compared with w1 and w2 ($P < 0.01$). On the non-stimulated side $SR_{\Delta}Coh_{\beta 1}$ decreased between w1 and w4 ($P < 0.05$). There were no effects on $SR_{\Delta}Pow_{\beta 1}$ (Table 1b).

Short-term effects: higher beta (21–29.9 Hz) coherence and power

No significant main effects or interactions were found in this frequency band (Table 1c). The color plots (Fig. 3) display the differential modulation of cortico-cortical coherence immediately after bifocal and monofocal stimulation on the stimulated (left) and non-stimulated (right) side.

Effect duration: alpha (8–12.9 Hz) coherence and power

The two-way rmANOVA on mean $SR_{\Delta}Coh_{\alpha}$ (Fig. 4A) revealed an interaction SIDE × BLOCK ($F_{4,60} = 2.508$, $P = 0.05$). Compared with w4 (90–150 s), $SR_{\Delta}Coh_{\alpha}$ did not significantly decrease after 10 min, but did after 20 min ($P < 0.05$) and 30 min ($P < 0.01$) on the stimulated side, indicating a return to baseline.

On the non-stimulated side, $SR_{\Delta}Coh_{\alpha}$ increased markedly after 10 min as compared with the initial decrease ($P < 0.01$) and returned to baseline 20 min after the end of stimulation ($P < 0.05$). Comparison with baseline revealed an increase of $SR_{\Delta}Coh_{\alpha}$ only at w4 (90–150 s) and only on the stimulated side. For band power values (Table 1a), the three-way rmANOVA (SIDE, ELECTRODES, BLOCK) on mean $SR_{\Delta}Pow_{\alpha}$ did not yield any significant main factors or interactions.

Effect duration: lower beta (13–20.9 Hz) coherence and power

The two-way rmANOVA with the factors BLOCK and SIDE on the mean $SR_{\Delta}Coh\text{-}beta_1$ (Fig. 4B) demonstrated an increase of coherence

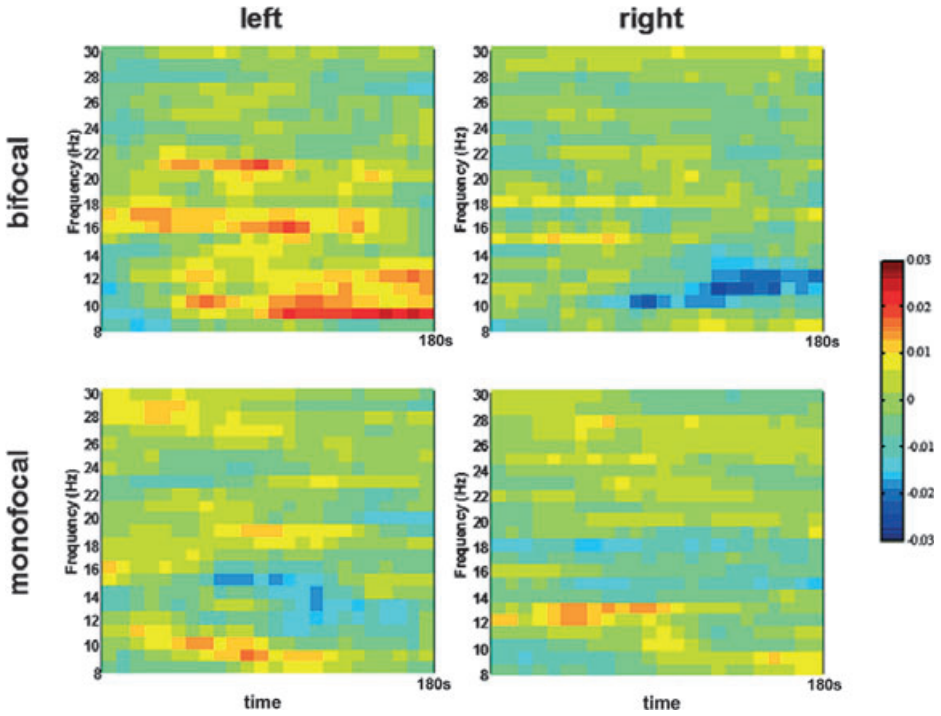

FIG. 3. Original data on SRCoh changes in the alpha and beta range (8–30 Hz) after stimulation, averaged across subjects and stimulations. A sliding window of 60 s was shifted in steps of 5 s. The colour bar represents the maximal range of SRCoh.

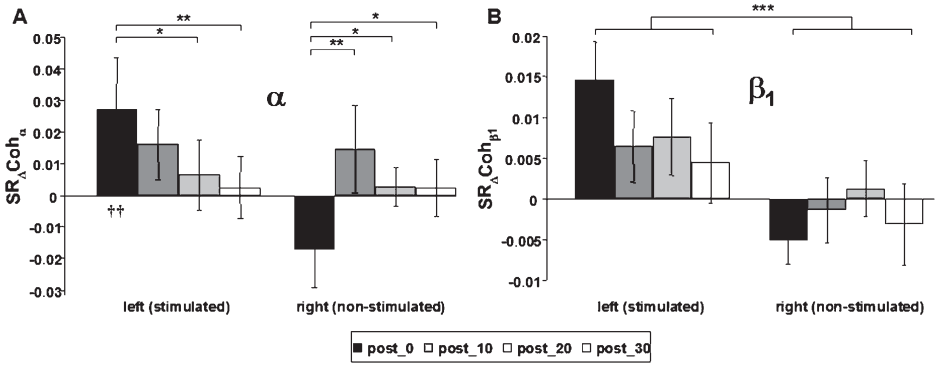

FIG. 4. Mean difference between SRCoh after bifocal and SRCoh after monofocal stimulation ($SR_{\Delta}Coh$), 90–150 s (post_0), 10 (post_10), 20 (post_20) and 30 min (post_30) in the (A) alpha (8–12.9 Hz) and (B) lower beta band (13–20.9 Hz). The latter showed higher $SR_{\Delta}Coh$ on the stimulated than on the non-stimulated side (***$P < 0.001$; rmANOVA). In the alpha band, 90–150 s after stimulation $SR_{\Delta}Coh$ is increased compared with baseline ($^{\dagger\dagger}P < 0.01$; Scheffé-test). Ten minutes after stimulation the increase of SRCoh on the left (stimulated) side is not significantly reduced, and is enhanced on the right (non-stimulated) side. Twenty and 30 min later, $SRCoh_{\alpha}$ returns to baseline on both sides (*$P < 0.05$, **$P < 0.01$; Scheffé-test). Error bars show the standard error of the mean (SEM).

after bifocal stimulation on the stimulated compared with the non-stimulated side ($F_{1,15} = 15.258$, $P < 0.001$). There was no effect of the factor BLOCK and no interaction BLOCK × SIDE. Thus, during the 30 min after stimulation a significant reduction of $SR_{\Delta}Coh\text{-}beta_1$ could not be detected.

The three-way rmANOVA (SIDE, ELECTRODES, BLOCK) on mean $SR_{\Delta}Pow_{\beta 1}$ did not show any significant main factors or interactions (Table 1b).

Effect duration: higher beta (21–29.9 Hz) coherence and power

Statistical analysis did not yield any significant effects (Table 1c).

Discussion

In the present study, we compared the effects of synchronous bifocal with monofocal rTMS on long-range synchronization of human brain activity. Stimulation was applied to the motor and visual cortex, respectively. The key finding was an increase of cortico-cortical coherence between the sensorimotor and the visual cortex on the stimulated side. This enhancement developed within 2 min after rTMS and persisted up to 10 min after stimulation. In contrast, EEG coherence remained unaffected by rTMS given to the primary motor cortex alone (Figs 3 and 4). These results support the hypothesis that coupling of oscillatory activity can selectively be enhanced by simultaneous rTMS to distinct brain areas.

Coherence of oscillatory brain activity has been regarded as an important neurophysiological feature, providing the basis for the formation of cooperating cell assemblies established by an associative-learning procedure (Miltner *et al*., 1999). Particularly, coherence of low-frequency oscillations has been shown to be associated with the constitution of large-scale networks, especially in motor (Andres & Gerloff, 1999) and visuomotor (Classen *et al*., 1998) integration tasks. A direct link between the magnitude of task-specific coherent oscillatory EEG activity and the degree of behavioral success was established in a visuotactile integration task (Hummel & Gerloff, 2005) and during memory encoding (Weiss & Rappelsberger, 2000). It has been suggested that interregional coherence is a neuronal mechanism underlying cognitive effort and performance in sensorimotor tasks (Hummel & Gerloff, 2005; Kay, 2005). With regard to the neuronal implementation of effective communication structures, coherent oscillation of neuronal groups has been put forward as a general mechanism for the preferential routing of selected signals (Fries, 2005; Womelsdorf *et al*., 2007). The present findings provide first evidence for selective enhancement of cortico-cortical coherence by non-invasive brain stimulation in human subjects.

The principle of associative plasticity has been confirmed previously using joint stimulations of convergent pathways in animal models (Baranyi & Feher, 1981) and human subjects (Stefan *et al*., 2000). Recently, functional reorganization was induced using *in vivo* spike activity at one site to trigger the stimulation of a second site (Jackson *et al*., 2006). In the present study, reorganization as indicated by changes in coherent oscillations of remote brain areas was induced by simultaneous co-stimulation of two cortical sites. Following Hebb's postulate, enhancement of synaptic efficacy by bifocal, associative cortical stimulation may reinforce the connections among cortical areas, as reflected by an increase of oscillatory coupling. However, there are no direct anatomical connections between visual and motor areas. Therefore, at least one intermediate synapse is necessary to connect these two areas. These intermediate links are possibly located in the dorsal parietal cortex or premotor and prefrontal cortices (Wise *et al*., 1997). It is known from behavioral studies in cats and human subjects that interareal synchronicity with zero time-lag is related to motor behavior in a visuomotor integration tasks (Roelfsema *et al*., 1997; Classen *et al*., 1998). We suggest that associative cortico-cortical stimulation by bifocal rTMS strengthens these communication pathways, resulting in enhanced coherence between the areas involved. The enhanced coupling between the visual and sensorimotor cortex that was observed could have been direct, mediated by one or more other regions or established by a third cortical or subcortical pacemaker. However, the asymmetric modulation of coherence argues against a single common pacemaker, e.g. in the thalamus. As the site of plasticity and the corresponding conduction delay is not known, the optimal timing of convergence of both stimuli will be subject to further studies. It might not necessarily be simultaneous TMS.

Of note, in the alpha band, the enhancement of coherence was established with a delay of approximately 60 s. This was surprising, as in most studies EEG effects of rTMS were found immideately after the end of stimulation and might be explained by complementary mechanisms. First, high-frequency stimulation is known to disrupt ongoing neuronal information processing by transient synchronization of spontaneous activity of cortical neurons (Siebner & Rothwell, 2003). This effect might have the potential to initially 'override' the physiological cortico-cortical coupling. Second, enhanced efficacy of the synaptic linkages induced by bifocal, associative stimulation may facilitate the gradual enhancement of functional coupling. This delayed enhancement and the fact that increases in the beta band are not plain upper harmonics of the 10-Hz induction frequency of rTMS argues against a technical artifact and a propagation of an imposed stimulation-driven frequency pattern or reset of oscillatory activity.

To our knowledge, this is the first study on the effects of non-invasive cortico-cortical stimulation on oscillatory coupling. However, earlier studies adopting associative learning procedures (Miltner *et al*., 1999) have accordingly demonstrated an increase of cortico-cortical coherence between brain regions that receive converging stimuli. In addition, the principle of associative stimulation was verified in multiple models applying convergent neuronal activation, e.g. by direct electrical stimulation, combination of physiological and artificial stimuli (Jackson *et al*., 2006), as well as magnetic and somatosensory stimuli (Stefan *et al*., 2000; Wolters *et al*., 2003).

Our observation of increasing coherence between visual and sensorimotor areas during the time window up to 150 s after stimulation was specific to the stimulated hemisphere. Transient decoupling on the contralateral side may indicate an essential role of temporal order of stimulation (Wolters *et al*., 2003) as the TMS input reaches the contralateral sensorimotor cortex with a delay of approximately 10 ms (Cracco *et al*., 1999). However, this finding may also reflect contralateral inhibition of M1 induced by high-frequency stimulation (Plewnia *et al*., 2003; Kobayashi *et al*., 2004).

Nevertheless, 10 min later, a bilateral enhancement of coherence was found. We can only speculate on the possible mechanisms of this effect. The propagation of coherence, i.e. expansion of coherence to the contralateral, non-activated side, has already been shown after unilateral finger movements (Leocani *et al*., 1997). At the neuronal level, visuomotor coherence could have been transmitted to the contralateral side via callosal cortico-cortical connections (Mima *et al*., 2000; Knyazeva *et al*., 2006) or cortico-thalamo-cortical loops (Destexhe *et al*., 1999).

Previous studies using monofocal rTMS given to the primary motor cortex (Strens *et al*., 2002; Oliviero *et al*., 2003), premotor cortex (Chen *et al*., 2003) and frontal cortex (Jing & Takigawa, 2000) have shown modifications of cortico-cortical coherence after stimulation with high and low frequencies. High-frequency stimulation (5 Hz) of the motor cortex with intensities below the resting motor threshold decreased intrahemispheric coherence between motor and premotor cortex in the alpha band (Oliviero *et al*., 2003), whereas this was increased with low-frequency stimulation (1 Hz) (Strens *et al*., 2002). These changes were linked to the excitability changes observed after rTMS of different frequencies. However, bifocal rTMS has not yet been used to modify cortico-cortical coherence. Moreover, changes of intrahemispheric coherence between motor and premotor cortex reported previously (Oliviero *et al*., 2003) cannot directly be related to our results demonstrating changes in long-range coherence between the visual and motor system.

In conclusion, our study extends the principle of non-invasive associative brain stimulation to a cortico-cortical stimulation paradigm. The demonstrated enhancement of cortico-cortical coupling by non-invasive brain stimulation techniques could provide a new method for the facilitation of behavioral relevant functional integration.

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Abbreviations

APB, abductor pollicis brevis; Coh, coherence; EEG, electroencephalography; M1, primary motor cortex; MEP, motor evoked potential; MT, motor threshold; Pow, power; PT, phosphene threshold; ROI, region of interest; TMS, transcranial magnetic stimulation; V1, primary visual cortex.

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