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RESEARCH ARTICLE

Improvement of tactile perception and enhancement of cortical excitability through intermittent theta burst rTMS over human primary somatosensory cortex

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Abstract Adopting the patterns of theta burst stimulation (TBS) used in brain-slice preparations, a novel and rapid method of conditioning the human brain has recently been introduced. Using short bursts of high-frequency (50 Hz) repetitive transcranial magnetic stimulation (rTMS) has been shown to induce lasting changes in brain physiology of the motor cortex. In the present study, we tested whether a few minutes of intermittent theta burst stimulation (iTBS) over left primary somatosensory cortex (SI) evokes excitability changes within the stimulated brain area and whether such changes are accompanied by changes in tactile discrimination behavior. As a measure of altered perception we assessed tactile discrimination thresholds on the right and left index fingers (d2) before and after iTBS. We found an improved discrimination performance on the right d2 that was present for at least 30 min after termination of iTBS. Similar improvements were found for the ring finger, while left d2 remained unaffected in all cases. As a control, iTBS over the tibialis anterior muscle representation within primary motor cortex had no effects on tactile discrimination. Recording somatosensory evoked potentials over left SI after median nerve stimulation revealed a reduction in paired-pulse inhibition after iTBS that was associated but not correlated with improved discrimination performance. No excitability changes could be found for SI contralateral to iTBS. Testing the performance of simple motor tasks revealed no alterations after iTBS was applied over left SI. Our results demonstrate that iTBS protocols resembling those used in slice preparations for the induction of long-term potentiation are also effective in driving lasting improvements of the perception of touch in human subjects together with an enhancement of cortical excitability.

Keywords Repetitive transcranial magnetic stimulation (rTMS) · Theta-burst stimulation · 2-Point discrimination · Somatosensory cortex · Motor cortex · Cortical plasticity · Perceptual

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Introduction

Based on animal experiments, long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission have been suggested to be key candidates for plastic reorganizational changes in the central nervous system (Bliss and Lomo 1973; Stanton and Sejnowski 1989). However, little is known how these phenomena affect perception when applied in the intact human brain. To date, the most promising technique for mimicking in humans procedures that are known to drive synaptic plasticity in animal models is transcranial magnetic stimulation (TMS). This non-invasive research tool introduced by Barker and colleagues in the mid 1980s (Barker et al. 1985) has been developed and refined to investigate mechanisms of brain function and cortical plasticity in humans [for review see Siebner and Rothwell (2003)]. TMS is capable of evoking changes in

excitability of cortical neurons depending on the choice of stimulus variables. For example, low-frequency repetitive TMS (rTMS) (<5 Hz) usually results in suppression of cortical excitability (Chen et al. 1997; Muellbacher et al. 2000; Romero et al. 2002), whereas high-frequency rTMS (≥5 Hz) is known to facilitate cortical excitability within the stimulated cortical area (Peinemann et al. 2000; Wu et al. 2000; Di Lazzaro et al. 2002; Ragert et al. 2004; for review see Siebner and Rothwell 2003).

Beyond these findings about rTMS-induced excitability changes, many reports demonstrated that rTMS also affects perceptual, behavioral and cognitive performance. Using low-frequency TMS several studies described suppressive effects on perception (Seyal et al. 1997; Knecht et al. 2003). On the other hand, using high-frequency rTMS beneficial effects are reported not only in the sensory and motor domain (Ragert et al. 2003; Kobayashi et al. 2004; Tegenthoff et al. 2005), but also for cognitive performance (Evers et al. 2001; Klimesch et al. 2003).

Recently, Huang and colleagues (Huang and Rothwell 2004; Huang et al. 2005) introduced a novel approach to apply transcranial magnetic stimulation called theta burst stimulation (TBS), a rapid method of conditioning the human motor cortex. They found that short bursts of high-frequency (50 Hz) rTMS pulses induced long-lasting changes in motor cortex physiology depending on the choice of stimulus pattern. For example, an application of intermittent theta burst stimulation (iTBS) over MI resulted in an enhancement of cortico-spinal excitability in MI, whereas a continuous application (cTBS) decreased motor evoked potentials (MEPs) (Huang et al. 2005). Remarkably, the short-lasting stimulation period of only 190 s induced a relatively long-lasting effect on motor cortex of about 60 min duration making this stimulation technique a promising tool to modulate cortical activity However, the behavioral relevance of iTBS application outside MI remains elusive.

Here we asked whether iTBS application over primary somatosensory cortex (SI) is able to induce excitability changes within SI and whether iTBS also evokes changes in tactile discrimination behavior. Paired-pulse behavior in SI using median nerve stimulation has been studied in detail by many groups (Frasson et al. 2001; Mochizuki et al. 2001). For example, cortical infarction due to photothrombosis led to a long-lasting and widespread reduction of $GABA_A$-receptor expression in the area surround the lesion, which was associated with an increased neuronal excitability as measured by paired-pulse behavior (Schiene et al. 1996). Given the LTP-like changes observed in human MI following iTBS we hypothesized that iTBS application over left SI is capable of enhancing cortical excitability and improve tactile discrimination performance. If this would be true, iTBS would qualify as a valuable alternative as compared to high-frequency TMS, which has been shown to drive corresponding alterations, yet based on a much longer stimulation time (Ragert et al. 2004; Tegenthoff et al. 2005). We hypothesized that iTBS application over left SI is capable of inducing neuroplasticity within SI, which is associated with an improvement in the perception of touch of the right hand. Furthermore, the spatial specificity of iTBS-induced changes in SI was evaluated by applying iTBS over the primary motor cortex (MI) while testing tactile discrimination behavior and simple motor tasks.

Materials and methods

Experimental procedures

Subjects

We tested a total number of 23 healthy subjects between 21 and 33 years of age [mean age 24.86 ± 2.56 years (SD)]. All subjects were naïve to the intention and purpose of the study. The protocol was approved by the local Ethics Committee of the Ruhr-University Bochum and was performed in accordance with the 1964 Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm). Subjects gave their written informed consent before participating. According to the Oldfield questionnaire for the assessment of handedness (Oldfield 1971), all subjects were right-handed. For an overview of participants' assignments to different tests see Table 1.

2-Point discrimination

Tactile 2-point discrimination on the fingers was assessed using the method of constant stimuli as described previ-

Table 1 Breakdown of 23 participants (numbered 1–23) in different experiments

	iTBS over SI	iTBS over SI	iTBS over SI	iTBS over MI
Total	Pre-post	Rec 30–90 min	Pre-post	Pre-post
23 subjects	Right/left d2 (right/left hand)	Right d2	Right d2/d4	Right/left d2
Tactile 2PD	1–9 (9)	10–14 (5)	15–19 (5)	20–23 (4)
Motor performance	1–9 (9)	N/A	N/A	N/A
SEP	1–9 (9)	N/A	N/A	N/A

Numbers in parentheses give the total number of participants for each set of experiments

ously (for an overview see Godde et al. 2000; Dinse et al. 2003; Pleger et al. 2003a). In brief, seven pairs of rounded needle-probes (diameter 200 μm) with separation distances ranging from 0.7 to 2.5 mm in 0.3-mm steps were used. For control, zero distance was tested with a single probe. The probes were mounted on a rotatable disc that allowed switching rapidly between distances. To accomplish a rather uniform and standardized type of stimulation the disc was installed in front of a plate that was movable up and down. The arm and fingers of the subjects were fixated on the plate and the subjects were then asked to move the arm down. The down movement was arrested by a stopper at a fixed position above the probes. The test finger was held in a hollow containing a small hole through which the distal phalanx of the finger came to touch the probes approximately at the same indentations in each trial. Each distance between probes was presented eight times in randomized order resulting in 64 single trials per session. Subjects were aware that there were single probes presented but not how often. The subjects had to decide immediately after touching the probes if he or she had the sensation of one or two tips by answering "one" or "two". After each session, individual discrimination thresholds as well as false alarm rates (the proportion of trials when the subject said "two" when there was a single needle) were calculated. The proportion of trials on which the subjects responded "two" for each probe were plotted against the tip distance as a psychometric function and were fitted with a logistic regression method (SPSS version 12.0.1). Thresholds as a marker for individual tactile performance were taken at that point at which 50% correct responses were reached. For a 2-point discrimination task using the method of constant stimuli, thresholds are obtained for 50% correct responses (Godde et al. 2000; Dinse et al. 2003; Pleger et al. 2003a).

Tapping task

During the tapping task, subjects were seated in a comfortable chair and were asked to hit a metal touch panel (8.5 × 8.5 cm) using a metal stick as fast as possible within a 60-s time frame. The touch panel and the metal stick were connected to a digital computer that counted and stored the hits every 20 s. The total number of hits and temporal variation of performance in three successive time windows of 20 s each were recorded (Ruff and Parker 1993).

Aiming task

During the aiming task, subjects were instructed to hold a metal stick with their right hand into a small hole of a metal block (diameter 6 mm) for 1 min with their forearm and upper arm held in right-angled position. Every contact with the edges or the bottom of the hole was counted as an error and registered with a digital computer.

Maximal grip force task

Subjects were asked to perform three maximum effort trials in a fist grip task starting with their right hand before and after iTBS. Each trial was separated by an interval of at least 30 s in order to avoid fatigue in both hand muscles. Subjects were asked to start from an initial state of relaxation, develop their maximum force as fast as possible and maintain it for 1–2 s. The emphasis of grip-force measurements was solely focused on maximum force.

Intermittent theta burst stimulation using repetitive transcranial magnetic stimulation

During the iTBS sessions, subjects were seated in a comfortable chair and were instructed to keep their eyes closed and relax. Subjects wore a tight-fitting cap with a coordinate system drawn on it (1 × 1 cm width), which was referenced to the vertex (Cz). Cz was identified as the intersection of the interaural line and the connection between nasion and inion. First, a MAGSTIM Rapid Stimulator that produced a biphasic waveform (Magstim, Whitland, Dyfed, UK) was connected to an 8-shaped coil (outside diameter 8.7 cm; peak magnetic filed strength 2.2 Tesla; peak electric field strength 660 V7m). Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseus (FDI) muscle by surface electromyography (EMG), using Ag–AgCl cup electrodes in a belly-tendon montage. The EMG raw signal was amplified and band-pass filtered (Neuropack 8, Nihon Kohden, Tokyo, Japan, 20Hz to 2 kHz). During searching the cortical FDI muscle representation, TMS stimuli were presented within a 2 × 2 cm array 5 cm away from Cz along the central sulcus. The "motor hot-spot" of the FDI muscle representation was identified at that scalp position, where single TMS pulses at slightly suprathreshold intensity induced the most consistent MEP amplitudes in the relaxed muscle. Resting and active motor thresholds were determined with the coil located over this scalp position. Resting motor threshold (RMT) was defined as the lowest intensity capable of evoking five out of ten MEPs with amplitudes of at least 50 μV in the relaxed muscle. Active motor threshold (AMT) was defined as the minimum stimulation intensity over the FDI muscle representation that could elicit MEPs of more than 200 μV in five out of ten trials during a grip-force measurement with 20% of the maximum force of the contralateral hand. After the determination of AMT, the coil was moved 1 cm posterior in parasagittal direction in order to stimulated the hand representation within the primary somatosensory cortex (SI) as described previously (Ragert et al.

2003; Ragert et al. 2004; Tegenthoff et al. 2005). The magnetic coil was held tangentially to the scalp at an angle of 45° to the midline with the handle pointing backwards. For repetitive transcranial magnetic stimulation over the left SI, we used an intermittent theta burst stimulation protocol (iTBS) introduced by Huang et al. (2005). iTBS consisted of bursts containing three pulses at 50 Hz repeated at 200 ms intervals. For the application of iTBS, a 2-s train of TBS is repeated every 10 s for a total of 190 s (600 pulses) and applied with an intensity of 80% AMT over SI. In order to study the spatial specificity of iTBS over SI, we applied the iTBS protocol to a remote location several centimeters apart as a control condition similar as described in a previous study (Tegenthoff et al. 2005). Furthermore, this control condition was carried out to exclude any unspecific stimulus-related effects that could affect tactile discrimination per se. However, here we chose the lower leg (tibialis anterior muscle) representation within primary motor cortex (MI), using a localization procedure as described above. First, the motor hot spot of the tibialis anterior muscle was identified at the position at which single-pulse TMS induced highest MEP amplitudes (approximately 1–2 cm lateral to Cz). Next, RMT was defined as the lowest intensity capable of evoking five out of ten MEPs with amplitudes of at least 50 μV in the relaxed muscle. In order to ensure that the relative difference in stimulation intensity of RMT and AMT for iTBS over MI was comparable to iTBS over SI, iTBS stimulation intensity was adjusted using the following formula: ($RMT_{lower\ leg}$ − percentage difference between RMT_{FDI} and AMT_{FDI}). In line with observations of Huang and colleagues (Huang and Rothwell 2004; Huang et al. 2005), iTBS application in the present study was well tolerated in all participants, and no adverse effects could be observed.

Paired-pulse somatosensory evoked potential recordings

Paired-pulse somatosensory evoked potential (paired-pulse SEP) recordings were performed as described previously (Ragert et al. 2004) in order to study inhibitory circuits within the stimulated (left) and non-stimulated (right) SI. In brief, we applied electrical median nerve stimulation with an inter-stimulus interval (ISI) of 30 ms to the wrist of the right and left hands in separate sessions. Median nerve stimulation (cathode proximal) was performed with a block electrode (electrical square-wave pulse of 0.2 ms duration, repetition rate of the paired stimuli 2 Hz).

Median nerve stimulation intensity was adjusted to 2.5 above individual sensory threshold and was kept constant for each subject before and after iTBS application. In all subjects, the chosen stimulation intensity induced a small muscular twitch in the thenar muscle of the thumb. During median nerve stimulation and SEP recordings, subjects were seated in a comfortable chair and were instructed to relax but to stay awake with their eyes closed. SEPs were recorded and stored for offline analysis with a Neuropack 8 equipment (Nihon Kohden, bandpass filter 2 Hz to 2 kHz, sensitivity 2 μV/devision, sampling rate of 5 kHz).

Paired pulse SEP recordings were made using a three-electrode array. Two electrodes (C3′ and C4′) were located over the left and right SI, 2 cm posterior to C3 and C4 according to the International 10–20 system. A reference electrode was placed over the midfrontal (Fz) position (bipolar recordings). The electrical potentials were recorded in epochs from 0 to 200 ms after the stimulus. A total number of 400 stimulus-related epochs were recorded. Latencies and peak-to-peak amplitude of the N20/P25 response that is generated in superficial and middle cortical layers of area 3b within SI (Allison et al. 1991) were measured and compared before and after iTBS. Furthermore, the amplitude of the N20 responses (baseline to peak) generated by paired-pulse SEPs was measured before and after iTBS. During iTBS application, SEP electrodes and block electrodes were removed; however, exact electrode positions were marked on the wrist and on the scull before removal. In addition to an analysis of the raw amplitude data, paired pulse suppression was expressed as a ratio (A2/A1) of the amplitudes of the second (A2) to the first N20/P25 peak (A1).

Experimental schedule

If not otherwise stated, the cortical hand representation in the left primary somatosensory cortex (SI) was used for iTBS (see Fig. 1). Behavioral tasks were performed on the same day before and after iTBS application in a randomized fashion. To achieve a stable baseline performance of tactile two-point discrimination, all subjects were tested on four consecutive sessions (s1–s4) on the right index finger (d2) before iTBS. In the fourth session, thresholds for the left index finger (left d2) were additionally tested and served as control. Additionally, individual motor performance (tapping, aiming and grip force) for the right hand was tested on three consecutive sessions before and after iTBS to assess the effects of iTBS on manual motor control. After application of iTBS, tactile performance was retested approximately 2 min after termination of iTBS (post iTBS condition = session s 5) and motor performance was retested starting about 10 min after the termination of iTBS. Recovery measurements (s6–s8) for performance changes in tactile discrimination were performed 30, 60 and 90 min after termination of iTBS for d2 of the right hand. Controls for the local specificity of iTBS-induced changes consisted of measuring discrimination thresholds on the right ring finger (d4) before and after the application of iTBS over left SI and of measuring discrimination thresholds of d2 before and after the application of iTBS

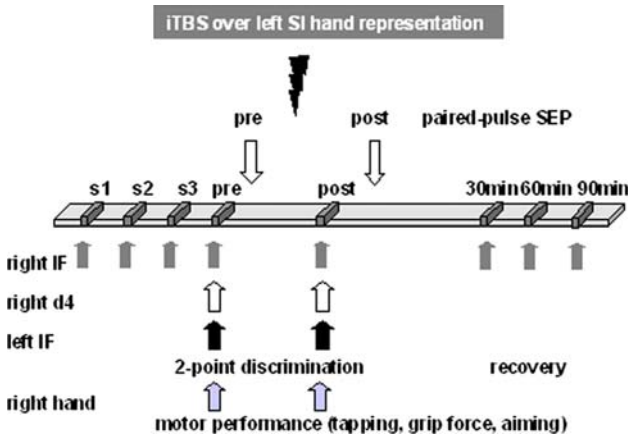

Fig. 1 Experimental setup and design. In order to obtain a stable baseline performance for the right IF, tactile discrimination thresholds were measured on four consecutive sessions (s1-pre (s4)) before iTBS was applied. d4 (right ring finger) and the left IF served as controls to assess spatial specificity of perceptual effects. Discrimination thresholds of d4 and left IF were tested only on session s4 (pre-condition) because effects of task familiarization are known to generalize across fingers. After session s4, paired-pulse SEPs (left and right SI) were recorded, and then iTBS was applied over left SI hand representation. After termination of iTBS, post paired-pulse SEP recordings were repeated. Then, performances on both index fingers (IFs) as well as d4 were measured. Sessions s6–s8 (termed 30, 60, 90 min) served to assess the recovery of iTBS-induced effects on tactile discrimination thresholds of the right IF. Apart from the assessment of tactile discrimination thresholds, aspects of basic motor performance (tapping, grip force, aiming) on the right hand was additionally tested before (s4) and after (s5) iTBS was applied over left SI

over the tibialis anterior muscle representation in MI. In order to evaluate iTBS-induced changes on paired-pulse inhibition we performed paired-pulse SEP recordings (Ragert et al. 2004) in a subgroup of participants.

Data analysis

Data were analyzed using the SPSS software package for Windows version 12.0.1. Repeated measures ANOVA (rmANOVA) and post hoc analysis (Bonferroni corrected) were used to compare tactile and motor performance before and after iTBS, and paired *t* tests were performed to compare the effect of iTBS on paired-pulse suppression in SI. All figures represent group data. Error bars refer to the standard error (sem) of the measurements. Asterisks represent a *P* value of <0.01.

Results

Effect of iTBS over SI on tactile discrimination thresholds of d2

Prior to iTBS, tactile acuity was assessed by measuring two-point discrimination thresholds on the tip of d2 in nine participants. To obtain a stable baseline performance, thresholds were tested in four consecutive sessions that revealed only little fluctuation in thresholds (rmANOVA with factor SESSION [d2, session (s) s1–s4], $F_{(3,24)} = 0.587$; $P = 0.630$, see Fig. 2). After the fourth session, iTBS was applied with a figure-8-coil positioned over the hand representation of left SI and tactile performance was re-tested around 10 min after termination of iTBS. We found an improved performance on d2 in all subjects indicated by a significant lowering of tactile discrimination thresholds of 0.24 ± 0.06 mm (mean ± sem) from 1.82 ± 0.03 mm before iTBS to 1.57 ± 0.04 mm after iTBS [rmANOVA pre vs. post, d2: $F_{(1,8)} = 14.533$; $P = 0.005$; posthoc test corrected for multiple comparisons (Bonferroni-corrected) of post-iTBS vs. s1–s4 (s4 = pre session); $0.047 \geq P \leq 0.006$]. We have repeatedly shown (Pleger et al. 2003b; Dinse et al. 2006) that performance improvements in 2-point discrimination were not associated with an increased false alarm (the proportion of trials when the subject said "two" when there was a single needle). In the present study, false alarm was zero in all subjects before and after iTBS application pointing to an increased hit rate that mediates performance improvements rather than an increased false alarm.

Time course of recovery of iTBS-induced behavioral gains

To obtain information about the duration of iTBS-induced effects on tactile performance, we performed measurements on d2 in a subgroup of participants ($n = 5$) every 30 min after termination of iTBS (termed rec30, rec60 and rec90). We found that iTBS-induced changes in tactile acuity persisted for at least 30 min after termination and recovered to baseline performance 90 min after termination of stimulation

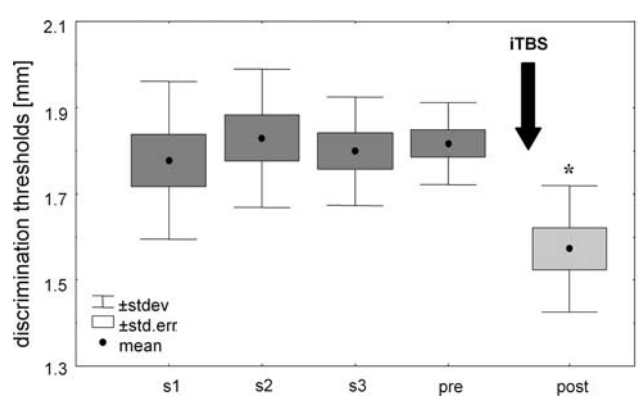

Fig. 2 Effects of iTBS on tactile discrimination thresholds of d2 of the right hand. Prior to iTBS, we found a stable baseline performance in 2-point discrimination on four consecutive sessions (s1–s4, s4 = pre-condition). After iTBS (s5 = post-condition) application, discrimination thresholds were significantly reduced. *Asterisks* indicate a $P \leq 0.05$

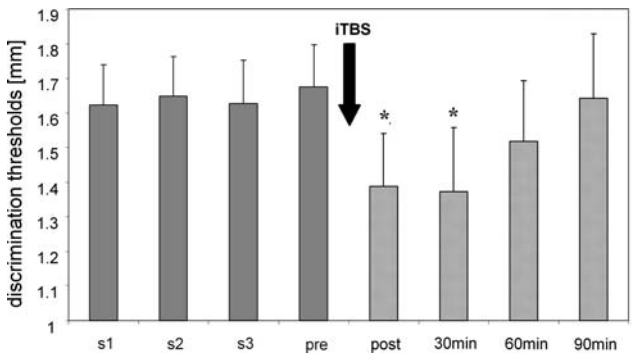

Fig. 3 Time course of recovery for tactile acuity improvement of d2 of the right hand. Reassessment of discrimination thresholds every 30 min after termination of iTBS revealed that the lowering in thresholds persisted for at least 30 min and recovered to a level of baseline performance only 90 min after termination of iTBS. These results demonstrate that iTBS can induce persistent effects on tactile discrimination thresholds that outlast the time of stimulation (3 min) by at least 30 min. *Asterisks* indicate a $P \leq 0.05$

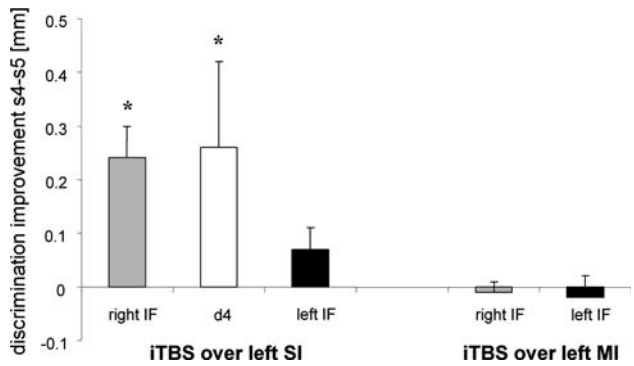

Fig. 4 Changes in tactile acuity evoked by iTBS application over SI hand representation and MI lower leg representation (control condition). Pre-post thresholds changes (s4–s5) are shown as differences for d2 (*gray*), d4 (*white*) of the right hand and for left d2 (*black*) after iTBS application over left SI hand representation and for the right and left d2 after iTBS application over left MI lower leg representation. A significant discrimination improvement was only found for the right IF and d4. *Asterisks* indicate a $P \leq 0.05$

[rmANOVA with factor SESSION (s1-rec90), $F_{(7,28)} = 7.519$; $P < 0.0001$; paired t test pre vs. post: $P = 0.0001$; paired t test pre vs. rec30: $P = 0.01$; paired t test pre vs. rec60: $P = 0.07$; paired t test pre vs. rec90: $P = 0.67$; see Fig. 3]. These results demonstrate that iTBS can induce a significant lowering of tactile discrimination threshold that outlasts the time of stimulation (3 min) by at least a factor of 10.

Spatial specificity of iTBS effects

In order to study the spatial specificity of iTBS-induced behavioral effects, a number of additional experiments were performed (see Table 1). As a first control, tactile discrimination thresholds on the index finger of the left hand (left d2; $n = 9$) ipsilateral to iTBS were measured immediately before (pre-condition) and after iTBS (post-condition) was applied. Baseline performance under pre-conditions between the left d2 and right d2 did not differ (paired t test: $P = 0.97$). After iTBS, thresholds on the left d2 showed only little fluctuations in thresholds of 0.04 ± 0.03 mm (rmANOVA with factor SESSION (pre vs. post): $F_{(1,8)} = 2.009$; $P = 0.194$) indicating that the effect of iTBS did not transfer to the finger of the other hand (see Fig. 4).

As a second control, we measured tactile discrimination thresholds on the right ring finger (d4) while iTBS was applied over the left SI hand representation. Baseline performance (pre-condition) on d4 was comparable to d2. After iTBS, we found also an improved performance on d4 (lowering of thresholds from 1.81 ± 0.06 to 1.56 ± 0.02 mm; see Fig. 4). Comparing the percentage changes in performance on d4 with that of d2 revealed a comparable magnitude of tactile discrimination improvement after iTBS ($13.64 \pm 3.70\%$ for d4 vs. $15.05 \pm 3.37\%$ for the right IF) indicating that stimulating the hand representation within SI from outside of the skull leads to an improved performance also on d4 of the right hand.

As a further control for specificity, iTBS was applied in additional participants ($n = 4$) over the tibialis anterior muscle representation within left MI, but tactile performance on d2 and left d2 was tested. The aim was to apply iTBS to a more remote brain area outside SI separated by a couple of centimeters with little or no direct connections to SI thereby serving as a control condition in which the finger representation in SI is not directly stimulated. Before iTBS, tactile performance on d2 of the right hand was 1.59 ± 0.09 mm and 1.79 ± 0.14 mm on d2 of the left hand. Applying iTBS over left MI caused no changes in tactile performance either on d2 of the right hand (1.59 ± 0.09 mm before iTBS vs. 1.60 ± 0.11 mm after iTBS) or on d2 of the left hand (1.79 ± 0.14 mm before iTBS vs. 1.80 ± 0.13 mm), indicating that iTBS over left MI did not affect tactile performance (see Fig. 4).

Effect of iTBS over SI on basic motor performance

Apart from measuring tactile performance, basic motor performance (grip force, tapping and aiming) was additionally tested in the same group ($n = 9$) of participants in counterbalanced order to test whether iTBS application over SI also leads to behavioral gains in basic motor tasks. We choose these motor tasks because they provide information about important features of simple motor performance such as maximum force, speed and accuracy.

Interestingly, no significant changes could be observed for the right hand, indicating that iTBS over SI did not

affect motor performance for the selected tasks [grip force, rmANOVA with factor SESSION (pre vs. post, right hand): $F_{(1,8)} = 1.229$; $P = 0.300$; tapping, rmANOVA with factor SESSION (pre vs. post, right hand): $F_{(1,8)} = 0.770$; $P = 0.406$; aiming, rmANOVA with factor SESSION (pre vs. post, right hand): $F_{(1,8)} = 0.370$; $P = 0.560$].

Effect of iTBS over SI on short-latency paired-pulse SEP components

In order to explore the nature of possible cortical changes that might develop in parallel to perceptual alterations we performed paired-pulse somatosensory evoked potential recordings (paired-pulse SEP) before and after iTBS using electrical median nerve stimulation of the right and left hand (cf. Ragert et al. 2004). Prior to iTBS, we found the typical paired-pulse inhibition within left and right SI. The amplitude of the first N20/P25 response complex (A1) was substantially larger than that evoked by the second stimulus (A2) (7.24 ± 1.47 μV (A1) vs. 4.28 ± 1.25 μV (A2) for left SI, paired *t* test: $P < 0.001$; 8.33 ± 1.83 μV (A1) vs. 4.67 ± 1.16 μV (A2) for the right SI, paired *t* test: $P = 0.004$) (Figs. 5, 6). The mean paired-pulse ratio (the amplitude of the second response amplitude normalized to the first amplitude (A2/A1)) of the N20/P25 complex was 0.52 ± 0.04 (left SI) and 0.54 ± 0.04 (right SI), indicating a paired-pulse inhibition of 47.10 ± 0.32% for the left SI and 45.83 ± 4.70% for the right SI (paired *t* test: $P = 0.79$). Reassessment of paired-pulse inhibition after iTBS revealed a significant reduction of the paired-pulse inhibition indicative of facilitation of cortical excitability only ipsilateral to iTBS (8.6 ± 1.96 μV (A1) vs. 6.48 ± 1.65 μV (A2) for left SI, paired *t* test: $P = 0.02$; see Figs. 5 and 6 for single subject data). Paired pulse inhibition was reduced by

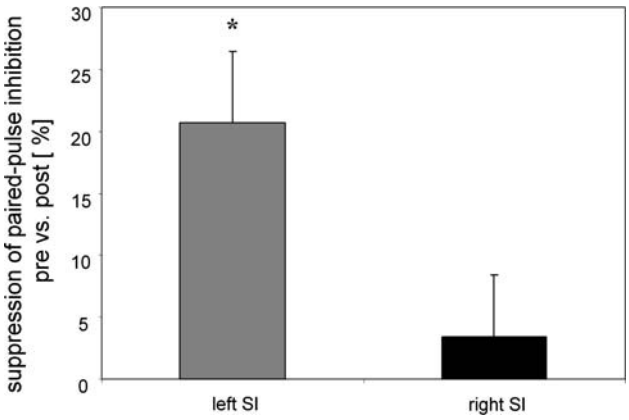

Fig. 5 Paired-pulse inhibition of the N20/P25 component as revealed by SEPs recorded in left and right SI before and after iTBS application over left SI. We found a significant suppression of paired-pulse inhibition after iTBS on the ipsilateral (left SI) but not on the contralateral (right SI) side. *Asterisks* indicate a $P \leq 0.05$

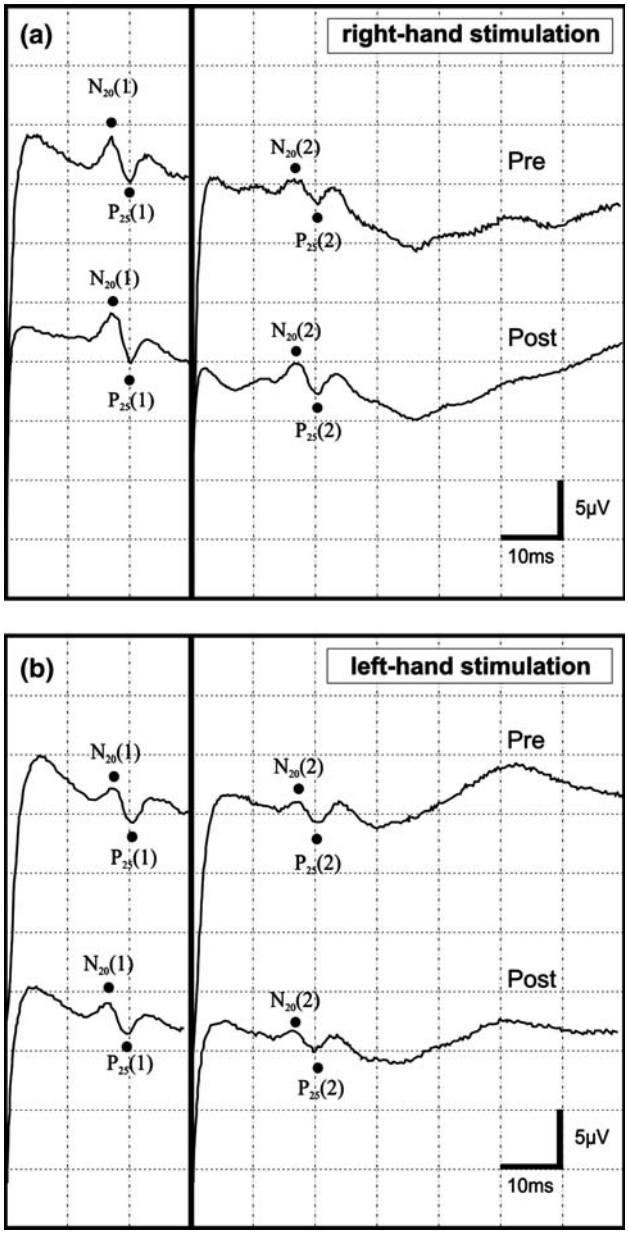

Fig. 6 Paired pulse behavior as revealed by single subject SEP recordings in SI over the left (**a**) and right (**b**) SI index-finger representation before (pre) and after (post) iTBS. Time and amplitude scales are given. Shown are the first (*1*) and second (*2*) N_{20}–P_{25} response components (*black dots*) following median nerve stimulation. Interstimulus interval of the paired median nerve stimulation was 30 ms

20.67 ± 6.07% for left SI (pre vs. post comparison, paired *t* test: $P = 0.009$) and by 1.68 ± 4.30% for right SI (pre vs. post comparison, paired *t* test: $P = 0.72$; paired *t* test left vs. right SI: $P = 0.03$). A linear correlation analysis (Pearson correlation) revealed no relationship between performance improvements (post-pre) and changes in paired-pulse inhibition ($r = 0.308$; $P = 0.419$). In general, latencies of the N20/P25 complex before and after iTBS did not differ. No

paired-pulse inhibition was found for the amplitude of the N20 response before and after iTBS [pre vs. post comparison right IF (A1/A2 ratio), paired *t* test: $P = 0.28$].

Discussion

Although there is evidence that conventional rTMS is capable of evoking transient changes in tactile performance (Knecht et al. 2003; Ragert et al. 2003; Satow et al. 2003; Tegenthoff et al. 2005), which is paralleled by changes in cortical somatosensory maps (Tegenthoff et al. 2005) the effect of iTBS over SI has so far not been investigated. Additionally, the effect of iTBS application on remote cortical areas is not known. To our knowledge, this is the first report testing the efficacy of iTBS application over the primary somatosensory cortex (SI) to induce tactile performance changes and changes in cortical sensory physiology. The aim of the present study was to examine two crucial issues that might improve our understanding of the effectiveness and local specificity of iTBS application: (a) is iTBS application over SI able to induce performance improvements that are paralleled by changes in cortical excitability and (b) does iTBS application over SI affect manual motor control.

In summary, the present study demonstrates that a brief period (190 s) of iTBS applied over the hand representation of left SI can induce perceptual changes as expressed by an improvement in tactile 2-point discrimination performance on d2 of the right hand.

On a cortical level, we found that excitability is altered in the stimulated SI as indicated by a suppression of the paired-pulse inhibition evoked by paired median nerve stimulation. In order to obtain information about the time course of iTBS-induced perceptual changes on d2 of the right hand, the assessment of two-point discrimination thresholds was repeated 30, 60 and 90 min after termination of iTBS. We found that tactile acuity changes persisted for at least 30 min after termination and recovered to baseline performance 90 min after termination of stimulation. In a previous study from our group, we found an improvement in tactile discrimination thresholds after two sessions of 5 Hz rTMS (duration approx. 10 min for each session) that outlasted the time of stimulation by a factor of approximately six. In contrast, iTBS application over SI with much lower stimulation time induced a significant lowering of tactile discrimination threshold that outlasts the time of stimulation (190 s) by at least a factor of 10. Depending on the number of transcranial magnetic pulses, after-effects in cortical excitability for iTBS application over MI could be observed for up to 1 h while the stimulation duration did not exceed more than 190 s (Huang et al. 2005).

Local specificity of iTBS-induced behavioral effects

Although there is evidence for far-reaching TMS-induced alterations of cortical processing (Schambra et al. 2003), high-frequency (5 Hz) rTMS in the somatosensory system seemed to provoke rather local and specific perceptual changes (Tegenthoff et al. 2005). To show that the iTBS-induced perceptual changes were similarly spatially specific to the stimulated cortical area, we performed a number of control experiment. For example, we found that tactile discrimination thresholds on d2 of the left hand ipsilateral to iTBS were unaffected. However, d4 of the right hand showed a similar improvement in performance relative to d2 while not affecting the opposite hemisphere. Furthermore, the application of iTBS over the tibialis anterior muscle representation within MI ("control site") revealed no changes in tactile discrimination behavior, either on the right, or on the left d2. These results are in line with a previous study using 5 Hz rTMS (Ragert et al. 2003; Tegenthoff et al. 2005) and provide further evidence that transcranial magnetic stimulation applied to a remote location outside SI results in no recordable changes in tactile performance.

Many studies in animals and humans provided intriguing evidence that the strict division between somatosensory and motor cortices might be less distinct than previously thought. For example, several imaging studies demonstrated activation of the somatosensory cortex during the execution of a motor task (Samuel et al. 1998; Andres et al. 1999). Furthermore, it has been shown that motor learning (Schwenkreis et al. 2001; Pleger et al. 2003b) can induce meaningful changes in associated cortical maps of the somatosensory cortex.

More recently, Pleger and colleagues demonstrated that 5 Hz rTMS over SI is able to induce a reconfiguration of activity patterns in the sensorimotor network, comprising the stimulated region (SI) and ipsilateral motor cortex (Pleger et al. 2006). Based on these findings, we hypothesized that iTBS application over SI could have changed motor cortex processing thereby evoking effects on certain aspects of motor performance. Therefore, we conducted an additional set of experiments where we tested the effect of iTBS over SI on basic motor performance (tapping, aiming, and grip force). Interestingly, we found no significant changes in performance either for the tapping task, or for aiming or grip force measures. However, as seen in Fig. 5, subjects showed some improvements, which, however, were not significant. It remains to be clarified if these changes were in fact due to the iTBS application, or alternatively resulted from simply practicing the task. One obvious explanation for the lack of behavioral effects in the motor domain might be, although not tested in this study, that the core effect of iTBS application over SI did not lead to a sufficient depolarization of cortical neurons in MI in

order to induce functional reorganizational changes that could be associated with improved motor behavior. In general, our findings do not rule out that iTBS over SI did not exert any effects on MI.

Cortical effects of iTBS over SI

For the quantification of iTBS-induced cortical excitability changes we used paired-pulse SEP recordings to study changes in the recovery functions in left (stimulated) and right SI. We found a significantly reduced paired-pulse inhibition for the left SI pointing to an enhanced iTBS-induced cortical excitability. Because we have no consistent data on possible changes of the P14/15 component the possibility remains that iTBS-induced excitability changes in SI have been generated subcortically, or that there is a subcortical contribution. However, analysis of our data revealed that the paired-pulse ratio (A1/A2) of only the N20 component, which is known to be generated in pyramidal cells in layer 4 (Allison et al. 1991) of area 3b in SI, remained unchanged after iTBS application. In contrast, the N20/P25 complex, which is generated in superficial and middle cortical layers of area 3b (Allison et al. 1991) showed a significant paired-pulse inhibition. The differential modulation of the N20 and the N20/P25 complex (see above) renders a subcortical origin of intracortical inhibition within SI and its modulation after iTBS rather unlikely. In support of this notion, enhanced excitability in SI has been shown after paired-associative stimulation (PAS) intervention with the absence of amplitude changes of the P14 component, indicating that PAS did not induce SEP changes arising at a subcortical level (Wolters et al. 2005).

In comparison to the iTBS-treated site, we found no effects on paired-pulse inhibition on the contralateral hemisphere. More importantly, we also found no performance changes on the left index-finger (IF) represented by SI contralateral to iTBS. This lack of contralateral effects provides evidence that iTBS-induced effects within the somatosensory system are rather confined and focused instead of affecting distant interconnected sites in the brain.

In contrast, several studies in the human motor domain have successfully used conventional rTMS protocols to reveal changes in corticospinal excitability within the opposite non-stimulated hemisphere (Wassermann et al. 1998) or more remote sites in the brain that are not directly stimulated (Siebner et al. 2000; Munchau et al. 2002). One explanation for the lack of iTBS-induced effects on the contralateral hemisphere might be that the threshold for producing effects at more distant sites requires higher intensities of stimulation (for review see Siebner and Rothwell (2003)). For example, in the hand area of motor cortex, corticospinal neurons seem to have a slightly lower threshold than transcallosally projecting neurons (Hanajima et al. 2001). Hence, it is conceivable to assume that the effect of iTBS in SI might be limited to the stimulated cortical area when applied with relatively low stimulus intensities but might spread to interconnected areas like SI contralateral to iTBS at high intensities. In fact, theta burst stimulation in the present study was applied using much lower intensity bursts (80% AMT) in comparison to conventional rTMS studies.

Previous findings revealed that iTBS application resulted in an increase in cortical excitability within MI as indicated by an increase in MEP size (Huang et al. 2005, 2007)and, at the same time, an increase in short-interval intracortical inhibition (SICI). ITBS-induced after-effects have been recently demonstrated to be NMDA receptor dependent and therefore are likely to involve LTP-like changes in the motor cortex (Huang et al. 2007).

By contrast, in the present study, applying the same intervention (iTBS) over SI resulted in a decrease of inhibition, as indicated by a reduction of paired-pulse suppression within the stimulated SI.

However, paired-pulse TMS-evoked SICI in MI and paired-pulse SEP suppression in SI cannot be directly compared. SICI is known to be mediated by $GABA_A$ receptors (Ziemann et al. 1996; Ilic et al. 2002), whereas the pharmacological basis of paired-pulse SEP suppression is not well investigated and might also rely on different neuronal circuits and might not be generated in the same cortical layers. It has been demonstrated that patients with a deficiency in GABAergic inhibition, such as focal hand dystonia (FHD) and myotonic dystrophy, show a reduction in paired-pulse SEP suppression at short interstimulus intervals ranging from 1 to 40 ms (Frasson et al. 2001; Mochizuki et al. 2001). We are therefore confident that paired-pulse SEP suppression reflects the activity of inhibitory circuits within SI. It remains to be clarified whether paired-pulse SEP suppression is mediated by $GABA_A$ or $GABA_B$ interneurons. Whatever the responsible mechanisms of the iTBS-induced paired-pulse SEP suppression are, based on the above mentioned findings, we conclude that SI is disinhibited or hyperexcitable after iTBS application.

Furthermore, high-frequency rTMS applied over M1 has been consistently shown to decrease paired-pulse TMS evoked SICI (for review see Fitzgerald et al. (2006)). These findings are in line with the current data. On the contrary, Huang et al. (2005) argued that iTBS over MI enhances activity in both excitatory and inhibitory circuits, but still their results are difficult to reconcile with the current rTMS literature.

In summary, our results provide compelling evidence that stimulation protocols resembling those used in slice preparations of brain tissue to induce LTP can also have meaningful behavioral effects on tactile perception in human subjects. Using short bursts of high-frequency

(50 Hz) rTMS pulses we could demonstrate that an improvement of tactile perception is associated with a disinhibition or hyperexcitability within SI. Therefore, our findings support the notion that iTBS application might be a promising technique in neurorehabilitation to modulate brain dysfunction after injury such as in chronic stroke patients. For example, TBS application might help to facilitate functional retraining after a loss in touch sensation that might occur in stroke patients.

Based on the information available in the literature it still remains to be determined whether iTBS has a greater likelihood for larger potential therapeutic effect in patients relative to conventional rTMS application. Although the behavioral outcome in the present study after 190 s of iTBS application are relatively short-lasting (30 min) one possible way to reach a more prominent behavioral and cortical effect might be a repeated application over several days or a combination of iTBS with available learning paradigms.

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