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Behavioural and neurophysiological markers reveal differential sensitivity to homeostatic interactions between centrally and peripherally applied passive stimulation

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Abstract

Repetitive transcranial magnetic stimulation (rTMS) is an effective tool for inducing functional plastic changes in the brain. rTMS can also potentiate the effects of other interventions such as tactile coactivation, a form of repetitive stimulation, when both are applied simultaneously. In this study, we investigated the interaction of these techniques in affecting tactile acuity and cortical excitability, measured with somatosensory evoked potentials after paired median nerve stimulation. We first applied a session of 5-Hz rTMS, followed by a session of tactile repetitive stimulation, consisting of intermittent high-frequency tactile stimulation (iHFS) to a group of 15 healthy volunteers ("rTMS + iHFS" group). In a second group ("rTMS w/o iHFS"), rTMS was applied without iHFS, with a third assessment performed after a similar wait period. In the rTMS w/o iHFS group, the 5-Hz rTMS induced an increase in cortical excitability that continued to build for at least 25 min after stimulation, with the effect on excitability after the wait period being inversely correlated to the baseline state. In the rTMS + iHFS group, the second intervention prevented the continued increase in excitability after rTMS. In contrast to the effect on cortical excitability, rTMS produced an improvement in tactile acuity that remained stable until the last assessment, independent of the presence or absence of iHFS. Our results show that these methods can interact homeostatically when used consecutively, and suggest that different measures of cortical plasticity are differentially susceptible to homeostatic interactions.

Introduction

The importance of previous brain activity in shaping the effect of an intervention designed to induce neural plasticity is becoming increasingly recognized. This shaping arising from the previous history of activity is usually interpreted in terms of homeostatic plasticity, which is supposed to provide the mechanisms for maintaining synaptic strength within a functionally relevant range. Within this context, the phenomenon of metaplasticity, i.e. a higher-order form of plasticity where the previous history of activity produces a change in the direction or magnitude of subsequent activity-dependent plasticity (Pérez-Otaño & Ehlers, 2005), has been extensively studied both *in vitro* and *in vivo*. Many researchers have attempted to elucidate how metaplasticity mechanisms influence the results of various interventions (Abraham & Bear, 1996; Abraham & Tate, 1997; Abraham, 2008). In practice, it is impossible to control the rate of neural activity of human subjects in a natural setting; therefore, a commonly utilized experimental approach consists of applying two interventions in sequence, where the first intervention (often called 'priming' or 'conditioning') constitutes the 'previous history', which can be directly observed and manipulated. Priming often does not itself produce observable changes, which is, however, not a defining feature of priming. Indeed, it is recognized that plastic changes in excitability are probably always accompanied by metaplasticity processes that will alter the effect of an intervention on a system that has already been stimulated, even if the first intervention itself also produced changes (cf. Lang *et al.*, 2004; Siebner *et al.*, 2004; Müller *et al.*, 2007).

Combinations of different stimulation methods such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) have also been shown to interact in a complex fashion. In one study, facilitative pre-conditioning with anodal tDCS enabled a subsequent application of low-intensity repetitive transcranial magnetic stimulation (rTMS) to the primary motor cortex (which had no effect when applied alone) to reduce corticospinal excitability to below-baseline levels. Conversely, inhibitory pre-conditioning with cathodal tDCS resulted in rTMS increasing corticospinal excitability (Siebner *et al.*, 2004). In another study, priming with facilitative anodal tDCS boosted the increase in cortical excitability produced by paired-associative stimulation (PAS), whereas inhibitory cathodal tDCS inverted the effect of PAS, causing PAS to produce inhibition when applied after the cathodal tDCS (Nitsche *et al.*, 2007). However, when both anodal tDCS and PAS were

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applied simultaneously, they interacted homeostatically, eliciting a decrease in excitability.

In the present study, we examined the interaction between a cortical and a peripheral stimulation method, when applied sequentially. Both methods alone are effective in producing plastic changes. rTMS (5 Hz) applied to the somatosensory cortex (SI) increases cortical excitability, as indicated by reduced paired-pulse suppression of the median nerve somatosensory evoked potential (SEP) (Ragert *et al.*, 2004), as well as improving tactile acuity of the index finger when applied on the approximate area of its cortical representation (Tegenthoff *et al.*, 2005). Intermittent high-frequency stimulation (iHFS), a form of repetitive peripheral tactile stimulation of the index finger, is similarly effective in improving tactile acuity (Ragert *et al.*, 2008) and, as we show here, also increases cortical excitability.

Ragert *et al.* (2003) demonstrated that rTMS and peripheral tactile stimulation can interact when applied simultaneously, with one potentiating the other's effect on tactile acuity, although their results suggested a potential ceiling limit to the combined effect, or a possible homeostatic mechanism controlling the possible range of plastic alterations. In this study, we aimed to investigate the extent to which these two interventions (rTMS and tactile iHFS) would interact when applied consecutively. Additionally, we sought to determine if two kinds of parameters, behavioural and neurophysiological, are affected by the interaction in similar ways.

Materials and methods

Subjects

We tested three groups, each with 15 subjects, who were all right-handed (20 females, aged 20–28 years; mean age, 24 years). Subjects gave their written informed consent prior to participating. The study protocol was approved by the local ethics committee of the Ruhr-University Bochum, and the project protocol was performed in accordance with the Declaration of Helsinki.

Median nerve somatosensory evoked potentials

To study changes in cortical excitability, we applied a paired-pulse protocol consisting of paired electrical median nerve stimulation, with an interstimulus interval (ISI) of 30 ms. Stimulation of the median nerve was selected in order to establish a link between the SEP recordings and the cortical representation of the right index finger selected for the two stimulation protocols (rTMS and iHFS). Nerve stimulation was performed using a block electrode placed on the wrist (pulse duration, 0.2 ms; repetition rate of the paired stimuli, 2 Hz; ISI between paired stimuli, 30 ms). The median nerve stimulation intensity was set at the motor threshold, defined as the intensity at which a visible contraction of the thenar muscles was detected, and was kept constant for each subject throughout the experiment. Subjects were asked to report a prickling phenomenon in the thumb, index and middle fingers of the stimulated hand in order to verify correct positioning of the stimulating block electrode. During median nerve stimulation and SEP recordings, subjects were seated in a comfortable chair, and were instructed to relax and to stay awake, with their eyes closed. SEPs were recorded and stored for offline analysis using a Schwarzer 8 apparatus (bandpass filter 2–2000 Hz). Paired-pulse SEP recordings were made using a two-electrode array. One electrode was located over the SI, 2 cm posterior to the C3 position (C3′), according to the International 10–20 system. A reference electrode was placed over the midfrontal (Fz) position. Electrical potentials were recorded in epochs from 0 to 200 ms after the stimulus. A total of 200 stimulus-related epochs were recorded for each measurement. Latencies and the peak-to-peak amplitude of the N20-P25 response component, which is assumed to be generated in the SI, were measured and compared before and after each intervention. In addition to an analysis of the raw amplitude data, paired-pulse suppression was expressed as a ratio of the amplitude (P2/P1) of the second peak (P2) over the amplitude of the first peak (P1) (Fig. 1).

Two-point discrimination

Tactile two-point discrimination of the index fingers was assessed using a method of constant stimuli, as described previously (Godde *et al.*, 2000; Pleger *et al.*, 2001; Dinse *et al.*, 2003b). We used a specifically designed apparatus that allows a standardized and objective form of testing. In brief, seven pairs of rounded needle probes (diameter 200 μm), with separation distances between 0.7 and 2.5 mm in 0.3-mm steps, were used. Each distance was presented eight times in a randomized order, resulting in 64 single trials per session. Subjects were aware that there were single needle-probe stimuli presented, but not how often they would be presented. As a control, zero distance was tested using only a single needle probe. The number of single-needle presentations was 1/8, i.e. eight presentations in one session.

The probes were mounted on a rotatable disc that allowed for rapid switching between distances. To accomplish a uniform and standardized stimulation, the disc was installed in front of a plate that could be moved up and down. The arm and fingers of subjects were fixed on the plate, which was moved up and down by the experimenter. The down movement was arrested by a stopper at a fixed position above the probes (Fig. 2A). The test finger (index finger, or d2) was held in a hollow containing a small hole (diameter, 15 mm), through which the distal phalanx of the finger came to touch the probes, at approximately the same indentations in each trial. The probes were always presented parallel to the fingertip. Subjects had to decide immediately

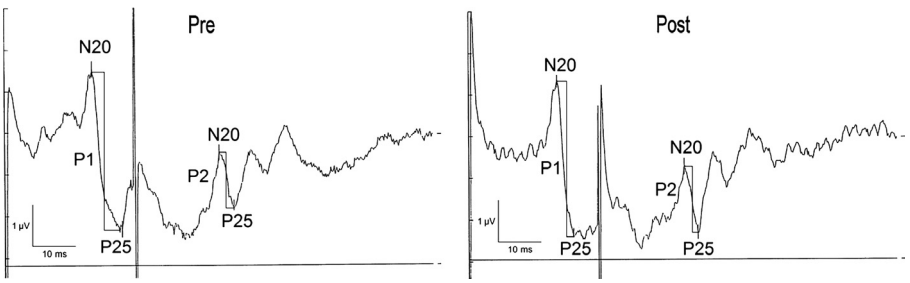

FIG. 1. Paired-pulse median nerve somatosensory evoked potentials. The N20-P25 component was measured from peak to peak. In addition to the analysis of raw amplitudes, the PPR was calculated dividing the amplitude of the second response by that of the first (P2/P1). The curve on the left represents the baseline condition (pre). The curve on the right (post) shows an increase in the amplitude of P2 after rTMS raising the PPR from 0.35 to 0.43.

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after touching the probes whether they had the sensation of touching one or two tips, simply by answering 'one' or 'two'. After each session, individual discrimination thresholds were calculated. The summed subject's responses ('1' for one tip and '2' for two tips) were plotted against the tip distance as a psychometric function, and were fitted with a logistic regression method (SPSS version 10.01). Thresholds as a marker for individual tactile performance were defined as the point at which a 50% correct response rate was obtained (Fig. 2B). In addition to analysing the two-point discrimination thresholds, we calculated the signal detection d′ index to control for response bias, which we report together with false alarm and hit rates.

Repetitive transcranial magnetic stimulation

A MAGSTIM Rapid Stimulator (Magstim, Whitland, Dyfed, UK) connected to a figure-of-eight-shaped coil was used for application of rTMS. During the rTMS sessions, subjects were seated in a comfortable chair, and were instructed to keep their eyes closed and try to relax. Subjects wore a tight-fitting cap with a 1-cm grid, referenced to the vertex. First, the subject's resting motor thresholds were measured at the relaxed first dorsal interosseous muscle of the right hand using surface silver–silver electrodes and single TMS pulses. While searching the cortical first dorsal interosseous muscle representation, TMS stimuli were presented within a 1 × 1-cm array, 5 cm lateral from the vertex. The first dorsal interosseous muscle "hot spot" was identified at the scalp position where TMS induced the highest amplitude motor evoked potentials (MEPs). The resting motor threshold was defined as the lowest intensity capable of evoking five out of 10 MEPs with an amplitude of at least 50 μV in the relaxed muscle. Next, the coil was positioned as close as possible to the right index finger representation in the primary SI as previously described (Ragert *et al.*, 2003, 2004; Tegenthoff *et al.*, 2005). For that purpose, from the "hot spot" of the contralateral first dorsal interosseous muscle, we moved the magnetic coil 2 cm posterior in the parasagittal direction. When stimulating this point, many subjects reported a sensation in an area of the hand and/or finger mostly including the index finger. After identifying the approximate location of the right index finger representation, the position of the figure-of-eight-shaped coil was fixed. This location is denoted as "SI right index finger" hereinafter. The rTMS intensity was set at 90% of the resting motor threshold. Although the focus of stimulation was clearly remote from the primary motor cortex, direct or indirect influences from primary motor cortex activation cannot be ruled out.

For rTMS, 50 trains of TMS pulses were applied through the tangentially oriented coil grip. A single train consisted of 50 single pulses of 5 Hz lasting 10 s, with an intertrain interval of 5 s. Five consecutive trains were grouped into one block. Between each block was a rest period of 1 min. The total stimulation time was 20 min and 40 s.

High-frequency tactile stimulation

The iHFS protocol was carried out as described by Ragert *et al.* (2008). iHFS consisted of tactile stimuli (10-ms duration) applied to the distal phalanx of the right index finger (d2). The pulse trains required to drive the stimulators were stored digitally, and played back via an MP3 player, allowing unrestricted mobility of the subjects during the stimulation period. To apply iHFS, a small solenoid (diameter, 8 mm) was taped to the tip of the right index finger, and transmitted the tactile stimuli of the iHFS protocol to the skin. Stimulation trains consisted of 20 single pulses with a frequency of 20 Hz for 1 s, with an intertrain interval of 5 s. The duration of stimulation was 20 min, resulting in a total of 4000 pulses.

Experimental schedule

We studied three experimental groups (Fig. 3). In all cases, initial assessment consisted of measuring two-point discrimination thresholds followed by recordings of paired SEPs after median nerve stimulation. rTMS was then applied in two of the three groups (Group 1, rTMS + iHFS; Group 2, rTMS w/o iHFS), whereas in the third group iHFS alone was applied instead. After this first intervention session, the tactile discrimination and SEP recordings were reassessed. After this second assessment, tactile iHFS was applied to Group 1 for 20 min, whereas in Group 2 a wait period was allowed to pass before the third assessment, but without applying the iHFS protocol. Then, in a third assessment, discrimination thresholds and SEPs were again recorded. The total time between the second and third assessments was approximately 25 min. In Group 3 only the iHFS protocol was applied. Two-point discrimination thresholds for each subject were measured once during the second and third assessment, but measured three times at the baseline assessment. This was to familiarize subjects with the discrimination tasks and to obtain a stable baseline performance.

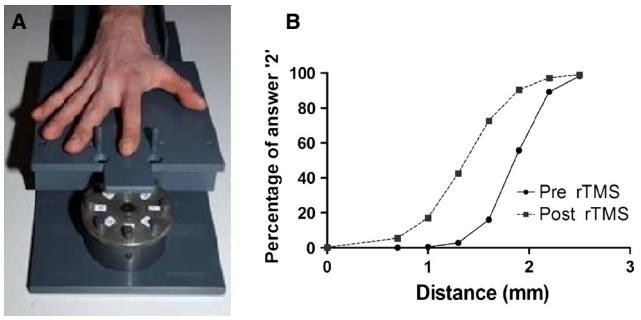

FIG. 2. (A) Pairs of needles separated by different distances were mounted on a rotating disc and presented to the tip of the index finger. (B) Psychometric curves built on the frequency with which the subjects report touching "two" needles. The probability of recognizing two needles as separate (Y axis) increases with greater distance between the needles (X axis). The solid line was obtained from an individual subject at baseline (pre). An improvement in tactile acuity after rTMS (dashed line) is represented by a shift towards smaller separation distances.

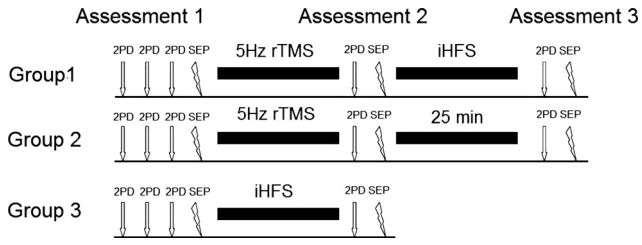

FIG. 3. Schematic representation of the experimental procedure. After a baseline assessment of two-point discrimination (2PD) and paired-pulse median nerve somatosensory evoked potentials, Groups 1 and 2 were treated with the same 5-Hz rTMS stimulation protocol. Following this, measurements were repeated. Subsequently, one group received iHFS, whereas the other received no further interventions. After an equivalent length of time for both groups, a final measurement of tactile discrimination and cortical excitability was made. In Group 3, after the initial measurements, iHFS alone was applied before a post-stimulation assessment. SEP, recording of paired-pulse SEPs.

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Data analysis

All statistical analyses, apart from calculation of two-point discrimination thresholds, were performed using Graphpad Prism v 5.0. All data are expressed as mean ± SEM. The change in SEP amplitude for P1 and P2, as well as the paired-pulse ratio (PPR) between the different time points, was tested with a one-way repeated-measures (RM)-ANOVA for Groups 1 and 2. The effect of iHFS alone on the PPR (Group 3) was tested with a paired Student's t-test.

In order to compare differences in the responses elicited by rTMS and iHFS between Groups 1 and 2, the ratios were normalized to the baseline condition, with the baseline value being expressed as 1. Data were analysed using a two-way ANOVA, using 'Time' (each of the three SEP measurements) as the within-subjects factor, and 'Group' (with or without iHFS) as the between-subjects factor. The same analyses were repeated to test the effect of rTMS/iHFS on two-point discrimination.

In order to investigate correlations between changes in the PPR across conditions, we used a Pearson correlation analysis plotting the change in the PPR for each subject between different conditions vs. the PPR in the baseline condition. These changes were expressed as percentage changes relative to the baseline PPR. The change in the PPR measured immediately after rTMS plotted against the baseline ratio assessment was denoted as 'Δ rTMS – baseline', and the PPR measured after iHFS in the rTMS + iHFS group, or after a 25-min wait period in the rTMS w/o iHFS group plotted against the baseline ratio assessment was denoted as 'Δ last – baseline'. In addition, to look for a possible correlation between changes in cortical excitability and tactile acuity, changes in the PPR were plotted against changes in two-point discrimination.

Results

Increased cortical excitability after repetitive transcranial magnetic stimulation and tactile intermittent high-frequency tactile stimulation

Comparison of the normalized PPRs of the rTMS + iHFS and rTMS w/o iHFS groups with two-way ANOVA (Fig. 4A) showed a significant interaction between the factors 'Time' and 'Group' ($F_{2,28}$ = 4.02, P = 0.02). The factor Time was itself statistically significant ($F_{2,28}$ = 16.47, P < 0.0001), whereas the factor Group was not ($F_{1,28}$ = 1.33, P = 0.25). Post-hoc comparison of the two groups showed a significant difference only in the last condition, i.e. after iHFS for 25 min (Bonferroni post-test, t = 2.83, P < 0.05, corrected for multiple comparisons).

The rTMS applied at 5 Hz for 20 min to the primary SI produced an increase in the averaged PPR. In the group that received only rTMS (Group 2), the PPR increased from a baseline level of 0.41 ± 0.04 to 0.53 ± 0.04, which represented a 29% increase from baseline. After a wait period without further intervention, there was a further increase to 0.67 ± 0.06, a 63% increase from baseline (RM-ANOVA, $F_{2,14}$ = 12.63, P = 0.0001). A post-hoc test between the second and third assessment showed that the increase was statistically significant (Bonferroni post-test, t = 2.7, P < 0.05). For the group that received rTMS + iHFS (Group 1), there was an increase in the PPR from a baseline of 0.42 ± 0.04 to 0.59 ± 0.098 (40% increase). In contrast to Group 2, rTMS followed by a second intervention of iHFS resulted in a decrease of the PPR to 0.55 ± 0.05 (RM-ANOVA, $F_{2,14}$ = 4.49, P = 0.02). A post-hoc test between the second and third assessment showed no statistically significant difference (Bonferroni post-test, t = 0.62, P > 0.05). Application of iHFS alone (Group 3) increased the PPR from a baseline value of 0.54 ± 0.03 to 0.63 ± 0.03 (17% increase, paired t-test, t = 5.7, P < 0.0001) (Fig. 4B).

Analysis of the amplitude of the first (P1) and second (P2) peaks revealed that, in all cases, the changes were dependent on the amplitude of P2. In Group 1, one-way RM-ANOVA revealed no change in the amplitude of P1 (RM-ANOVA, $F_{2,14}$ = 1.01, P = 0.38), whereas there was a significant increase in the amplitude of P2 (RM-ANOVA, $F_{2,14}$ = 5.3, P = 0.01). In Group 2, a similar pattern was found (RM-ANOVA, $F_{2,14}$ = 0.58, P = 0.56 for P1; $F_{2,14}$ = 7.98, P = 0.002 for P2). The same was found for Group 3 (paired t-test, t = 0.17, P = 0.86 for P1 and t = 2.54, P = 0.02 for P2) (Fig. 5).

The effect of repetitive transcranial magnetic stimulation on the paired-pulse ratio depends on baseline excitability

In order to discover if the effects of rTMS and iHFS depend on the baseline state of excitability, we performed a Pearson correlation

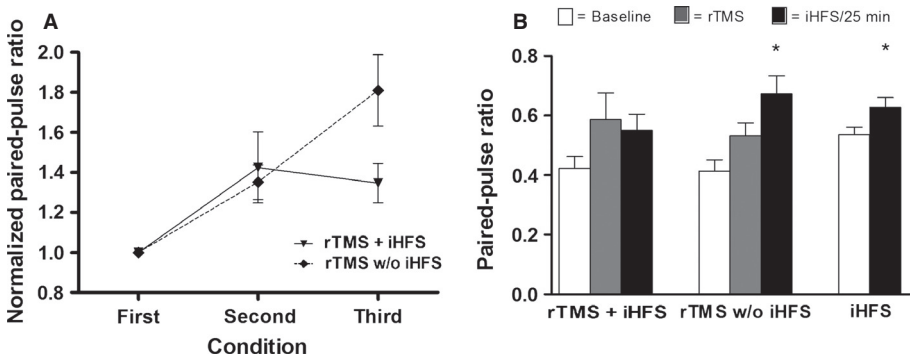

FIG. 4. (A) Normalized PPR. In order to compare the two groups, the data were normalized for each subject with respect to the first ratio (baseline condition), which was expressed as '1'. Two-way ANOVA showed a significant effect of the factor 'Time' ($F_{2,28}$ = 16.47, P < 0.0001) but not Group ($F_{1,28}$ = 1.33, P = 0.25). There was, however, a statistically significant interaction between the two factors ($F_{2,28}$ = 4.02, P = 0.02). Post-hoc comparison of the two groups showed a significant difference only in the last condition, i.e. after iHFS/25 min (Bonferroni post-test, t = 2.83, P < 0.05, corrected for multiple comparisons). (B) Changes in the PPR. In Groups 1 and 2, rTMS produced an initial increase in the PPR. This was partially reversed by the subsequent application of iHFS, although the overall effect remained significant (RM-ANOVA, $F_{2,14}$ = 4.49, P = 0.02). In the group without iHFS, the increase in the PPR continued to build up (RM-ANOVA, $F_{2,14}$ = 12.63, P = 0.0001) after the stimulation protocol had been completed. Post-hoc analysis showed that the difference between the final ratio and that immediately after rTMS was significantly different in this group (Bonferroni post-test, t = 2.7, P < 0.05). This was, however, not the case for the group that received iHFS (Bonferroni post-test, t = 0.62, P > 0.05). In the iHFS alone group, paired t-test showed a significant increase of the PPR (paired t-test, P < 0.0001), asterisks indicate significance P < 0.05.

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analysis between the baseline PPR and the percentage change after rTMS (Δ rTMS – baseline), and between baseline and the percentage change recorded at the last measurement (Δ last – baseline) for each group separately. After rTMS, there was no correlation between the percentage change in the PPR compared with baseline for either Group 1 (r = −0.2115, $P = 0.3996$) or Group 2 (r = −0.3417, $P = 0.1652$). In contrast, after the wait period (Δ last – baseline), there was a significant negative correlation for Group 2 (r = −0.748, $P = 0.0001$) between baseline ratios and those obtained in the last assessment. In Group 1, this correlation was not significant (r = −0.439, $P = 0.0684$) (Fig. 6). The difference between the two correlation coefficients obtained for each group was tested for significance using a Fisher r-to-z transform test. The difference was not statistically significant in either case, although there was a trend in Group 2 (z = 1.5, $P = 0.13$) that was not present in Group 1 (z = 0.63, $P = 0.52$). The baseline PPR did not correlate with the percentage change in the group that only received iHFS (r = −0.16, $P = 0.57$). Pearson's correlation test showed no relationship between the changes in the PPR and the changes in two-point discrimination in any condition.

Improvement of tactile acuity after repetitive transcranial magnetic stimulation and intermittent high-frequency tactile stimulation

One-way RM-ANOVA comparing the three initial measurements of two-point discrimination used to establish baseline performance, pooling all subjects ($n = 45$), showed no significant difference, thus confirming the stability of performance for each subject (RM-ANOVA, $F_{2,43} = 1.26$, $P = 0.28$).

Groups 1 and 2 showed a significant improvement in tactile acuity after rTMS, which remained essentially unchanged in the last measurement in both cases (i.e. after either iHFS or a 25-min wait period). Comparison of the normalized thresholds with two-way ANOVA showed no interaction between the factors 'Time' and 'Group' ($F_{2,28} = 0.9$, $P = 0.4$). The factor Time was statistically

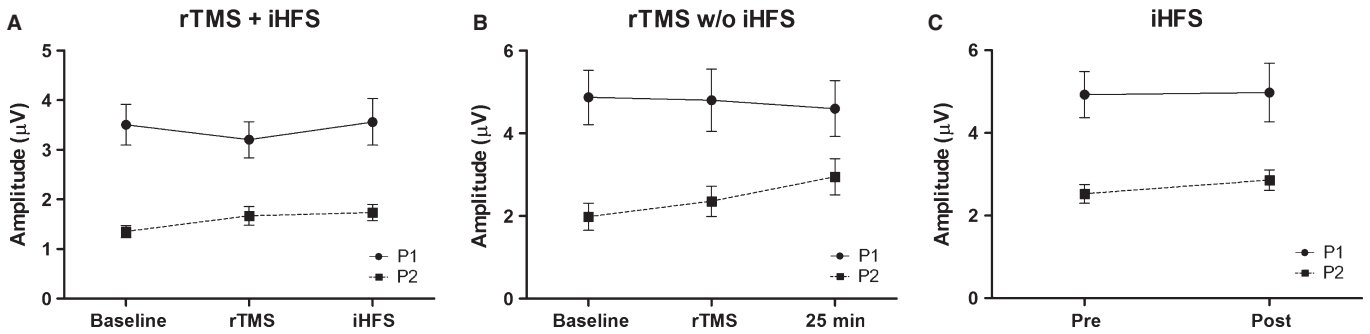

FIG. 5. The amplitudes of the first (P1) and second (P2) peaks were analysed separately for differences between conditions. (A) In the group that received iHFS, one-way RM-ANOVA showed no change in the amplitude of P1 (ANOVA, $F_{2,14} = 1.01$, $P = 0.38$), whereas there was a significant increase in the amplitude of P2 (ANOVA, $F_{2,14} = 5.3$, $P = 0.01$). (B) Similarly, the group without iHFS showed a significant increase in P2 (ANOVA, $F_{2,14} = 7.98$, $P = 0.002$), whereas P1 remained unchanged (ANOVA, $F_{2,14} = 0.58$, $P = 0.56$). (C) In the group that received iHFS alone, there was no change in P1 (paired t-test, $t = 0.15$, $P = 0.8835$), whereas P2 showed a significant increase in amplitude (paired t-test, $t = 2.62$, $P = 0.0199$).

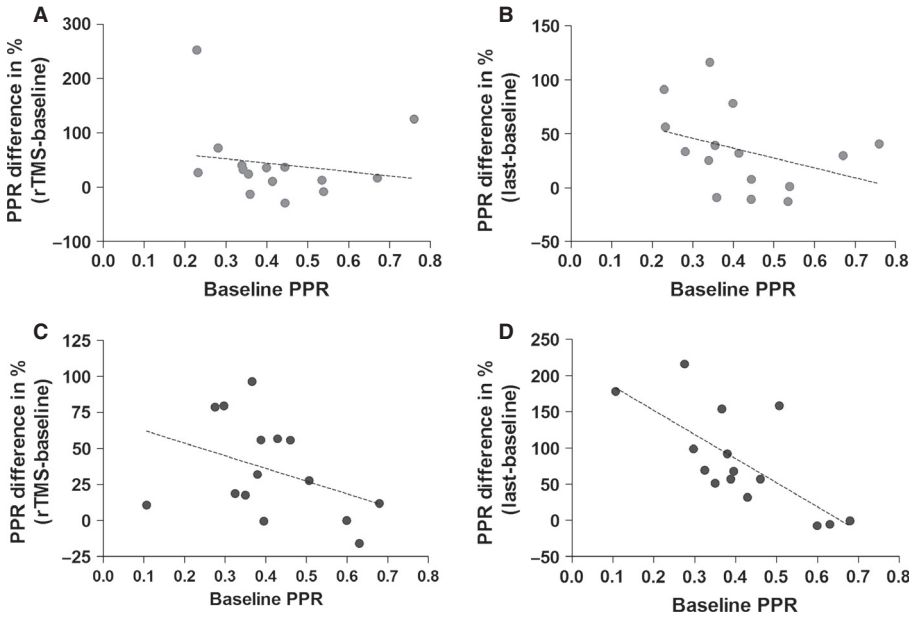

FIG. 6. Changes in the PPR depend on the baseline level of excitability. After rTMS, neither group showed a correlation between changes in the PPR and the baseline state (A and C). At the final measurement, however (Δ last - baseline), there was a significant negative correlation (D) in Group 2 (Pearson's correlation test, r = −0.748, $P = 0.0001$). For Group 1 (B), this correlation fell short of reaching statistical significance (Pearson's correlation test, r = −0.439, $P = 0.0684$).

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significant ($F_{2,28}$ = 25.7, P < 0.0001), whereas the factor Group was not ($F_{1,28}$ = 0.43, P = 0.51).

In Group 1, the two-point discrimination threshold went from a baseline value of 1.58 ± 0.06 mm to 1.34 ± 0.07 mm after rTMS. After the second iHFS intervention, there was a further, non-significant reduction to 1.27 ± 0.05 mm (RM-ANOVA, $F_{2,14}$ = 9.9, P = 0.0005). In Group 2, the threshold for two-point discrimination decreased from a value of 1.69 ± 0.06 mm in the baseline condition to 1.4 ± 0.06 mm after rTMS. After a 25-min wait period, the threshold was 1.46 ± 0.6 mm (RM-ANOVA, $F_{2,14}$ = 16.85, P < 0.0001). In both groups, post-hoc analysis showed that there was no significant difference between the discrimination threshold after rTMS, and that obtained in the final measurement. In Group 3 (Fig. 7), the two-point discrimination threshold decreased from a baseline of 1.55 ± 0.04 to 1.47 ± 0.05 (paired t-test, t = 3.5, P = 0.0021).

Additionally, we calculated the bias-free d′ signal detection index for Groups 1 and 2. Two-way ANOVA showed no interaction between the factors Time and Group ($F_{2,28}$ = 1.3, P = 0.32), a significant effect of Time ($F_{2,28}$ = 4.7, P = 0.01), and no effect of the factor Group ($F_{1,28}$ = 0.7, P = 0.4). This change in d′ was determined by a similar change in the hit rate (two-way ANOVA; interaction, $F_{2,28}$ = 1.72, P = 0.18; Time, $F_{2,28}$ = 14.77, P < 0.0001; Group, $F_{1,28}$ = 0.07, P = 0.8), whereas the false alarm rate remained unchanged (two-way ANOVA; interaction, $F_{2,28}$ = 0.27, P = 0.76; Time, $F_{2,28}$ = 0.12, P = 0.87; Group, $F_{1,28}$ = 1.4, P = 0.25).

Discussion

In the present experiment, we set out to investigate the combined effects of high-frequency rTMS and peripheral iHFS. Immediately after the 5-Hz rTMS, subjects showed a significant increase in cortical excitability as measured by a decrease in intracortical suppression, which continued to build up for at least 25 min after stimulation. When peripheral iHFS was applied, however, this continued increase was prevented. In contrast, rTMS produced an improvement in tactile acuity, which remained stable for at least 25 min after the end of stimulation, and was not affected by the additional application of iHFS.

Paired-pulse suppression of the median nerve somatosensory evoked potential

During the last few years, stimulation with pairs of stimuli in close succession (paired-pulse stimulation) has become a common tool to investigate short-term plasticity. This is a useful technique to investigate changes in, and the balance between, cortical excitation and intracortical inhibition. Paired-pulse suppression describes the phenomenon that, at short ISIs, neuronal responses to the second stimulus are significantly reduced. Paired-pulse suppression is quantified in terms of the ratio of the amplitude of the second response divided by the first. That means that large ratios are associated with reduced paired-pulse suppression, and small amplitude ratios are associated with stronger paired-pulse suppression. The fact that the second response of two stimuli given in short succession is strongly suppressed has often been denoted as a special form of short-term plasticity, which describes changes of neural behaviour resulting from prior activity (Zucker, 1989; Zucker & Regehr, 2002). Paired magnetic stimulation of the human motor cortex is frequently used to characterize different forms of intracortical inhibition and facilitation (Kujirai *et al.*, 1993; Chen, 2004; Di Lazzaro *et al.*, 2005). In these studies, GABAergic interneurons have been suggested as mediators of paired-pulse inhibition. However, the cellular mechanism underlying paired-pulse suppression of SEPs is not yet fully understood. According to *in vitro* studies, GABAergic inhibition appears to also play an important role in paired-pulse suppression (Porter & Nieves, 2004; Torres-Escalante *et al.*, 2004). Höffken *et al.* (2010) reported that, with an ISI of 30 ms, there is no paired-pulse suppression of potentials originating in the cranial medulla, suggesting that, at this ISI, paired-pulse suppression must occur at least at the level of the thalamus or intracortically.

Effects of repetitive transcranial magnetic stimulation and intermittent high-frequency tactile stimulation on cortical excitability

The increase in cortical excitability after the 5-Hz rTMS stimulation was similar for both groups. This finding is consistent with previously published results, where this effect was seen after a similar rTMS application (Ragert *et al.*, 2004). Furthermore, there was a significant further increase in excitability demonstrated in the last measurement for the group that did not receive iHFS. This suggests that there is a time window in which the effect of rTMS on cortical excitability continues to build up, even after stimulation has ceased, before it begins to return to baseline. Similar findings have been reported elsewhere, e.g. Peinemann *et al.* (2004). In their study, 1800 pulses of rTMS applied to the primary motor cortex, also at a rate of 5 Hz, produced an increase in MEP amplitude that continued to build up after the stimulation ceased, as demonstrated by a

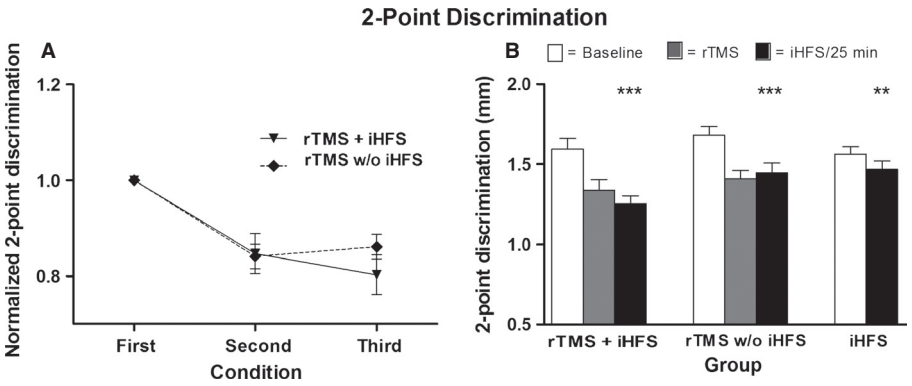

FIG. 7. Effect of rTMS and iHFS on tactile acuity. (A) illustrates normalized 2-point thresholds, (B) shows thresholds in mm. In both groups, rTMS produced an improvement in tactile acuity (RM-ANOVA, $F_{2,14}$ = 9.9, P = 0.0005 for the group with iHFS; $F_{2,14}$ = 16.85, P < 0.0001 for the group without iHFS). After the initial effect of rTMS, the PPR remained essentially stable, without significant changes between the last measurement and that made immediately after rTMS, independent of the application of iHFS, as revealed by Bonferroni's multiple comparisons test, asterisks indicate significance levels (** P < 0.0001, *** P > 0.00001).

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second measurement taken 15 min after the end of the stimulation session. Conceivably, this observation might reflect a common finding in rTMS studies, in which repeated post-stimulation assessments have been performed. The data from Peinemann *et al.* (2004) suggest that the amount of stimulation used might play a crucial role in determining the time course. It is possible that, depending on the stimulation, different populations of neurons are involved, which react with different time courses due to saturation effects. It should be noted that, in *in vitro* synaptic plasticity experiments, which use much higher frequencies (e.g. 100 Hz), typically maximal effects are observed immediately after the stimulation.

In our study, application of iHFS clearly cancelled this further increase in cortical excitability. Both groups exhibited an almost identical increase in excitability immediately after rTMS (Δbaseline – rTMS), but the last measurement (Δbaseline – last) demonstrated a marked difference between them (Fig. 4B).

Other studies have shown such interactions between tTMS stimulation and subsequent interventions. Delvendahl *et al.* (2010) showed that pre-treatment with very low-frequency rTMS at 0.1 Hz inhibits the effects of subsequent PAS, whether in its excitatory or inhibitory form. A further study has described a similar effect of 5-Hz rTMS on the subsequent application of either continuous or intermittent theta burst stimulation (Iezzi *et al.*, 2011). In these two studies, the effects of priming are attributed in one case to "antigating" (Delvendahl *et al.*, 2010) and in the other to another non-homeostatic form of interaction (Iezzi *et al.*, 2011). Our experiment resembles these studies in that 5-Hz rTMS effectively abolished the effect of subsequent iHFS on cortical excitability. However, our study differs in that our "priming" intervention produced a strong effect in excitability, the temporal course of which was altered by subsequent iHFS, in a way that might indicate a homeostatic interaction.

Influence of the baseline state of excitability

In the group without iHFS, the change in paired-pulse suppression seen at the end of the experiment (Δ last – baseline) was strongly dependent on the baseline state of excitability, as demonstrated by a highly significant inverse correlation (Fig. 6D) between the final change in the PPR and the naive state values. Taking this into account, it is possible that normal fluctuations in the population in terms of their state of cortical excitability could explain the observed variability in responses to interventions such as rTMS. The importance of the baseline state of excitability of the brain in shaping the effect of an intervention such as rTMS is becoming increasingly recognized (Silvanto & Pascual-Leone, 2008; Silvanto *et al.*, 2008). Indeed, the main goal of homeostatic plasticity studies is to control this directly by means of a 'priming' stimulus, as opposed to letting it vary normally, so as to optimize any effect of an intervention protocol (Fricke *et al.*, 2011).

The correlation between the change in the PPR and baseline state was not evident in the measurement taken immediately after rTMS, although the average increase in the PPR even at that point was statistically significant. This is notable as it indicates that the influence of the baseline state of excitability on the response to rTMS is not present immediately after the stimulation has ended, but rather requires a time lapse to build up. This may indicate that the changes observed in the final measurement represent something closer to a 'final' size of response, before the effect begins to fade. However, this cannot be ascertained without a more prolonged period of post-stimulation testing.

In the group that also received iHFS, this correlation between the baseline condition and the final measurement was not present, indicating that iHFS had a disruptive effect on the normal time course of the response to rTMS. It is important to note that, in the group that received rTMS alone (Group 2), the PPR increased significantly after 25 min compared with the values obtained immediately after rTMS. This makes it unlikely that the lack of further increase in the PPR after iHFS in Group 1 was simply due to a ceiling effect, as after rTMS the PPR value was almost identical for both groups. Furthermore, in the group that received iHFS alone (Group 3), the baseline value of the PPR approximated the value found in the other two groups after rTMS. This did not prevent iHFS from producing a significant increase in the PPR, suggesting that the lack of effect of iHFS in Group 1 depended on the previous history of activity rather than on the value of the PPR at the time of stimulation.

Effects on tactile acuity

In contrast to the results obtained for cortical excitability, rTMS and iHFS showed no significant interaction in their effect on tactile acuity. Both groups experienced a significant improvement in two-point discrimination immediately following rTMS, which remained unchanged in the last assessment, with or without iHFS.

A previous report, in which a similar rTMS protocol was used, also showed that the induced change in tactile acuity was strongest immediately after stimulation, and slowly reverted to baseline values over the following hours (Tegenthoff *et al.*, 2005). This represents a marked difference from the effect of rTMS on cortical excitability, which, as was shown above, is considerably stronger 25 min after the end of stimulation than immediately after. In addition, the effect on the PPR was highly sensitive to iHFS, whereas iHFS had almost no influence on the rTMS-induced change in tactile acuity.

It seems likely that this difference is due in part to the fact that two-point discrimination is a complex perceptual task that engages many brain areas outside the SI and, although the latter is involved in the two-point discrimination task, other areas play important roles. The results do, however, suggest that the rules governing the effect of plasticity-inducing interventions, and especially interactions between them, are complex, and depend on what type of data is considered to be indicative of plasticity (e.g. behavioural vs. neurophysiological). A similar dissociation between changes of excitability and behavioural measures has been described for the SI following PAS (Litvak *et al.*, 2007). In these experiments, a gain in tactile acuity depended on whether TMS applied to the SI was near-synchronous to afferent signals containing either mechanoreceptive or proprioceptive information. In the latter case, acuity remained unchanged despite changes in excitability, which questions a simple relation between enhancement of synaptic efficacy and behavioural gain. In another study, facilitative PAS has been reported to inhibit motor learning (homeostatic interaction), only if 90 min were allowed to elapse between PAS and motor practice (Jung & Ziemann, 2009). If motor practice was carried out immediately after PAS, then PAS actually improved learning (non-homeostatic interaction). In contrast, studies that explore homeostatic plasticity using MEPs as an indicator often find that such effects develop immediately. Furthermore, the time window during which homeostatic plasticity can be demonstrated using this paradigm appears to be relatively short, as revealed by studies in which short priming interventions were used. In such cases, even a 5- or 10-min interval between interventions is sufficient to abolish homeostatic interaction (Huang *et al.*, 2010; Iezzi *et al.*, 2011).

The lack of significant influence of iHFS on tactile acuity when applied after rTMS contrasts with the results previously reported by Ragert *et al.* (2003), in which the two types of stimuli produced an additive effect. This shows that the manner in which the two

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interventions interact might be dependent on their timing. In a previous study (Nitsche *et al.*, 2007), it was shown that the same two plasticity-inducing techniques (tDCS and PAS) interact homeostatically when applied simultaneously and synergistically when applied in succession. This, as the authors point out, contradicts previous results combining tDCS and rTMS (Lang *et al.*, 2004; Siebner *et al.*, 2004), which showed a homeostatic interaction after sequential application. This indicates that the mode of interaction between two interventions (i.e. homeostatic or synergistic) may also depend on the specific form of stimulation used. However, once a certain plasticity process is underway, it may exhibit a degree of immunity to further changes induced by additional interventions. Such an explanation has been put forward by Jung & Ziemann (2009) in connection with the above-mentioned finding that was based, perhaps significantly, on functional parameters (motor learning) and not on neurophysiological parameters (e.g. MEPs).

Pearson's analysis showed that there was no correlation between the changes in two-point discrimination and changes in the PPR after either rTMS (Groups 1 and 29) or iHFS (Group 3). Significant correlations between perceptual changes and neural changes have been robustly demonstrated for blood oxygenation level dependent signals and dipole changes (Pleger *et al.*, 2001, 2003; Dinse *et al.*, 2003a,b), whereas a correlation with excitability measures has so far been described only once (Höffken *et al.*, 2007), offering a greater dynamic range of changes, which facilitates the detection of correlations. We therefore assume that, in the present study, because of the large observed fluctuation in the PPR, together with smaller acuity effects, a correlation between the two parameters did not emerge.

Site of effect and interaction

The fact that sequentially applied rTMS and iHFS showed an interaction can be regarded as an indication that the two interventions probably affect, at least in part, the same population of neurones. When one intervention affects the outcome of a second intervention, this is taken to indicate changes in the threshold for the induction of plasticity induced by the first intervention (see e.g. Sale *et al.*, 2011). This is particularly interesting in view of the fact that rTMS and iHFS represent completely different methods of stimulation, with the former activating cortical networks directly, and the latter making use of the sensory pathway to reach the cortex.

The rTMS has the advantage of allowing for localized stimulation of the brain tissue that lies directly under the coil. Although it is not clear exactly which cell populations are predominantly activated during TMS, modelling studies suggest that the induced electric fields are particularly strong around the gyral crowns and lips, and are less likely to extend deep into the sulcal walls (Opitz *et al.*, 2011; Thielscher *et al.*, 2011). In terms of the primary SI in the post-central gyrus, this corresponds broadly with Brodmann area 1. This is, furthermore, the proposed origin of the N20-P25 component of the median nerve SEP, according to many studies (Arezzo *et al.*, 1979; Allison *et al.*, 1989; McCarthy *et al.*, 1991;.) It is thus highly probable that the homeostatic interaction occurred in a neuronal population located on the crown of the post-central gyrus as a result of the two interventions used, rTMS and tactile coactivation, as the latter has been previously shown to effect changes in the same SEP component (N20-P25) that originates in this area (Höffken *et al.*, 2007). However, from our experimental design it cannot be ruled out that interactions between iHFS and rTMS can also occur outside the primary SI. For example, recent data showed that inter-regional interactions can be induced via premotor-to-motor inputs (Pötter-Nerger *et al.*, 2009).

Separate analysis of the raw amplitudes of P1 and P2 for both groups showed that all changes in the PPR were mediated primarily by an increase in the amplitude of P2, whereas P1 underwent no significant change. A similar finding was reported by Ragert *et al.* (2004) after a similar application of rTMS over the S1. In fact, of numerous studies that have used rTMS applied directly over the primary SI, none has found changes in the early components of the SEP when measured as single pulses (single-pulse SEPs), that could be considered as analogous to the first peak of a paired-pulse paradigm (Enomoto *et al.*, 2001; Restuccia *et al.*, 2007; Nakatani-Enomoto *et al.*, 2012). This indicates that the effect of rTMS is focused on the mechanism responsible for paired-pulse suppression, rather than the excitability of thalamocortical afferents. In contrast, the related technique of PAS applied over the S1 has proven capable of modulating the amplitude of single-pulse SEPs (Wolters *et al.*, 2005; Pellicciari *et al.*, 2009), although this effect has not been consistently reproducible (Bliem *et al.*, 2008; Tamura *et al.*, 2009).

Conclusion

Our results demonstrate that two different plasticity-inducing interventions, rTMS and iHFS, interact homeostatically, indicating that the two are, at least partially, acting on the same neuronal population. Our data also emphasize the importance of timing on the way in which different interventions interact, as the same two techniques were seen to have an additive effect when used simultaneously. Furthermore, the final effect of rTMS, when allowed to run its time course undisturbed, was found to be dependent on the baseline state of cortical excitability, demonstrating the dependence of such interventions on the previous brain state. Finally, the interaction between rTMS and iHFS adhered to a homeostatic rule only as far as neurophysiological measures were concerned, and this did not extend to psychophysics. This might indicate that the rules governing changes in measures of brain excitability do not necessarily apply in the same simple form for the functional outcomes, which are more likely to depend on complex effects, probably involving networks distributed across several brain areas.

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Abbreviations

iHFS, intermittent high-frequency tactile stimulation; ISI, interstimulus interval; MEP, motor evoked potential; PAS, paired-associative stimulation; PPR, paired-pulse ratio; RM, repeated-measures; rTMS, repetitive transcranial magnetic stimulation; SEP, somatosensory evoked potential; SI, somatosensory cortex; tDCS, transcranial direct current stimulation; TMS, transcranial magnetic stimulation.

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