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Combination of 5 Hz repetitive transcranial magnetic stimulation (rTMS) and tactile coactivation boosts tactile discrimination in humans

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Abstract

A combination of 5 Hz repetitive transcranial magnetic stimulation (rTMS) over the left primary somatosensory cortex together with tactile coactivation applied to the right index-finger representation (coac + rTMS) boosted tactile discrimination ability tested on the right index-finger. Applying coactivation alone caused a 0.25 mm lowering in tactile discrimination thresholds. In contrast, after coac + rTMS we found a significant further improvement of discrimination thresholds in comparison to the coactivation-induced perceptual changes alone. We demonstrate that the individual further improvement after coac + rTMS depended on the effectiveness of the coactivation protocol when applied alone. Subjects, who showed little gain in tactile performance after coactivation alone, showed the largest improvement after coac + rTMS implying that the combined application was selective for poor learners. The selective effects of coac + rTMS are discussed in respect to *N*-methyl-D-aspartate receptor activation.

Keywords: Perceptual learning; Coactivation; Repetitive transcranial magnetic stimulation (rTMS); Plasticity; Reorganization; Tactile acuity; Discrimination performance; Somatosensory cortex; Long term potentiation

Many studies have demonstrated that repetitive transcranial magnetic stimulation (rTMS) is capable to evoke changes of intracortical excitability that persist after termination of rTMS [1,9,10,14,16]. Muellbacher et al. [9] showed that low frequency rTMS of 1 Hz depressed motor excitability. In contrast, high-frequency rTMS application (from 5 to 20 Hz) is known to facilitate excitability of the stimulated motor cortex [2,11,19]. Five Hz rTMS applied over primary motor cortex is accompanied by persistent changes in neuronal activation measured with positron emission tomography (PET) [15].

It has been repeatedly demonstrated that a so-called coactivation applied to the tip of the index-finger (IF) induced tactile discrimination improvements paralleled by plastic reorganizational changes within primary and secondary somatosensory cortex [4,5,12]. Coactivation is a task-free, passive stimulation protocol without invoking cognitive factors such as attention or reinforcement. It was developed to study systematically the impact of altered input statistics on plastic capacities by simultaneously stimulating skin portions of the IF. In this way, a large number of receptive fields were coactivated in a Hebbian manner to strengthen their mutual interconnectedness [5, 12]. Here we asked whether enhancement of tactile acuity induced by coactivation is subject to further improvement by a combination of coactivation with 5 Hz rTMS applied to the hand representation in left primary somatosensory cortex in close vicinity of the right IF.

We tested 12 right handed healthy subjects (nine female, three male, mean age 22 years, range 19–25 years). All subjects gave their written informed consent before participating. The study was approved by the Ethics Committee of the Ruhr-University of Bochum and was performed in accordance with the 1964 Declaration of Helsinki.

Measurement of 2-point discrimination thresholds: The measurement of discrimination thresholds served as a marker of perceptual learning processes induced by

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coactivation or combined application of coactivation and 5 Hz rTMS (coac + rTMS). Spatial 2-point discrimination thresholds were tested in a two-alternative forced choice discrimination task [5,12]. Seven pairs of needles, separated by 0.7, 1.0, 1.3, 1.6, 1.9, 2.2, and 2.5 mm, were mounted on a rotatable disc that allowed to switch rapidly between distances. In addition, zero distance was tested with a single needle. For details of the apparatus see refs. [5,12]. Each distance of the needles was tested ten times in randomized order resulting in 80 single trials per session, which lasted about 15 min. The subject had to decide immediately if he had the sensation of one or two tips by answering 'one' or 'two'. The summed responses were plotted against distance as a psychometric function for absolute threshold, fitted by a binary logistic regression (SPSS©, SPSS Inc.). Threshold was taken from the fit at the distance where 50% correct responses was reached.

Coactivation: The coactivation protocol was the same as in our previous studies [5,12]. Coactivation-stimuli were randomly presented at different interstimulus-intervals between 100 to 3000 ms; average frequency was 1 Hz, pulse duration was 10 ms. Pulses were recorded on tape and were played back via portable tape recorders allowing unrestrained mobility of the subjects during coactivation. To apply coactivation, a small solenoid with a diameter of 8 mm was taped to the tip of the right index finger and transmitted the tactile stimuli of the coactivation protocol to the skin with a mean stimulation duration of 3 h.

Repetitive transcranial magnetic stimulation (rTMS): A MAGSTIM Rapid Stimulator (Magstim, Whitland, Dyfed, UK) connected to an 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-fitted cap with a 1 cm grid referenced to the vertex (Cz). First, subject's motor thresholds (MT) were measured at the relaxed first dorsal interosseous (FDI) muscle of the right hand using single pulse TMS. 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 FDI muscle representation was identified at that scalp position, where TMS induced highest motor evoked potentials (MEPs). MT was defined as the lowest intensity capable of evoking five out of ten MEPs with an amplitude of at least 50 μV. Next, to position the coil as close as possible to the right IF representation in the left primary somatosensory cortex, we used the coordinates of the sensorimotor representations of the fingers provided by Maldjian et al. [8]. To this end, from the point of maximum stimulation of the contralateral FDI muscle we moved the magnetic coil 1–2 cm posterior in parasagittal direction to that position, where subjects reported sensible sensations in their right IF induced by single pulse TMS. After having encircled the location of the right IF representation, the position of the figure-of-eight coil was fixed. This location is denoted $SI_{right\ IF}$ in the following. rTMS intensity was set at 90% of the MT. During subsequent rTMS stimulation, surface electromyographys (EMGs) were recorded from the FDI muscle of the right hand using silver-silver electrodes.

For rTMS, 25 trains of TMS pulses were applied through the tangentially oriented coil grip backwards positioned over $SI_{right\ IF}$. A single train consisted of 50 single pulses of 5 Hz lasting 10 s with an inter-train interval of 5 s. Five consecutive trains were grouped into one block. Between each block was a rest period of 1 min. Forty-five min after the termination of this rTMS session, $SI_{right\ IF}$ stimulation was repeated in a second rTMS session, with stimulation intensity, magnetic coil position and parameter settings kept constant. To be able to apply 2500 TMS pulses, rTMS sessions were separated by 45 min to ensure that TMS stimulation was well tolerated.

Experimental schedule: In experiment 1, tactile discrimination thresholds were tested for all subjects on five consecutive sessions (one session per day; s1 to s5, s5 = pre1) on the right IF in order to obtain a stable baseline performance (see also Fig. 1). Sessions were statistically analyzed for stability (repeated measures and univariate analysis of variance (ANOVA) or Student's *t*-test). In the 5th session (pre 1), the thresholds of the left IF were additionally measured. Previous studies had shown that the effect of baseline training was transferred to the IF of the left hand [5,12]. After the 5th session, the coactivation-protocol was applied to the tip of the right IF for 3 h. Reassessment of tactile discrimination thresholds of the right and left IF (post 1) was performed approximately 15 min after termination of coactivation. The left (control) IF was tested to confirm previous findings about the local specificity of coactivation induced changes [5,12]. Two weeks after application of coactivation alone, discrimination behaviour was retested for the left and right IF (pre 2). In contrast to experiment 1, rTMS was additionally applied in two sessions separated by 45 min during the time coactivation was applied to the right IF (coac + rTMS). The first rTMS application started 1.5 h after the onset of coactivation, the second 45 min after the termination of the first one. Each rTMS session lasted about 10 min. Reassessment of tactile discrimination thresholds of the

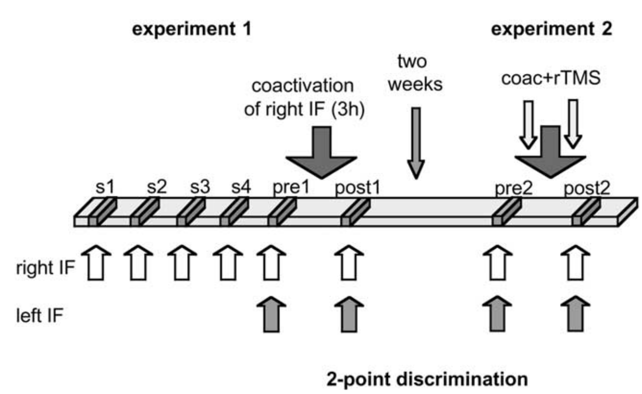

Fig. 1. Experimental setup and design. For details see text.

right and left IF (post 2) was performed approximately 15 min after termination of coac + rTMS (cf. Fig. 1).

During experiment 1, all subjects achieved a stable baseline performance during the initial training period s 1-pre 1 (repeated measures ANOVA (right IF; s 1-pre 1): $F_{(5,55)} = 1.082$, $P = 0.381$). Before coactivation was applied (pre 1), the mean discrimination threshold was 1.65 ± 0.06 (SEM) mm for the right, and 1.72 ± 0.09 (SEM) mm for the left IF. The thresholds of the right and left IF did not differ (t-test: $P = 0.561$). After coactivation, discrimination performance of the right IF improved for all subjects as indicated by a significant lowering of discrimination thresholds of about 0.25 mm, from 1.65 ± 0.06 (SEM) to 1.40 ± 0.08 (SEM) mm (repeated measures ANOVA (right IF; s 1-post 1): $F_{(6,66)} = 2.457$, $P = 0.03$, pre 1 versus post 1; t-test: $P < 0.001$; Fig. 2A). In contrast, the left non-coactivated control IF remained unchanged, confirming the local specificity of the coactivation induced changes (t-test: $P = 0.333$).

For experiment 2, which was performed about 2 weeks after experiment 1, reassessment of discrimination thresholds (pre 2) showed no differences to pre 1, indicating a stable tactile behaviour over a period of 2 weeks (pre 1/pre 2 difference t-test: $P = 0.85$; Fig. 2A). After coac + rTMS we found a further improvement of discrimination thresholds in comparison to the coactivation-induced perceptual changes alone. No side-effects due to rTMS were observed and the rTMS sessions were well tolerated by all subjects. After coac + rTMS, the gain in tactile discrimination performance was 0.35 ± 0.05 (SEM) mm (22.19%), whereas coactivation improvement without rTMS was only 15.73% (repeated measures ANOVA (right IF; pre/post difference coac versus coac + rTMS): $F_{(1,11)} = 6.876$; $P = 0.024$; factor pre/post $F_{(1,11)} = 37.266$; $P < 0.0001$; Fig. 2A).

We then asked whether coac + rTMS leads to an unspecific improvement in all subjects independent of their gain in performance induced by coactivation alone. We

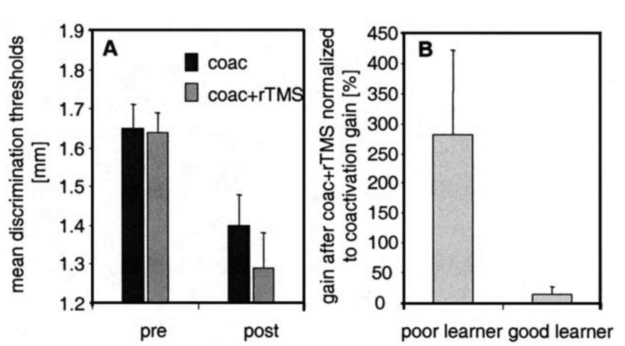

Fig. 2. (A) Effect of coactivation alone and of coac + rTMS on mean (± SEM) discrimination thresholds ($n = 12$). Note decrease in thresholds after coactivation alone, but significantly larger effects after coac + rTMS (repeated measures ANOVA: $F_{(1,11)} = 6.876$; $P = 0.024$). (B) Comparison of the effectiveness of coac + rTMS in poor (gain in performance < 0.25 mm) and good learners (gain in performance > 0.25 mm) normalized to the improvement found after coactivation alone.

therefore correlated the individual gain after coac + rTMS normalized to the gain found after coactivation alone with the gain observed after coactivation in experiment 1 revealing a strong negative relationship ($r = -0.693$, $P = 0.012$). Accordingly, subjects that benefited much after coactivation alone did show only minor effects after coac + rTMS. In contrast, subjects with only little gain in performance after coactivation, improved substantially after coac + rTMS. This finding was corroborated in an additional analysis where subjects were divided according to the average gain (0.25 mm) found after coactivation alone into poor (gain in performance < 0.25 mm, $n = 6$) and good learners (gain in performance > 0.25 mm, $n = 6$). This analysis revealed that poor learners showed a huge tactile further improvement in performance of 0.13 ± 0.06 (SEM) mm (mean ± SEM $282.81 \pm 138.95\%$) after coac + rTMS. In contrast, good learners showed only small additional discrimination improvements of 0.06 ± 0.04 (SEM) mm after coac + rTMS ($15.90 \pm 11.02\%$; univariate ANOVA for percentage improvements after coac + rTMS (right IF): $F_{(1,10)} = 6.889$; $P = 0.025$; Fig. 2B). Thus, despite the large improvement in poor learners after coac + rTMS, their absolute thresholds were still higher than those found in good learners.

In summary, we found that the combined application of coac + rTMS is capable to boost discrimination improvement in a tactile spatial 2-point discrimination task as compared to coactivation alone. Most intriguingly, we could demonstrate that the individual further improvement after coac + rTMS was dependent on the effectiveness of the coactivation protocol applied alone. Subjects, who showed little learning after experiment 1, showed the largest improvement after coac + rTMS implying that the combined application was selective for poor learners. Previously we had shown that application of rTMS alone using the same stimulation parameters as in the present study was effective in improving spatial discrimination performance. The average lowering of thresholds was comparable to the gain observed after coactivation alone [13].

The baseline performance (pre 2) of the subjects in experiment 2 was on approximately the same level as compared to pre 1 (Fig. 2A). Previous studies have stressed the reversibility of the coactivation-induced threshold lowering within 24 h [5,12]. Furthermore, these findings provided evidence that not only the thresholds of naïve subjects are comparable to non-naïve subjects, but also the threshold changes induced by coactivation were in the same range [5]. We therefore assume that the repeated testing with combined coac + rTMS 2 weeks after coactivation did not interfere with learning processes evoked during experiment 1. Instead, comparing the effects of coactivation and coac + rTMS in the same subjects was instrumental in reducing interindividual variability.

Based on animal data, rTMS has been reported to induce long term potentiation (LTP)-like or long term depression (LTD)-like mechanisms depending on the stimulation frequency used [18]. In rats, in vitro-autoradiography revealed that rTMS exposure results in persistent effects on *N*-methyl-D-aspartate (NMDA) binding sites [7]. Ziemann et al. [20] provided evidence that during ischemic nerve block low-frequency rTMS application in humans also includes NMDA-receptor activation known to be involved in LTP-like processes. Using a non-competitive NMDA-antagonist, we have recently demonstrated that the coactivation-induced perceptual learning and cortical reorganization of the right IF can be completely blocked, but not the performance of the left, non-coactivated IF. These findings indicate that NMDA receptor activation is required for the manifestation of coactivation induced perceptual changes [3]. We therefore assume that both experiments 1 and 2 induce learning processes that depend on NMDA-receptor activation. This notion is compatible with the repeatedly reported enhancement of cortical excitability after rTMS [2,11,19].

To be operative, NMDA receptors require sufficient depolarization, thus, the balance of excitation and inhibition plays an important role in controlling plasticity. Therefore, to explain the selective effect of coac + rTMS on poor learners, we suggest that in some subjects the coactivation-induced activation failed to induce LTP presumably due to insufficient depolarization. As a result, no or only little improvement of discrimination occurred in these subjects. In contrast, the combined application of coac + rTMS was sufficient to overcome the prevailing inhibition in these subjects. Blind Braille readers were shown to have far lower discrimination thresholds than normal subjects [17], which suggests that the limited further improvement found in good learners are most unlikely due to ceiling effects. Further studies have to be designed to study the effectiveness of rTMS alone in poor learners.

Recently, Knecht and coworkers [6] demonstrated that low-frequency (1 Hz) rTMS can provoke sustained disruption of tactile perception. In contrast, to our knowledge the present study is the first attempt to use high-frequency (5 Hz) rTMS as a non-invasive tool to boost learning processes within the somatosensory system.

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