Voluntary movement and repetitive transcranial magnetic stimulation over human motor cortex

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Abstract

Repetitive transcranial magnetic stimulation (rTMS) can induce short-term reorganisation of human motor cortex. Here, we investigated the effect of rTMS during relaxation and weak voluntary muscle contraction on motor cortex excitability and hand function. Subjects (n=60) participated in one of four studies. Single transcranial magnetic stimuli were delivered over the motor area of the first dorsal interosseus for measurement of motor evoked potential (MEP) size before and after real or sham rTMS delivered at an intensity of 80% of active motor threshold. rTMS involved trains of stimuli applied at 6 Hz for 5 s and repeated every 30 s for 10 minutes. Resting MEP size was suppressed for 15 mins after rTMS during relaxation. However, MEP suppression was abolished when additional brief voluntary contractions were performed before and after rTMS (Study one). Resting MEP size was suppressed for 30 mins after rTMS during weak voluntary contraction. MEP suppression was present even though voluntary contractions were performed before and after rTMS (Study two). The MEP suppression most likely reflects a decrease in motor cortical excitability. Surprisingly, rTMS during voluntary contraction did not alter maximal finger tapping speed or performance on a Grooved Pegboard test, object grip and lift task (Study three), and visuomotor tracking task (Study four). These studies document the complex relationship between voluntary movement and rTMS-induced plasticity in motor cortex. This work has implications for the optimisation of rTMS parameters for improved efficacy and potential therapeutic applications.

Key words: plasticity, motor evoked potential, voluntary muscle contraction, hand function.
rTMS and voluntary movement

Introduction

Repetitive transcranial magnetic stimulation (rTMS) has been used to induce short-term reorganisation of the cerebral cortex in humans. The reorganization is thought to arise from changes in synaptic efficacy within the cortex that are brought about by processes such as long-term potentiation (LTP) and long-term depression (LTD). These phenomena are activity-dependent processes that involve strengthening (LTP) or weakening (LTD) of synaptic transmission and can persist for a substantial time (for review see 21). In motor cortex, reorganisation can be reflected by a change in motor cortical excitability, examined by measurement of the electromyographic response (motor evoked potential: MEP) to single-pulse transcranial magnetic stimulation (TMS). It is interesting that rTMS-induced changes in motor cortical excitability do not necessarily translate into changes in actual motor function (16). Performance on relatively simple tasks are not affected by low-frequency rTMS protocols that reduce motor cortical excitability (6, c.f. 15, 19, 20) nor is voluntary force and acceleration during maximal efforts (16). However, performance of more complex tasks can be disrupted after high-frequency rTMS (18). In addition, low-frequency rTMS can disrupt motor learning (5) and early motor consolidation (17). The modest functional correlates reported so far may be related to voluntary movement disrupting the rTMS induced changes in motor cortical excitability (12, 22, 23) or compensatory mechanisms in voluntarily activated motor networks that adapt to altered basal excitability (20). An additional factor to consider is that rTMS-induced changes in motor cortical excitability are usually evoked when the target muscles are relaxed: that is, there is no functional context for the change in cortical activity.
In the current study, we investigated the effect of rTMS application during relaxation and voluntary muscle contraction on motor cortical excitability and hand function. We used trains of subthreshold 6 Hz rTMS, a paradigm that is known to suppress motor cortical excitability for approximately 30 mins after the end of stimulation when delivered during relaxation (22). For rTMS delivered during relaxation, our hypothesis was that subsequent voluntary contractions would disrupt the effect of rTMS on motor cortical excitability. For rTMS delivered during voluntary contraction, our hypotheses were that a) the paradigm would reduce motor cortical excitability, b) the reduction in motor cortical excitability would persist beyond future movements, and c) the paradigm would alter performance during tests of simple (maximal finger tapping speed) and complex hand function (Grooved Pegboard test, object grip and lift task, and a visuomotor tracking task). This work will provide the basis for optimisation of rTMS parameters to improve efficacy which is important given its potential application as a therapeutic intervention in a range of neurological conditions.

**Materials and methods**

Subjects (n=60, 22±5 yrs; 31 female, 29 male) participated in one of four studies to investigate the effect of rTMS application during voluntary contraction (note: one female subject participated in two studies). All experimental procedures were undertaken with approval of The University of Adelaide Human Research Ethics Committee. Experiments were conducted according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained.

*Electromyographic and force recordings*
Electromyographic activity (EMG), force, and potentiometer signals were recorded using a data acquisition system (CED 1902 and 1401 Power Interface with Signal software, Cambridge Electronic Design, Cambridge, UK). EMG was recorded with surface electrodes (Ag-AgCl, 10 mm diameter) overlying the first dorsal interosseus (FDI) of the dominant hand. Surface EMG signals were amplified (X 300 or X 1000), filtered (20-1000 Hz), and sampled at 2000 Hz or 5000 Hz. Isometric index finger and thumb opposition force was measured with the use of a linear strain gauge (width: 3.5 cm; Model MLP-25, Transducer Techniques, Temecula, USA). Isometric index finger abduction force was measured with a linear strain gauge (LC1205-K020, A & D Co. Ltd, Tokyo, Japan) positioned at the proximal interphalangeal joint. Force signals were amplified (X 1000), filtered (low-pass 100 Hz), and sampled at 400 Hz or 2000 Hz. Medial-lateral movement of the index finger was recorded with a potentiometer (Model 157, Vishay, NSW, Australia). The axis of rotation of the potentiometer was positioned over the metacarpophalangeal (MCP) joint. Potentiometer signals were sampled at 2000 Hz.

**Tests of hand function**

Surface EMG electrodes were not removed during the assessment of hand function but subjects were disconnected from the equipment to enable free hand movement. Four tests of hand function were performed on the dominant hand: maximum finger tapping speed, Grooved Pegboard test, object grip and lift task, and a visuomotor tracking task. 

**Maximum finger tapping speed**

Subjects were instructed to tap a linear strain gauge (Model MLP-25, Transducer Techniques, Temecula, USA) as fast as possible with their index finger. The number of
taps was counted over a 4 s interval. Three trials, separated by at least 15 s of rest, were performed before and after rTMS.

**Grooved Pegboard test**

Complex sensorimotor function was assessed using the Grooved Pegboard test (Model 32025, Lafayette Instrument Company, Lafayette, USA). This test involves key shaped pegs that must be rotated appropriately to match the groove in the corresponding hole. Subjects were instructed to place 25 pegs into corresponding holes as fast as possible and in a set sequence. The time taken to complete the test was recorded. Three trials, separated by 1 min of rest, were performed before and after rTMS.

**Object grip and lift task**

Performance during the grip and lift task was assessed with the use of a light-weight manipulandum that is similar in concept to that described by Westling and Johansson (1984) (24). Subjects were instructed to lift, hold, and then replace the manipulandum on a table using the index finger and thumb in a precision grip. The manipulandum consisted of two linear strain gauges (model MLP-100, Transducer Techniques, Temecula, USA) for measurement of horizontal grip force and vertical lift force. The grip strain gauge was mounted between two polished brass disks, 35 mm apart. The grip strain gauge was positioned above and at 90 degrees to the lift strain gauge. An accelerometer (ADXL311JE, RS Components Pty Ltd, Smithfield, Australia) was also attached to the apparatus. The total weight of the manipulandum was 342 g. Force and acceleration signals were amplified (force: X 1000; acceleration: X 3), filtered (low-pass 100 Hz), and sampled (400 Hz) using a data acquisition system (CED 1902 and 1401 interface with Signal software, Cambridge Electronic Design, Cambridge, UK). Standardised verbal instruction and demonstration were provided prior to the first
attempt at lifting the manipulandum. Subjects were instructed to ‘lift the device off the table to the height indicated (10 cm). Hold the device there for 3 s and then replace it on the table.’ Five trials, separated by approximately 3 s of rest, were performed before and after rTMS.

**Visuomotor tracking task**

The visuomotor tracking task involved subjects matching MCP joint angle of the right index finger with a moving target on a computer screen. The moving target consisted of 18 unique 10-s frames (e.g. Fig. 6A). The target moved across the screen while making unpredictable upward and downward movements. Subjects received visual feedback of MCP joint angle relative to the target and were instructed to follow the target as closely as possible. Abduction of the finger moved the feedback line downward while adduction moved the feedback line upward. The maximum MCP joint angle movement was ±10° from neutral.

**Transcranial Magnetic Stimulation**

Single transcranial magnetic stimuli were delivered using a Magstim 200<sup>2</sup> stimulator (Magstim Co., Whitland, UK) with a figure-of-eight focal coil (9 cm external diameter of wings). Stimuli were applied to the FDI motor area of the hemisphere contralateral to the dominant hand. The coil was held at approximately 45 degrees to the mid-line with the handle pointing posteriorly. This coil orientation induces a current in the brain that flows in a posterior to anterior direction and approximately perpendicular to the central sulcus, an orientation that is optimal for activating the hand region of the motor cortex. Resting motor threshold was defined as the minimum stimulus intensity at which 5 out of 10 consecutive stimuli evoked an MEP of at least 50 µV in amplitude in the relaxed FDI muscle. To determine threshold, stimuli were first delivered at a suprathreshold
intensity. The stimulus intensity was then reduced in small increments until it was clearly below threshold. For investigating changes in MEP size over time, the intensity of TMS was set to evoke baseline MEPs of approximately 1-2 mV in amplitude in both the relaxed and contracting FDI.

The rTMS was delivered with the use of a Magstim Super Rapid stimulator (Magstim Co. Whitland, UK). Trains of stimuli were applied at a frequency of 6 Hz for 5 s and repeated every 30 s for 10 minutes (total of 600 stimuli). This stimulation paradigm is based on that previously employed by our group (22) and others (14). The magnetic stimulus had a biphasic waveform with a pulse width of ~400 μs. The stimulus intensity was set at 80% of active motor threshold. Active motor threshold was determined with single stimuli during weak index finger and thumb opposition. Active motor threshold was defined as the minimum stimulus intensity required to evoke MEPs of at least 100 μV in amplitude in 5 out of 10 successive trials.

**Sham transcranial magnetic stimulation**

Sham stimulation was applied using a coil (placebo coil, PN 3285-00, Magstim Co. Ltd, Whitland, UK) that is similar in appearance and operation to the normal figure of eight coil. The placebo coil provides minimal scalp sensation and a sound similar to that made by the normal coil, but without stimulating the cortex.

**Experimental protocol**

**Study one**

Subjects (n=14) participated in two experiments to investigate the effect of voluntary contraction on rTMS-induced changes in resting motor cortical excitability. The experiments were performed in a pseudorandom order on separate days, at least 48
hours apart. The first experiment started with measurement of resting motor threshold and baseline resting MEP size (Fig. 1A, top panel). Resting MEP size was recorded during a set of 15 single stimuli delivered at approximately 0.2 Hz. Subjects then performed a weak precision grip voluntary contraction (i.e. index finger and thumb opposition) at approximately 10% of maximal voluntary contraction to determine active motor threshold (using the Super Rapid stimulator). Subjects then received intermittent real rTMS during relaxation. Resting MEP size was again measured at 0, 15, and 30 mins post-rTMS. The second experiment commenced with 3 brief maximal precision grip voluntary contractions. The duration of each contraction was 2-3 s and contractions were separated by at least 30 s of relaxation. The root mean square (RMS) EMG was measured (over a 1 s interval). The average RMS EMG for FDI was averaged and a 20% target was set on an oscilloscope. Baseline resting motor threshold and resting MEP size were then measured (Fig. 1A, lower panel). Subjects then performed a 5 s precision grip voluntary contraction at the target level. Resting MEP size was measured approximately 2 mins after completion of the contraction. Active motor threshold was determined (during contractions of approximately 10% of maximal voluntary contraction) and intermittent real rTMS was delivered during relaxation. Resting MEP size was again measured before and after a 5 s precision grip contraction (at the target level) at 0, 15, and 30 mins post-rTMS.

Study two

Subjects (n=11) participated in two experiments to assess the effect of rTMS application during voluntary contraction on motor cortical excitability. The experiments were performed in a pseudorandom order on separate days, at least 48 hours apart. The first experiment started with measurement of resting motor threshold and baseline resting
MEP size (Fig. 1B). Resting MEP size was recorded during a set of 15 single stimuli delivered at approximately 0.2 Hz. Subjects then performed 3 brief (2-3 s duration) maximal voluntary precision grip contractions. The FDI RMS EMG amplitude was measured during each maximal contraction. The average FDI RMS EMG was calculated and a 20% target was set on an oscilloscope. Subjects were instructed to contract up to the target EMG level whilst 15 single-pulse TMS were delivered to determine baseline active MEP size (contraction duration approximately 75 s). Active motor threshold was also calculated during this task for determination of rTMS stimulus intensity (using the Super Rapid stimulator). Subjects then received intermittent real rTMS whilst maintaining the target voluntary contraction. Subjects were instructed to contract up to the target EMG level for 5 s before each train of rTMS and to relax at the end of each train to avoid fatigue. This cycle was repeated for 10 mins. Following rTMS, resting and active MEP size and resting motor threshold were again measured at 0, 15, and 30 mins post-rTMS. Resting measures were always obtained before active measures. The second experiment was similar to the first except that sham rTMS was delivered instead of real rTMS.

**Study three**

Subjects (n=10) participated in three experiments to investigate the effect of rTMS application during voluntary contraction on hand function. The experiments were performed on separate days, at least 48 hours apart, and in a pseudorandom order. The first experiment was identical to the first experiment in Study two except that MEP measures were obtained at 0, 18, and 30 mins post-rTMS instead of 0, 15, and 30 mins (Fig. 1C, upper panel). In the second and third experiments, hand function was assessed before and after real or sham rTMS during voluntary contraction. Assessment of hand
function involved 5 trials of the grip and lift task, 3 trials of maximal finger tapping, and 3 trials of the Grooved Pegboard test. The timing of hand function assessment in relation to MEP measures and rTMS application is shown in Figure 1C (lower panel).

**Study four**

Subjects participated in one experiment to investigate the effect of rTMS during voluntary contraction on performance during a visuomotor tracking task. The experiment started with measurement of resting motor threshold and baseline resting MEP size (Fig. 1D). Subjects then performed 3 brief (2-3 s duration) maximal voluntary isometric index finger abductions. The FDI RMS EMG amplitude was measured during each maximal contraction. The average FDI RMS EMG was calculated and a 20% target was set on an oscilloscope. Subjects were instructed to contract up to the target EMG level whilst active motor threshold was calculated for determination of rTMS stimulus intensity (using the Super Rapid stimulator). Subjects then performed a 3-min visuomotor tracking task. Subjects then received intermittent real (n=13) or sham (n=13) rTMS whilst maintaining the target isometric voluntary contraction. Resting MEP size was measured then the 3-min visuomotor tracking task was repeated.

**Data analysis**

The area, peak-to-peak amplitude, and latency of MEPs were measured in each trial (e.g. Fig. 2A). For measurements obtained at rest, trials in which MEPs were preceded by EMG activity were excluded from the analysis. Mean force and RMS EMG amplitude were measured over a 100 ms interval immediately prior to single-pulse TMS and over a 1 s interval during maximal voluntary contractions (MVCs).
the silent period following single-pulse TMS was measured by cursor and was taken as the interval from the stimulus to the return of continuous voluntary EMG.

In preliminary experiments involving rTMS application during voluntary contraction, we noticed a suppression of voluntary EMG activity after each subthreshold stimulus in the rTMS train. We used a modified cumulative sum (CUSUM) technique to quantify the EMG suppression (4). This technique allows for calculation of the latency, duration, and strength of the EMG suppression in absolute units for each subject. Analysis involved rectifying the EMG signal, extracting a defined time period from around the stimuli (50 ms pre- and post-stimuli), averaging the signals in 2 min epochs (120 stimuli), subtraction of the noise level in the system (i.e. minimum average rectified EMG recorded during relaxation), normalisation of the data to the average pre-stimulus rectified EMG, followed by zero-phase 11-bin moving average filtering. The average pre-stimulus rectified EMG was subtracted from the data before CUSUM calculation. A significant event in the CUSUM was categorised if the vertical distance between two consecutive CUSUM turning points (i.e. EMG that crosses the pre-stimulus average) was more than 100% of the maximum pre-stimulus variation (see Fig. 2B). The latency of a significant event in the CUSUM was defined as the time of the turning point that initiated the significant event. The duration of the significant event was the horizontal distance between the latency and the next turning point (see Fig. 2B). The strength of a significant event corresponded to the vertical distance between two CUSUM turning points and was expressed as a percentage of the largest possible significant event (4). This definition indicates the total amount of EMG activity change rather than the maximum or minimum value. The characteristics of non-significant CUSUM events
were also measured for inclusion in a repeated measures analysis of variance (ANOVA).

The lift phase and hold phase of the grip and lift task were analysed separately. The lift phase was defined as the period from 0 s (lift onset) to 1.5 s and the hold phase was defined as the period from 1.5 s to 2.5 s (Fig. 3A). The following ten parameters were measured during the lift phase: grip onset relative to lift onset (termed ‘preload duration’), peak grip force, time-to-peak grip force (from lift onset), ratio of grip force to lift force at peak grip force, maximum rate of change in grip force (maximum dGF/dt; Fig. 3B), peak acceleration, time-to-peak acceleration (relative to lift onset), and minimum lift (degree of downward force application before lifting the object). The temporal relation between grip force and lift force was also assessed by cross-correlating the rate of change in grip force (dGF/dt) and lift force (dLF/dt) (Fig. 3C; SPSS 13.0 for Windows, SPSS Inc., Chicago, USA). That is, dLF/dt was shifted in increments of 2.5 ms (sampling resolution) relative to dGF/dt until the maximum cross-correlation coefficient was obtained (11). The time shift required to achieve the maximum cross-correlation coefficient represents the time difference between the change in lift force relative to grip force and is an index of whether the grip strategy was primarily anticipatory (negative time shift) or reactive (positive time shift). The following three parameters were measured during the hold phase: mean grip force, mean grip force relative to mean lift force, and standard deviation of grip force. Data collected during the grip and lift task are reported as the mean of 5 trials performed before and after rTMS application.
Performance during the visuomotor tracking task was assessed in 30 s epochs. For each epoch, the target line was cross-correlated with MCP joint angle (e.g. Fig. 6A and B). The maximum cross-correlation coefficient and the lag time to achieve the maximum cross-correlation coefficient were calculated. Tracking error was calculated by subtraction of MCP joint angle from the target time at each sample point. The mean absolute tracking error for each epoch is reported.

Data from each study were analysed separately. In the text, group data are presented as the mean ± standard deviation (SD), whereas in figures, mean ± standard error of the mean are shown. For Study one, group MEP data were analysed with two-way repeated measures analysis of variance (ANOVA) for comparison between experiment (relaxation, relaxation + contraction) and time (baseline, 0, 15, 30 mins post-rTMS). Group post-activation facilitation of MEP size (post-contraction MEP/pre-contraction MEP*100; %) was analysed with one-way repeated measures ANOVA. For Study two, group MEP data were analysed with two-way repeated measures analysis of variance (ANOVA) for comparison between experiment (real rTMS, sham rTMS) and time (baseline, 0, 15, 30 mins post-rTMS). An additional two-way repeated measures ANOVA was performed for comparison of group MEP data between studies (Study one: experiment two, Study two: experiment one; between subject factor) and time (baseline, 0, 15, 30 mins post-rTMS; within subject factor). Mauchly’s test of sphericity was performed and the Greenhouse-Geisser method was used to correct for non-sphericity (SPSS 15.0 for Windows, SPSS Inc., Chicago, USA). Group EMG suppression data during real rTMS were analysed with one-way repeated measures ANOVA for comparison between time points (2, 4, 6, 8, 10 mins). For Study three,
group data for experiment one (real rTMS) was analysed separately to that of experiments two (real rTMS and hand function) and three (sham rTMS and hand function). Group MEP data for experiment one was analysed with one-way repeated measures ANOVA for comparison between time points (baseline, 0, 18, 30 mins). Group MEP data for experiment two and three were analysed with two-way repeated measures ANOVA for comparison between experiment (rTMS and hand function, sham rTMS and hand function) and time (baseline, 0, 18, 30 mins post-rTMS). Group hand function data were analysed with two-way repeated measures ANOVA for comparison between experiment (rTMS and hand function, sham rTMS and hand function) and time (average baseline, average post-rTMS). Non-parametric data were transformed to ranks and repeated measures ANOVA on ranks were performed. Post-hoc discrimination between means was made with Student-Newman Keuls procedure (Sigmapstat 3.11, Systat Software Inc, Point Richmond, USA). For Study four, group MEP data were analysed with two-way repeated measures ANOVA for comparison between experiment (real rTMS, sham rTMS; between-subject factor) and time (baseline, post-rTMS; within-subject factor). Group performance data were analysed with three-way repeated measures ANOVA for comparison between experiment (real rTMS, sham rTMS; between-subject factor), tracking task (pre- and post-rTMS; within-subject factor) and time (0, 30, 60, 90, 120, and 150 s epochs; within-subject factor). Mauchly’s test of sphericity was performed and the Greenhouse-Geisser method was used to correct for non-sphericity (SPSS 15.0 for Windows, SPSS Inc., Chicago, USA). Post-hoc discrimination between means was made with Student-Newman Keuls procedure (Sigmapstat 3.11, Systat Software Inc, Point Richmond, USA). Statistical significance was set at 5%.
Results

Study one: The effect of voluntary contraction on rTMS during relaxation

Motor cortical excitability was assessed before and after rTMS application during relaxation by measurement of MEP size and resting motor threshold. For comparison between experiments, resting MEPs in experiment one were compared to pre-contraction resting MEPs in experiment two. Baseline resting MEP area, MEP amplitude, and resting motor threshold were not significantly different between experiments. The average baseline resting MEP area and amplitude was 4.5±1.5 mV.ms and 1.1±0.4 mV, respectively. The average resting motor threshold was 43±7% of stimulator output. There was a significant main effect of time on MEP area (F3,39=7.87; P<0.001) and amplitude (F3,39=6.39; P<0.001). There was no significant main effect of experiment on MEP area or amplitude. However, there was a significant experiment-by-time interaction (F3,39=2.84; P=0.05) on MEP area and a strong trend for an experiment-by-time interaction on MEP amplitude (F3,39=2.49; P=0.075). Resting MEP area was reduced for 15 mins after rTMS in experiment one (P<0.011) and there was a trend for a reduction at 30 mins (P=0.064). MEP area remained unchanged after rTMS in experiment two (Fig. 4A). The only difference in protocol between experiments was the inclusion of additional voluntary contractions in experiment two (i.e. three maximal voluntary contractions before rTMS, one submaximal contraction before rTMS, and three submaximal contractions after rTMS).

In experiment two, baseline post-contraction MEP area and amplitude was 113.2±36.5% and 117.0±36.3% of baseline pre-contraction MEP area and amplitude,
respectively. Post-activation facilitation of resting MEPs remained unchanged after rTMS.

**Study two: The effect of rTMS application during voluntary contraction**

Motor cortical excitability was assessed before and after rTMS application during voluntary contraction by measurement of MEP size and resting motor threshold. Motor cortical excitability was also assessed during rTMS application by measurement of the EMG suppression after each subthreshold stimulus. Baseline resting MEP area, resting MEP amplitude, and resting motor threshold were not significantly different between experiments. The average baseline resting MEP area and amplitude was 4.7±1.3 mV.ms and 1.2±0.3 mV, respectively. The average resting motor threshold was 45±11% of stimulator output. There was a significant main effect of time on MEP area (F3,30=4.62; P=0.009; Fig. 4B) and amplitude (F3,30=3.21; P=0.027). Resting MEP area and amplitude decreased over time (P<0.05). There was a significant main effect of experiment (F1,30=5.27; P=0.045) on resting MEP area (Fig. 4B) and a strong trend for a main effect of experiment on resting MEP amplitude (F1,30=4.67; P=0.056). Resting MEP area was approximately 30% smaller in the real rTMS experiment than in the sham rTMS experiment. For resting motor threshold, there was a significant main effect of time (F3,20=4.24; P=0.013) but not experiment. Across experiments, resting motor threshold increased by an average of 1.3±1.5% of stimulator output after rTMS (P<0.047).

Across experiments, the average baseline active MEP area, amplitude, and latency were 5.7±1.7 mV.ms, 1.6±0.5 mV, and 21.8±1.7 ms, respectively. The average baseline grip force was 14.8±4.7% MVC and the average duration of the silent period was 70.3±22.3
ms. There were no significant main effects of experiment or time on voluntary RMS EMG amplitude, grip force, silent period duration, active MEP area, or active MEP amplitude.

During real rTMS application, each subthreshold stimulus suppressed voluntary EMG for a short period of time (for example see Fig. 2B). Across subjects (n=11) and time points (n=5), there were 40 instances of significant EMG suppression (of a possible 55). Significant EMG suppression was categorised by the vertical distance between two consecutive CUSUM turning points (i.e. EMG that crosses the pre-stimulus average) exceeding the maximum pre-stimulus variation by more than 100%. At the beginning of rTMS, 9 of the 11 subjects had significant EMG suppression with an average latency of 25.9±3.0 ms (range: 21.4-29.8 ms), 4.4±1.9 ms longer than the latency of baseline active MEPs. Voluntary EMG was suppressed by 20.8±6.7% for 12.4±4.9 ms. The time course of the EMG suppression throughout the rTMS protocol was investigated in all 11 subjects using repeated measures ANOVA. There was no significant effect of time on the latency, duration, and strength of the EMG suppression. During sham rTMS application, there were 4 instances of significant EMG suppression (of a possible 55) across all subjects (n=11) and time points (n=5). A significant EMG suppression occurred once in two subjects, and twice in one subject.

**Study one versus Study two: Comparison of rTMS application during relaxation and voluntary contraction**

For comparison of the effects of rTMS delivered during relaxation and voluntary contraction, resting MEPs from Study one (experiment two) were compared to resting MEPs measured in Study two (experiment one). The experimental protocol was similar
except for the level of muscle activity during rTMS (relaxation or voluntary contraction) and the duration of pre- and post-rTMS voluntary contractions (5 s versus 75 s). There was a significant main effect of time on MEP area ($F_{3,92}=3.31; P=0.047$) and a trend for a main effect of time on MEP amplitude ($F_{3,92}=2.61; P=0.09$). There was no significant main effect of experiment on MEP area and amplitude. However, there was a significant experiment-by-time interaction on MEP area ($F_{3,92}=2.98; P=0.037$) and amplitude ($F_{3,92}=3.58; P=0.018$). MEP area and amplitude was significantly reduced for 30 mins after rTMS during voluntary contraction ($P<0.01$) but not after rTMS during relaxation.

**Study three: The effect of rTMS application during voluntary contraction on hand function**

The effect of real rTMS on resting motor cortical excitability was investigated again in experiment one with a new subject cohort. As expected, and consistent with the results of the previous study, there was a significant main effect of time on resting MEP area ($F_{3,27}=3.84, P=0.021$) and amplitude ($F_{3,27}=4.10, P=0.016$). Resting MEP area (Fig. 4C) and amplitude was suppressed for 30 mins after real rTMS application during voluntary contraction ($P<0.05$).

The effect of real and sham rTMS on hand function was investigated in experiments two and three, respectively. Across experiments, the average baseline maximum finger tapping speed and performance on the Grooved Pegboard test was $27\pm3$ taps and $55.5\pm4.8$ s, respectively. Table 1 shows the average baseline values across experiments for parameters measured during the object grip and lift task. We were primarily interested in significant experiment-by-time interactions on measures of hand function. There was a trend for an experiment-by-time interaction on time shift during the lift
phase ($F_{1,9}=4.63; P=0.06$; Fig. 5A) and mean grip force relative to mean lift force during
the hold phase ($F_{1,9}=3.41; P=0.098$; Fig. 5B) of the object grip and lift task. There were
no significant experiment-by-time interactions on maximum finger tapping speed,
performance on the Grooved Pegboard test, nor the remaining parameters measured
during the lift phase and hold phase of the object grip and lift task. We performed an
additional two-way repeated measures ANOVA for comparison of experiment (rTMS
and hand function, sham rTMS and hand function) and lift (lift 5 pre-rTMS, lift 1 post-
rTMS) for the grip and lift task. There was no significant experiment-by-lift interaction
on any parameter measured during the grip and lift task.

In the experiments with tests of hand function (experiments two and three), there was no
main effect of experiment or time on resting MEP area (Fig. 4C) but there was a
significant main effect of experiment on resting MEP amplitude ($F_{1,27}=5.11; P=0.05$).
Resting MEP amplitude was larger in the sham rTMS experiment than in the real rTMS
experiment ($P=0.05$). There was no main effect of experiment or time on active MEP
area or silent period duration.

**Study four: The effect of rTMS application during voluntary contraction on
performance during a visuomotor tracking task**

Motor cortical excitability and performance on a visuomotor tracking task was assessed
before and after real (n=13) or sham (n=13) rTMS during voluntary contraction. Across
groups, the average baseline maximum cross-correlation coefficient, lag time, and
tracking error was $0.50 \pm 0.19$, $317.1 \pm 141.9$ ms, and $4.0 \pm 1.1$ degrees, respectively. There
was a significant main effect of task and time on the maximum cross-correlation
coefficient (task: $F_{1,125}=105.65; P<0.001$; time: $F_{5,125}=41.73; P<0.001$; Fig. 6D), lag
time (task: $F_{1,125}=36.91; P<0.001$; time: $F_{5,125}=3.83; P=0.017$; Fig. 6E), and tracking error (task: $F_{1,125}=102.38; P<0.001$; time: $F_{5,125}=26.36; P<0.001$; Fig. 6C). Performance improved over time within each task and performance was greater in the second task than in the first task. There was also a significant task-by-time interaction for each parameter (maximum cross-correlation coefficient: $F_{5,125}=21.01; P<0.001$; lag time: $F_{5,125}=4.38; P=0.012$; tracking error: $F_{5,125}=10.93; P<0.001$). There was no significant main effect of experiment on performance.

There was a significant main effect of time on resting MEP amplitude ($F_{1,25}=4.39$, $P=0.047$) and a strong trend for a main effect of time on resting MEP area ($F_{1,25}=3.87$, $P=0.061$). There was also a significant experiment-by-time interaction on resting MEP area ($F_{1,26}=8.44, P=0.008$) and amplitude ($F_{1,26}=7.29, P=0.013$). Resting MEP area (P=0.009) and amplitude (P=0.017) decreased after real rTMS (baseline area: 5.0±2.0 mV.ms; post-rTMS area: 3.4±1.9 mV.ms; baseline amplitude: 1.1±0.5 mV; post-rTMS amplitude: 0.8±0.5 mV). Resting MEP area and amplitude remained unchanged after sham rTMS (baseline area: 4.6±1.8 mV.ms; post-rTMS area: 5.0±2.8 mV.ms; baseline amplitude: 1.1±0.4 mV; post-rTMS amplitude: 1.2±0.6 mV).

**Discussion**

In the present study, we investigated the effect of rTMS application during relaxation and voluntary contraction on motor cortical excitability and hand function. The four main findings were a) inhibition of MEPs induced by rTMS during relaxation is disrupted by voluntary contractions performed before and after rTMS, b) MEPs are inhibited for 30 mins after rTMS during voluntary contraction, c) inhibition of MEPs after rTMS during voluntary contraction persists beyond subsequent movements, and d)
rTMS application during voluntary contraction does not significantly alter performance on four tests of simple and complex hand function.

**rTMS during relaxation (Study one)**

As expected, intermittent 6 Hz rTMS during relaxation suppressed MEP size for 15 mins after the end of stimulation. The duration of MEP suppression (15 mins) was shorter than that reported previously by our group (30 mins) (22). The inhibition of resting MEPs most likely reflects a decrease in motor cortical excitability. Although not tested in the current study, there are several pieces of evidence that support this view. First, rTMS at 80% of active motor threshold is unlikely to induce descending activity in corticospinal neurones because the threshold for evoking descending activity is close to active motor threshold (10). Second, inhibition of resting MEPs induced by a low intensity rTMS protocol delivered during relaxation does not alter H-reflexes (13). Third, recordings of corticospinal activity in conscious humans show that inhibitory continuous theta burst stimulation (at 80% active motor threshold) and inhibitory suprathreshold 1 Hz rTMS decrease the amplitude of I-waves (8, 9). Lastly, suprathreshold rTMS delivered during relaxation modulates TMS evoked resting MEPs but not the response to transcranial electrical stimulation (2). This suggests that, even at higher intensities, the effects of rTMS on resting MEP size are due largely to changes within the motor cortex. Interestingly, MEP suppression was disrupted by voluntary contractions performed before and after rTMS during relaxation. The disruption was clearly demonstrated in the present study and has been reported previously by our group (22) and others (12, 23). Voluntary contractions performed prior to rTMS may induce metaplasticity (12), a phenomena where the prior synaptic history of a pathway can affect the subsequent induction of long-term potentiation or depression. Voluntary
contractions performed after rTMS may normalise (or reset) excitability in the cortical circuits responsible for MEP generation or disrupt long-term depression like changes in synaptic efficacy. In vivo cellular recordings in rat hippocampus (25) and in the developing Xenopus retinotectal system (26) show that long-term potentiation and depression can be reversed by spontaneous or behaviourally related activity.

**rTMS during voluntary contraction (Study two, three, and four)**

Resting MEPs were inhibited by approximately 30% for 30 mins after real rTMS during voluntary contraction (Study two and three). Inhibition of resting MEPs was reproducible (Study two versus Study three) and the inhibition persisted beyond voluntary contractions of the target muscle. The persistence of MEP inhibition after voluntary contraction is interesting given that voluntary contraction disrupted the MEP inhibition induced by rTMS during relaxation (Study one). The inhibition of resting MEPs most likely reflects a decrease in motor cortical excitability because our rTMS paradigm caused suppression of voluntary EMG after each subthreshold stimulus.

At subthreshold intensities, TMS can suppress voluntary EMG for a short period of time (7). In Study two, significant suppression of voluntary EMG occurred after each subthreshold stimulus in the real rTMS paradigm. In the first 2-min epoch of real rTMS, significant EMG suppression was evident in 9 out of 11 subjects. The latency of the suppression was ~4.4 ms longer than the short latency excitation produced by suprathreshold TMS during the same weak voluntary contraction. The characteristics of the EMG suppression were similar to those observed in other hand muscles using single subthreshold stimuli and surface EMG (7). The latency, duration, and strength of the EMG suppression did not change throughout the course of subthreshold rTMS. This
suggests that our rTMS protocol did not induce long-term change in the activity of the intracortical inhibitory circuits responsible for this short lasting suppression of voluntary EMG.

The size of active MEPs and the duration of the silent period were unchanged after rTMS application during voluntary contraction. However, there was a strong trend for an experiment-by-time interaction on active MEP amplitude (P=0.053). This suggests that our rTMS paradigm induces a change in basal excitability that is partially overcome or compensated for during voluntary activation. Similar results have been reported using functional imaging techniques where low frequency rTMS has been shown to modulate movement-related preparatory brain activity (19) and regional cerebral blood flow in area 4p (20) without affecting performance in a finger tapping task. However, a paired-pulse rTMS paradigm delivered during voluntary contraction can significantly increase active MEP size and improve performance during fatiguing exercise of the hand (1). The contradictory results involving low frequency rTMS and paired-pulse rTMS could be due to task-specificity and the magnitude and direction of the rTMS-induced change in excitability.

We hypothesised that rTMS application during voluntary contraction would alter performance during tests of simple and complex hand function. However, this was not the case. Our rTMS paradigm did not significantly alter performance on four tests of hand function. Although inhibitory rTMS protocols, like the one used here, have been shown to consistently reduce resting motor cortical excitability, it has proved more difficult to demonstrate associated functional effects. For example, low-frequency
inhibitory rTMS protocols delivered during relaxation do not affect relatively simple motor tasks such as self-paced finger tapping (6, 19, 20) or voluntary force generation and acceleration during maximal efforts (16). Although, a high-frequency inhibitory rTMS paradigm (continuous theta burst stimulation) can prolong reaction time in the hand (13). There is some limited evidence that inhibitory rTMS protocols can also affect more complex sensorimotor tasks. Nowak and colleagues demonstrated that maximum grip force and the ratio between grip force and lift force is disrupted after inhibitory theta burst stimulation delivered during relaxation (3, 18). This suggests that rTMS may have disrupted predictive grip force scaling. A strong trend for an experiment-by-time interaction on time shift (P=0.06) in the current study provides some limited support that rTMS during voluntary contraction may also disrupt predictive grip force planning. Ipsilateral or contralateral low-frequency rTMS during relaxation can also disrupt motor learning (5) and early motor consolidation (17). However, motor learning and early motor consolidation during our visuomotor tracking task was not affected by rTMS during voluntary contraction. In general, the differential effects of rTMS application on resting motor cortical excitability and motor function may reflect a capacity of the motor system to compensate for some forms of rTMS-induced alterations in basal motor cortical excitability but not others. The potential compensatory behaviour of the motor system is supported by unchanged motor cortical excitability during weak isometric voluntary contractions following rTMS application in the current study.

In summary, rTMS delivered during voluntary contraction suppressed resting motor cortical excitability for at least 30 mins after the end of stimulation. The suppression of
resting motor cortical excitability was reproducible and persisted beyond subsequent movements but did not translate to altered performance on tests of simple and complex hand function.
Grants

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References


Table 1. Average baseline value across experiments for parameters measured during the object grip and lift task.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
</tr>
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<tbody>
<tr>
<td><strong>Lift phase</strong></td>
<td></td>
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<tr>
<td>Preload duration (ms)</td>
<td>73.1 ± 47.0</td>
</tr>
<tr>
<td>Peak grip force (N)</td>
<td>5.36 ± 3.39</td>
</tr>
<tr>
<td>Time-to-peak grip force (ms)</td>
<td>516.0 ± 185.2</td>
</tr>
<tr>
<td>Grip force relative to lift force at peak grip force (N)</td>
<td>1.47 ± 0.91</td>
</tr>
<tr>
<td>Minimum lift (N)</td>
<td>-0.20 ± 0.12</td>
</tr>
<tr>
<td>Maximum dGF/dt (N.s⁻¹)</td>
<td>22.71 ± 14.95</td>
</tr>
<tr>
<td>Peak acceleration (g)</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>Time-to-peak acceleration (ms)</td>
<td>368.6 ± 90.6</td>
</tr>
<tr>
<td>Maximum cross-correlation coefficient (r)</td>
<td>0.80 ± 0.08</td>
</tr>
<tr>
<td>Time shift (ms)</td>
<td>-13.2 ± 18.3</td>
</tr>
<tr>
<td><strong>Hold phase</strong></td>
<td></td>
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<tr>
<td>Mean grip force (N)</td>
<td>4.23 ± 2.95</td>
</tr>
<tr>
<td>Mean grip force relative to mean lift force</td>
<td>1.26 ± 0.89</td>
</tr>
<tr>
<td>Standard deviation of grip force (N)</td>
<td>0.20 ± 0.17</td>
</tr>
</tbody>
</table>

Data are mean ± STDEV.
Figure legend

Figure 1. Experimental protocol. A, Study one. B, Study two. C, Study three. D, Study four. Closed and open arrows show the timing of blocks of single transcranial magnetic stimuli for measurement of motor evoked potential (MEP) size and motor threshold, respectively. Grey boxes represent the timing of weak voluntary contractions. Black boxes represent the timing of a set of 3 brief maximal voluntary contractions. The duration of the silent period was also measured when single stimuli were applied during voluntary contraction. Real or sham repetitive transcranial magnetic stimulation (rTMS) was delivered during relaxation (white box) or weak voluntary contraction (grey box). rTMS involved trains of stimuli applied at 6 Hz for 5 s and repeated every 30 s for 10 minutes. White boxes represent tests of hand function. G, Grip and lift task. F, Maximum finger tapping speed. P, Grooved Pegboard test. V, Visuomotor tracking task.

Figure 2. Average EMG traces and CUSUM characteristics from a typical subject in Study two. A, Relaxed and active motor evoked potentials at baseline and 30 mins after real repetitive transcranial magnetic stimulation (rTMS) delivered during a voluntary contraction. Each trace is the average of 15 individual trials. Single ended arrows indicate the timing of single transcranial magnetic stimuli (TMS). B, Average EMG and CUSUM characteristics during the first 2-min epoch of rTMS during voluntary contraction. A CUSUM event was significant if the vertical distance between two consecutive CUSUM turning points (i.e. EMG that crosses the pre-stimulus average; lower horizontal dotted line) was more than 100% of the maximum pre-stimulus variation (upper two horizontal dotted lines). The latency of the significant CUSUM
event was defined as the time of the turning point that initiated the significant event. The duration of the significant CUSUM event corresponded to the horizontal distance between the latency and the next turning point. The strength of a significant CUSUM event corresponded to the vertical distance between two CUSUM turning points and was expressed as a percentage of the largest possible significant event (dashed line).

Figure 3. Raw and processed force and acceleration traces in one subject during a grip and lift trial. A, Raw lift (upper panel), acceleration (middle panel), and grip (lower panel) traces. Vertical dotted lines represent the boundary of the lift phase and hold phase. B, Rate of change in lift force (dLF/dt; upper panel) and grip force (dGF/dt; lower panel). C, The temporal relation between grip force and lift force was assessed by cross-correlating dGF/dt and dLF/dt. Vertical dotted line represents the time shift required to achieve the maximum cross-correlation coefficient.

Figure 4. Group data showing the average resting motor evoked potential (MEP) area before and after repetitive transcranial magnetic stimulation (rTMS). A, Study one (rTMS during relaxation). In experiment one, MEP area was significantly reduced for 15 mins after rTMS (black triangles; P<0.011). In experiment two, MEP area was measured prior to voluntary contraction (white triangles) and remained unchanged after rTMS. B, Study two (rTMS during voluntary contraction). MEP area was significantly larger in the sham rTMS experiment (experiment two; white circles) than in the real rTMS experiment (experiment one; black circles; P=0.045). C, Study three (rTMS during voluntary contraction). Without functional tasks, MEP area was suppressed for 30 mins after real rTMS (experiment one; black circles; P<0.05). With functional tasks,
MEP area was unchanged after real rTMS (experiment two; black squares) and sham rTMS (experiment three; white squares). MEPs are expressed as a percentage of baseline (B).

Figure 5. Group data for Study three showing two parameters measured during the object grip and lift task. The grip and lift task was performed before (white bars) and after (black bars) real or sham repetitive transcranial magnetic brain stimulation (rTMS) during voluntary contraction. A, Time shift required to achieve the maximum cross-correlation coefficient during the lift phase of the object grip and lift task. The time shift represents the time difference between the change in lift force relative to grip force and is an index of whether the grip strategy was primarily anticipatory (negative time shift) or reactive (positive time shift). There was a trend for an experiment-by-time interaction on time shift (P=0.06). B, Mean grip force relative to mean lift force during the hold phase of the object grip and lift task. There was a trend for an experiment-by-time interaction on mean grip force relative to lift force (P=0.098).

Figure 6. Single subject and group data showing performance during the visuomotor tracking task. A, Raw metacarpophalangeal (MCP) joint angle trace (dotted line) and target line (solid line) in one subject during a single 10 s frame. B, Corresponding cross-correlogram for the same subject and 10 s frame. Dashed line represents maximum cross-correlation coefficient and resultant lag time. C-E, Group data showing performance during the visuomotor tracking task performed before and after real (black circles) or sham (white circles) rTMS during voluntary contraction. C, Absolute tracking error (degrees). D, Maximum cross-correlation coefficient. E, Lag time.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.