Preparatory suppression of the human primary motor cortex induced by repetition of simple and choice reaction time tasks: A transcranial magnetic stimulation study

Hiroshi Kinoshita\textsuperscript{a,*}, Susumu Yahagi\textsuperscript{b}, Tatsuya Kasai\textsuperscript{c}

\textsuperscript{a}Graduate School of Medicine, University of Osaka, 1-17 Machikaneyama-cho Toyonaka City, Osaka 560-0043, Japan
\textsuperscript{b}Department of Sports Sciences, Hiroshima Shudo University, Hiroshima, 731-3195, Japan
\textsuperscript{c}Division of Sports and Health Sciences, Graduate School for International Development and Cooperation, Hiroshima University, 1-5-1 Kagamiyama, Higashihiroshima 739-8529, Japan

\textbf{Abstract}

Transcranial magnetic stimulation was performed to investigate preparatory suppression of activity in the human primary motor cortex (M1) in relation to trial repetition of simple (SRT) and Go/NoGo choice RT (CRT) tasks. These tasks were performed in such a way that after a warning signal, the subjects (N=16) maintained 5\% MVC isometric finger force against the force sensor to secure a facilitated state of M1. A response signal to generate pulsed force came at 2 s after the warning signal. TMS was given 1.5 s after the warning signal, and the amplitudes of motor-evoked potentials (MEPs) in the first dorsal interosseous muscle were evaluated during 30 repetitive trials over 3 sessions for each subject. For the SRT task, the MEP amplitude was significantly decreased from baseline values in all trials of the three sessions. For the CRT task, on the other hand, there was a clear decreasing trend of the MEP amplitude with trial at the first and second sessions. The mean MEP amplitude at the first session was clearly higher than the baseline while it decreased significantly and reached the value below the baseline at the third session. The findings indicate that active suppression of M1 activity is involved in the preparatory state for RT tasks and that the degree of this suppression can relate to trial experience. The effect is thus most likely a consequence of a rapid adaptive change with the central nervous system in optimizing the preparatory state of M1 for the upcoming motor response.

\section{Introduction}

The primary motor cortex (M1) has been considered to play some role(s) in motor preparation. Findings from monkey experiments indicated that while the monkey was waiting for an imperative signal to quickly respond with a simple limb movement after a warning signal, activity of neurons located in M1 could be detected without any overt muscular activity (Alexander and Crutcher, 1990; Boussaoud and Wise, 1993; Riehle, 2005). Investigation of human M1 activity and associated spinal excitability during the foreperiod or preparatory period (PP) for simple reaction time (SRT) and choice reaction time (CRT) tasks has been done by single pulse transcranial magnetic stimulation (TMS) to the completely relaxed target
muscle (Hasbroucq et al., 1997, 1999; Touge et al., 1998) and spinal H-reflex measurement (Brunia et al., 1982; Touge et al., 1998). Touge et al. (1998) compared TMS and H-reflex records during PP and reported that changes in the motor-evoked potential (MEP) of the agonist muscle largely reflected the level of cortical, rather than spinal, activation. The findings from human TMS studies commonly indicated that preparation for a short duration (0.5 s) PP involved a decreased state rather than an increased state of corticospinal excitability, and thus it differed from effects observed during animal studies. Touge et

Fig. 1 – Results of the SRT and NR experiments. Representative MEP specimen records of four (1st, 3rd, 6th, and 9th) trials in a single subject in each session (A). Changes in the mean and standard deviation values of normalized MEP amplitude over the ten trials in the first session for all subjects (B) and means and standard deviations of the normalized MEP amplitude (C) and background EMG (D) for all subjects in each session.
al. also reported that when the PP was extended to 2.0 s, amplitudes of the MEP and the H-reflex were unchanged, though these results were based on the data from only 2 subjects. Brunia et al. (1982) also reported no modulation of the H-reflex during a 4-s PP. Hasbroucq et al. (1999) postulated that the decreased MEP amplitude during the PP for RT tasks reflected an adaptive mechanism to increase the sensitivity of the corticospinal pathway to the forthcoming voluntary command.

One problem with this assumption is that none of the previous researchers has demonstrated the adaptive changes in cortical excitability during PP for SRT and CRT tasks. Using single-pulse TMS on normal human subjects, we attempted to address this problem by examining changes in MEP amplitude during PP in relation to trial repetition of SRT and Go/NoGo CRT isometric abduction force production tasks performed by the index finger. These tasks were performed in such a way that after a warning signal, the subjects maintained isometric abduction force at the level of 5% of maximum voluntary contraction (MVC) to induce contraction of the first interosseous (FDI) muscle. This differentiated from the completely resting muscular condition during the PP in the previous studies (Hasbroucq et al., 1997, 1999; Touge et al., 1998). Earlier Di Lazzaro et al. (1998) as well as Stedman et al. (1998) demonstrated that at a lower level of contraction by the FDI muscle, the MEP modulations demonstrated the level of supra-spinal activation and predominately reflected M1 excitability (see also Abbruzzese and Trompetto, 2002). The maintenance of M1 excitability secured at a similar level above the threshold should provide a stable baseline of preparatory M1 activity in every trial. It would also allow monitoring of a wider range of possible adaptive reduction in MEP amplitude from an initial naive state to the adapted and optimized state after repetitions of the trials. The effect of repetitive trials in a task with no overt motor response (NR) following PP was also examined in the present study to test if TMS alone could modulate the MEPs.

2. Results

2.1. SRT and NR tasks

Fig. 1A shows representative MEP specimen records of separate four trials from one subject at the first, second and third sessions for the SRT and NR tasks. Note that there were nearly no changes in MEP amplitude for both tasks across all three sessions. To show the changes in MEP amplitude of each trial within a session, we plotted trial means and SDs for all subjects at the first session in Fig. 1B. Again, there was no clear trend of changes in the MEP amplitude across trials.

Table 1 presents the mean values of MEP amplitude and background EMG both in millivolts for each condition. In Figs. 1C and D, the mean values of normalized MEP amplitude and background EMG for all subjects for each session are plotted, respectively. The normalized MEP amplitude for the SRT was around 80% of the baseline (control) value, and it was nearly constant across the sessions. The normalized MEP amplitude for the NR task was close to the control value at all three sessions. These normalized data were used for the subsequent statistical analysis. Two-way ANOVA with repeated measures revealed a significant task difference (SRT<NR) in the MEP amplitude (F_{2,14}=38.13, p<0.001). Neither session×task (F_{2,14}=0.76, p=0.49) nor session (F_{2,14}=0.40, p=0.67) effect was significant. Background EMG did not differ among the sessions for the SRT (F_{2,14}=0.79, p=0.47) and NR (F_{2,14}=0.45, p=0.64) tasks.

The mean values of RT for all subjects for the SRT task at the first, second and third sessions were 250±46 (SD), 247±26 ms and 240±30 ms, respectively. No statistical difference was found among these means (F_{2,14}=0.15, p=0.86).

2.2. CRT task

Typical examples of MEP records for the CRT task in one subject at the three sessions are shown in Fig. 2A. These records clearly show that MEP amplitude decreased with an advance in session. The mean trial data for all subjects in each of the three sessions are plotted in Fig. 2B. Note that the very first trial had the largest mean value, which gradually decreased with trial in the first and second sessions. Within each session, one-way ANOVA with repeated measures was performed, which revealed significant differences among the trial means for the first (F_{9,63}=2.86, p=0.007) and second (F_{9,63}=4.81, p<0.001) sessions, but not for the third session (F_{9,63}=0.69, p=0.71). A Tukey’s post hoc comparison was performed to determine the difference in the mean values within each session. For the first session, the initial trial mean was significantly higher than the 4th, 5th, 7th, 9th and 10th means, and the 2nd trial mean was also higher than the 5th and 9th trial means (Fig. 2B). For the second session, the initial trial mean was significantly higher than all the subsequent trial means except for the 5th trial mean.

The mean values of normalized MEP amplitude for all subjects for the three sessions are shown in Fig. 2C, and their non-normalized means in millivolts are also given in Table 1. The mean MEP amplitude at the first session was 1.3 mV, and the corresponding normalized value was 130%. For the second session, the initial trial mean was significantly higher than all the subsequent trial means except for the 5th trial mean.

Table 1 - Means and standard deviations of MEP amplitude and background EMG in millivolts for each session

<table>
<thead>
<tr>
<th>Task</th>
<th>Variable</th>
<th>First session</th>
<th>Second session</th>
<th>Third session</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRT</td>
<td>MEP amplitude</td>
<td>0.65±0.14</td>
<td>0.68±0.19</td>
<td>0.64±0.23</td>
</tr>
<tr>
<td></td>
<td>Background EMG</td>
<td>5.99±1.13</td>
<td>6.00±1.00</td>
<td>5.88±0.75</td>
</tr>
<tr>
<td>NR</td>
<td>MEP amplitude</td>
<td>1.03±0.60</td>
<td>1.08±0.40</td>
<td>1.14±0.59</td>
</tr>
<tr>
<td></td>
<td>Background EMG</td>
<td>5.21±0.98</td>
<td>5.51±1.46</td>
<td>5.38±1.12</td>
</tr>
<tr>
<td>CRT</td>
<td>MEP amplitude</td>
<td>1.31±0.64</td>
<td>1.18±0.81</td>
<td>0.84±0.36</td>
</tr>
<tr>
<td></td>
<td>Background EMG</td>
<td>5.93±2.39</td>
<td>5.46±2.86</td>
<td>5.54±2.45</td>
</tr>
</tbody>
</table>
the normalized data of the MEP amplitude and background EMG as two dependent variables and session as an independent variable. There was a significant session effect (Wilks’ lambda = 0.43, approximated $F_{2,26} = 3.32$, $p = 0.025$). Separate one-way ANOVAs with repeated measures were then performed for each of the background EMG and normalized MEP amplitude data. It was found that the session effect was insignificant for the background EMG ($F_{2,14} = 1.58$, $p = 0.24$). For the MEP amplitude, on the other hand, the session effect was significant ($F_{2,14} = 6.11$, $p = 0.01$). A Tukey’s post hoc test for this variable confirmed that only the difference between the first and third sessions was significant ($p = 0.01$).

**Fig. 2** – Results of the CRT experiments. Representative MEP specimen records from four (1st, 3rd, 6th, and 9th) trials in a single subject in each session (A). Changes in mean and standard deviation values of normalized MEP amplitude over the 30 trials in the three sessions for all subjects (B). Trials from 1st to 10th, from 11th to 20th, and from 21st to 30th correspond to the first, second and third sessions, respectively. The asterisks indicate the results of Tukey’s post hoc comparisons ($p < 0.05$). Changes in the means and standard deviations of the normalized MEP amplitude (C) and background EMG (D) for all subjects in each session. The asterisk indicates the results of ANOVA ($p < 0.05$).
The RT could be obtained only for the Go condition. The mean values for all subjects were 445 ± 116, 472 ± 117 and 457 ± 117 ms at the first, second and third sessions. ANOVA revealed no difference among these means.

3. Discussion

Touge et al. (1998) earlier used the resting MEP amplitude as a baseline to examine time-dependent changes in MEP amplitude during short 0.5-s PP for the SRT and CRT wrist movement tasks. They found a clear reduction in MEP amplitude when stimuli were delivered at least 250 ms after the warning signal. They also tested the effect of longer warning period (2 s) in two of the subjects and found no changes in MEP amplitudes measured at every 0.5 s from the warning signal. They postulated that this was due to a greater difficulty of precisely estimating the response time compared to that for the 0.5-s PP. When the task was changed so that their subjects did not know whether to flex or extend the wrist until the imperative signal was given (i.e. a CRT task), then there was also very little change in the MEP in flexor or extensor muscles during the course of the warning period. They stated that the decrease of M1 excitability during PP could be dependent on the combination of knowing what to do and precisely when to do it. Similarly, Takei et al. (2005) have recently reported that the MEP amplitude decreased from rest during predictable and semi-predictable TMS timing, while the amplitude did not decrease when the timing of TMS was totally unpredictable. Hasbroucq et al. (1997) hypothesized that the decrease of MEP amplitude during the PP could reflect an adaptive mechanism that includes the sensitivity of the corticospinal tract to the forthcoming voluntary command. This mechanism therefore allows filtering of task-unrelated afferents to motor structures, thereby facilitating the interpretation of voluntary commands by increasing the signal-to-noise ratio.

In the present study, we investigated the adaptive reduction of MEP amplitude in the weakly activated target FDI muscle during the 2-s PP for the SRT and Go/NoGo CRT tasks. It was found that from the initial SRT trial, the MEP amplitude was already lower than the pre-activated level. There was no such reduction of MEP amplitude under the NR condition where the subjects did not make an effort at motor preparation. The findings of these experiments suggest that the subjects had already learned the timing of the response signal before the start of the actual SRT experiment. The sources of learning can be then a few times of the preparatory practice trials and can also be the observation of demonstration trials. Whatever the reasons, the storage of response timing for the SRT task seems to occur very quickly.

A clear reduction in MEP amplitude between the SRT and NR tasks confirmed that M1 activity had been strongly suppressed during the PP for upcoming motor response. The results of the CRT experiment, which presented a more attention- and decision-demanding task, and therefore less prepared initially than the SRT task, showed that MEP amplitude was indeed higher during the initial trials compared to the baseline. This effect, however, gradually decreased over 30 repetitions of the same CRT task, and therefore the mean value of MEP amplitude at the third session was significantly smaller than that at the first session. This indicates that with an adequate number of trials, a complex RT task is no exception to show a clear reduction in M1 excitation during PP. Interestingly, the relative value of the MEP amplitude at the third session regressed toward the level similar to that observed for the SRT experiment performed by the other group of the subjects, indicating that some optimized level of the sensitivity of M1 to the forthcoming motor response may present.

The background EMG at the initial session was slightly elevated compared to the subsequent sessions. Therefore, there was a decreasing trend of their means over the three sessions though these differences were insignificant. A small decrease in the background EMG upon repetition of a similar finger abduction task has also been reported by Yahagi et al. (2005). It is well known that the level of voluntary contraction of a target muscle relates closely to the magnitude of MEP amplitude (Hess et al., 1987; Yahagi et al., 2005). It is also known that at a lower level of voluntary contraction of the FDI muscle, modulations of MEP amplitude mainly reflect M1 excitability (Di Lazzaro et al., 1998, Stedman et al., 1998). A portion of the larger MEP amplitude observed at the initial session in the present study thus could have been attributed to this M1 activity due to a slightly elevated background muscular contraction.

A functional role of M1 in motor preparation has been suggested in numerous studies with monkeys (e.g., Alexander and Crutcher, 1990; Churchland et al., 2006; Riehle, 2005) as well as humans (e.g., Connolly et al., 2007; Cunnington et al., 2005; Deiber et al., 1996; Lee et al., 1999; Leuthold and Jentsch, 2001; Rushworth et al., 2003; Tandonnet et al., 2003). In monkeys, Alexander and Crutcher (1990) found motor preparatory-related activity in neurons of the anterior M1 and a fewer number of neurons in the posterior M1. In humans, Geyer et al. (1996) reported that M1 has anterior and posterior subdivisions with different cytoarchitectures and distributions of transmitter-binding sites. These separate M1 regions appear to process different motor-associated functions in parallel while communicating with the relevant cortical and subcortical areas. These may include the anterior supplementary motor area (pre-SMA) and the cingulate motor area (CMA) that assemble motor programs for their implementation by M1 in concert with the posterior SMA and premotor cortex (PMC) (e.g., Lee et al., 1999; Picard and Strick, 1996), as well as areas in the frontal and parietal cortices responsible for visual, auditory and motor attention (Rowe et al., 2002; Rushworth and Taylor, 2006). The observed gradual MEP amplitude reduction with a repetition of trials suggests an active allotment of these functions from M1 to the other cortical areas as learning of the spatiotemporal features of the present Go/NoGo task proceeds (Connolly et al., 2007). Findings from the electroencephalograph and functional MRI studies have suggested that the candidate cortical areas are the anterior SMA and CMA (Cunnington et al., 2005; Weilke et al., 2001). Improved predictability for the timing of the response signal inevitably occurs with trial repetitions, and thus these secondary motor areas may play a greater role during PP upon learning of the CRT task.

An essential mechanism that could have been involved in the suppression of M1 excitability during the PP is the pathway from the inferior frontal cortex, via the basal ganglia, to intra-
cortical inhibitory networks within M1 (Aron and Poldrack, 2006; Coxon et al., 2006). Using functional MRI technique, Aron and Poldrack (2006) have recently shown that the subthalamic nucleus in the basal ganglia exerts a top-down influence for effective inhibitory control of the direct fronto-striatal pathway that is activated by response initiation. The decrease in M1 excitability with trial repetition thus could be related to optimization of these mechanisms to support rapid prevention of the prepared movement when required.

In conclusion, a clear reduction of preparatory MEP amplitude occurred within a relatively small number (~30) of the trials for the time-fixed RT tasks performed from the pre-activated state of the FDI muscle in humans. The findings thus indicate trial-dependent adaptive reduction in M1 excitability during PP. The preparatory MEP amplitude in both SRT and CRT tasks regressed toward some value clearly below the baseline, suggesting that there is some level of optimally reduced excitability in M1 when preparing for the upcoming motor response. Further research, using pair-pulse TMS technique, should be conducted to assess the effects of intracortical inhibition and facilitation on this short-term adaptive suppression of M1 excitation during PP. A study by Zogihi et al. (2003) suggests that if the conditioning stimulus intensity is below the active motor threshold, it is possible to measure intracortical inhibition in M1 from the pre-activated intrinsic hand muscle.

4. Experimental procedure

4.1. Subjects

The SRT and NR tasks and CRT task were tested in separate experiments using two groups of right-handed subjects who were free from neurological or orthopedic problems. None of the subjects had previously participated in either a TMS study or RT experiment. The first group consisted of six males and two females (23–45 years old), and they were assigned to the
SRT and NR experiments. The second group consisted of seven males and one female (24–32 years old), and they did the CRT experiment. An Edinburgh MRC Handedness Inventory was performed for each of the subjects to confirm their hand dominance (Oldfield, 1971). The subjects were informed as to the purpose of the research and experimental procedures in advance, and all gave their written informed consent, as required by the Helsinki Declaration. The study was then approved by the Ethics Committees of Hiroshima University.

4.2. Experimental apparatus

The experimental setup consisted of a Magstim 200 stimulator (Magstim co., UK) with a figure-of-eight shaped magnetic coil (diameter of each wing 9.5 cm), a load cell (LM-5KA, Kyowa co., Japan), a strain amplifier (DPM 6-1A Kyowa co., Japan), an electromyography (EMG) amplifier (AB-621G, Nihonkohden co., Japan) and an analogue–digital converter interfaced with an NEC personal computer. The load cell was firmly fixed at the tip of a metal rod, and its height could be adjusted so that the tip of the index finger could be matched to the load cell center for each subject (see Fig. 3A). The load cell had a measurement range of 0 to 100 N within a 1% error in linearity with resolution of 0.025 N. Using this equipment, changes in the finger force and EMG activity were recorded during the experiments.

4.3. EMG recordings

Surface EMG activity was recorded from the right FDI muscle using disposable electrodes and the standard surface EMG recording method. The active electrodes were mounted on the muscle belly, while the inactive electrode was placed over the base of the metacarpophangeal joint of the thumb (Fig. 3A). Raw EMG signals were bandpass-filtered between 5 Hz and 3 kHz and sampled at 5 kHz.

4.4. Cortical stimulation and MEP recording

Using the stimulator, focal single pulse TMS was performed by one of the present authors. The coil of the stimulator was held tangentially to the skull with the handle positioned backwards. Each subject wore a swimming cap so that the optimal scalp position could be marked in response to TMS. Stimulation intensity was then set sufficiently to evoke MEP responses in the right FDI muscle. The resting motor threshold was determined at the optimal site as the stimulator intensity needed to produce a response of 50 μV in the relaxed FDI muscle in at least five of ten consecutive trials at a resolution of 2–3% of the maximal stimulator output. This TMS intensity was referred to as the motor threshold at rest (1.0× MT). Thereafter, the stimulator intensity sufficient to evoke peak-to-peak amplitudes of 1.0–1.5 mV in the pre-contraction of the FDI muscle was determined for testing (see Ziemann et al., 1996).

4.5. Preparatory period and TMS time

In the present study, the duration of PP (i.e.; the interval between a warning signal and the response signal) was fixed to 2 s in all trials (Fig. 3B). This duration was chosen based on the findings of our previous study that examined the effect of varied durations of PP on SRT (Yuanhui and Kasai, 1993). As for the TMS timing during PP, Keller and Heckhausen (1990) reported that the readiness potentials associated with preparation for voluntary movement commonly start approximately 1 s prior to the onset of movement. We therefore recorded MEPs 500 ms before the Go signal was generated (Fig. 3B). The amplitude of MEP was computed from the obtained data for each trial for each subject. RT was determined by the duration between the response signal and force onset (1 SD above the mean force during 100 ms after the response signal).

4.6. Data acquisition

During all experimental sessions, the subjects were seated in a height-adjustable chair and faced a test table. The subject’s right upper arm was parallel with their torso, and the forearm was extended anteriorly. The hand was held in a half-prone position, and the tip of the right index finger lightly touched the surface of the load cell. Under these postural conditions, maximum isometric pinch force against the load cell for 3 s was first measured for each subject. The force level corresponding to 5% of the maximum voluntary contraction (5% MVC) was then determined for each subject. The subjects practiced maintaining this level of index finger abduction force by viewing an oscilloscope placed in front of them until they were confident of performing this preparatory force production.

4.6.1. SRT and NR experiments

The subjects in the first group performed the SRT and NR tasks and the task order was randomized for each subject. Following the observation of several demonstration trials performed by one of the experimenters, practice trials (~2 trials) without TMS were performed by each subject. A hot-spot for TMS was then determined for each subject. In the SRT task, the subjects were instructed to generate a 5% MVC preparatory force after hearing the warning signal (500 Hz, 600 ms duration). Two seconds after this signal, an auditory “Go signal” (500 Hz, 300 ms duration) was given for the subjects to generate a target force (10% MVC) isometrically in a step mode (Fig. 3B). After the practice trials, the subjects rested for about 3 min, and they performed 30 trials in three sessions. There were about 8 s between trials and a 2-min rest between sessions.

In the NR task, the subjects were also instructed to generate an index finger abduction force at 5% MVC after hearing the warning signal (500 Hz, 600 ms duration), but this time there was no “Go signal”. The subjects maintained the same force level for at least 3 s after the warning signal until the experimenter gave an oral command to terminate the force generation. This task was also performed over 30 trials in three sessions by each subject.

4.6.2. CRT experiment

In the CRT experiment, a Go/NoGo paradigm was introduced (Fig. 3C). After generating an index finger abduction force at 5% MVC during PP, one of two different warning sound signals (High tone or Low tone, H; 500 Hz and 600 ms duration or L; 166 Hz and 300 ms duration, respectively) was given. When the response signal was presented as the same tone as the warning
signal, the subjects had to respond to the prepared motor response as quick and accurately as possible (Go task). If the response signal was different from the preceding warning signal, the subject had to remain in a pre-contraction state with no motor response (NoGo task) (see Fig. 3C). The appearance of probability in the Go and NoGo tasks was set at 50% each. Following one or two practice trials and a rest of about 3 min, data of 30 trials in three sessions were collected for each subject. There were about 8 s between trials and a 2-min rest between sessions. Trials in which an incorrect response was reported by the subject or the experimenter’s visual inspection of the collected data were excluded, and a new trial was added.

### 4.6.3. Normalization of the MEP data

For both the SRT/NR and CRT experiments, in order to normalize the inter-subject variation, control data of MEP amplitude and background EMG were obtained from 5 to 8 trials with 5% MVC without response signals prior to the first session as well as after the third session (see “control” in Fig. 3A). All MEP and background EMG data were then normalized for each subject by dividing by the corresponding control data.

### 4.6.4. Reaction time

The reaction time was taken as the interval between the onset of the response signal and the onset of finger force (a threshold = ±1 SD of the values during PP).

### 4.7. Statistical analysis

Depending of the purpose of the comparison, we used one-way, two-way ANOVA with repeated measures, and MANOVA. Dependent variables used were MEP amplitude or RT, and independent variables were session or trial. When the omnibus F-value was significant, Tukey’s post hoc multiple comparisons were performed to allocate the position(s) of the significant difference between the two means. Statistical significance was accepted at p < 0.05.

### REFERENCES


