Excitability changes in the primary motor cortex just prior to voluntary muscle relaxation

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16 **Abstract**

17 We postulated that primary motor cortex (M1) activity does not just decrease immediately prior
18 to voluntary muscle relaxation; rather, it is dynamic and acts as an active cortical process. Thus, we
19 investigated the detailed time course of M1 excitability changes during muscle relaxation. Ten healthy
20 participants performed a simple reaction-time task. After the go signal, they rapidly terminated isometric
21 abduction of the right index finger from a constant muscle force output of 20% of their maximal voluntary
22 contraction force and performed voluntary muscle relaxation. Transcranial magnetic stimulation pulses
23 were randomly delivered before and after the go signal, and motor evoked potentials (MEPs) were
24 recorded from the right first dorsal interosseous muscle. We selected the time course relative to an
25 appropriate reference point, the onset of voluntary relaxation, to detect excitability changes in M1. MEP
26 amplitude from 80 to 60 ms before the estimated electromyographic offset was significantly greater than
27 that in other intervals. Dynamic excitability changes in M1 just prior to quick voluntary muscle relaxation
28 indicate that cortical control of muscle relaxation is established through active processing, and not by
29 simple cessation of activity. The cortical mechanisms underlying muscle relaxation need to be reconsidered
30 in light of such dynamics.

31

32 ***Keywords:***

33 Transcranial magnetic stimulation, muscle relaxation, motor cortex, motor evoked potential,
34 electromyography

35

36 **INTRODUCTION**

37 The control of coordinated movement requires that muscle contraction and relaxation be
38 smoothly and repetitively alternated. That is, appropriate muscle relaxation is a prerequisite for smooth
39 muscular action and is an important factor in motor control as well as in muscle contraction. Nevertheless,
40 there are fewer studies on muscle relaxation than on muscle contraction, and the cortical mechanism
41 underlying muscle relaxation is unclear.

42 A number of clinical conditions featuring disordered control of muscle relaxation exist, for
43 example, hypertonia accompanying central nervous system disorders. In patients with hemiplegia,
44 voluntary movement can be disabled by increased muscle tone (spasticity) and compensatory adaptation
45 can produce unwanted activation of the antagonist muscles (co-contraction) and synkinetic movements
46 (Burke et al. 2013). Clarifying the mechanism controlling muscle relaxation will aid in preventing such
47 pathological muscle contraction.

48 Electroencephalographic (EEG) studies to investigate the physiology of muscle relaxation
49 control have shown that cortical activation similar to that for voluntary muscle contraction occurs prior to
50 voluntary muscle relaxation (Labyt et al. 2006; Terada et al. 1995; Terada et al. 1999; Yazawa et al. 1998),
51 and that EEG activity during muscle relaxation partly depends on the particular relaxation task used
52 (Rothwell et al. 1998).

53 Additionally, an event-related functional magnetic resonance imaging (fMRI) study suggested
54 that the primary motor cortex (M1) contralateral to the effector and bilateral supplementary motor areas

55 (SMA) are commonly activated in preparation and execution phases of both muscle relaxation and
56 contraction (Toma et al. 1999). Thus, it is believed that, like muscle contraction, voluntary muscle
57 relaxation is controlled by an active cortical process.

58 However, these techniques could not be used to closely investigate the time course of any
59 facilitatory or inhibitory changes in focal brain regions during motor control because of the limited
60 temporal resolution of fMRI and the limited spatial resolution of EEG. In contrast, transcranial magnetic
61 stimulation (TMS) has good temporal resolution and lends itself to analyzing cortical activation changes
62 (particularly M1 changes) at intervals of milliseconds, from the presentation of the go signal to the
63 execution of muscle relaxation.

64 A previous study using paired-pulse TMS techniques showed that before the onset of relaxation,
65 M1 activity started to decline and an increase in short-interval intracortical inhibition (SICI) occurred
66 (Buccolieri et al. 2004a). This pattern of changes is contrary to that for muscle contraction (Reynolds and
67 Ashby 1999; Soto et al. 2010; Starr et al. 1988). Conversely, Begum et al. (2005) reported a decrease in
68 SICI prior to muscle relaxation; the differences in the results of these two studies may be due to the
69 different relaxation tasks employed. Recently, Motawar et al. (2012) reviewed these two studies and
70 revealed that the disparity is partly due to the different paired-pulse TMS techniques used, and further
71 reported that SICI gradually increased along with the progress of muscle relaxation (i.e., not prior to
72 muscle relaxation).

73 However, these time course studies analyzed long and different periods in the transition from

74 muscle contraction to relaxation. Hence, their findings are insufficient for clarifying phasic M1 excitability

75 changes related to voluntary muscle relaxation.

76 Our focus in this study was motor control just prior to muscle relaxation. We previously reported

77 that M1 was temporarily activated prior to muscle relaxation; however, we did not analyze M1 excitability

78 changes in detail (i.e., with a narrow time window) (Sugawara et al. 2009). Additionally, we had often

79 observed relatively large motor evoked potential (MEP) amplitudes when the TMS pulse had been

80 delivered just prior to muscle relaxation (Sugawara K, unpublished observations).

81 From this viewpoint, we hypothesized that during muscle relaxation, M1 activity does not only

82 gradually decrease to attenuate muscle contraction, but also dynamically changes, thus acting as an active

83 cortical process that evokes the transition from contraction to relaxation. The discrepancy between

84 previous studies may be due to the dynamic state just prior to muscle relaxation not having been

85 sufficiently investigated. Therefore, this study analyzed the detailed time course of M1 excitability changes

86 just prior to voluntary muscle relaxation to understand cortical control during this time.

87

88 **METHODS**

89 *Participants*

90 The participants were 10 students (five men and five women aged 20–23 years) from Kanagawa

91 University of Human Services. All participants were right-handed according to their scores on a

92 handedness questionnaire (Chapman and Chapman 1987). The mean score was 13.3 and the standard

93 deviation was 0.7. None of the participants had any history of neuromuscular or physical functional
94 impairment that may have affected task performance. All participants gave their informed consent before
95 the experiment. This study was conducted with the approval of the Research Ethics Committee of
96 Kanagawa University of Human Services.
97
98 *Experimental Paradigm*
99 In this study, we used a simple reaction time (RT) paradigm for a voluntary muscle relaxation
100 task. The participants sat comfortably on a chair with their right forearm pronated and digits extended on a
101 table. The distal interphalangeal joint of the right index finger was positioned at the middle of a hard metal
102 plate. A strain gauge (Kyowa Electronic Instruments Co., Tokyo, Japan) was mounted on the vertically
103 bent portion of this plate (Figure 1). The analog signal was amplified (SA-250 STRAIN AMPLIFIER;
104 TEAC, Tokyo, Japan), filtered, and digitized (NI USB-6229 BNC; National Instruments Corp., Austin,
105 Texas, USA). These data were entered into a laboratory computer and presented as the cursor on a liquid
106 crystal display monitor in front of the participant, using LabVIEW (LabVIEW2009; National Instruments
107 Corp.). In short, the cursor was moved in real time by the in-progress force output against the strain gauge.
108 Before initiating the experiment, we measured the abduction force exerted against the plate by
109 each subject's maximal voluntary contraction (MVC) of the first dorsal interosseous muscle (FDI). At the
110 onset of an acoustic warning signal, the participants were required to perform an isometric abduction of the
111 right index finger at 20% of MVC, pressing steadily against the plate while self-controlling their

112 performance by observing the cursor and the target line (20% MVC for each individual). After an acoustic
113 go signal was presented, the participants were required to terminate isometric contraction (that is, initiate
114 muscle relaxation) as quickly as possible. The interval between the warning and go signals was 3000–5000
115 ms, which was randomized using LabVIEW. The participants were instructed to press the plate with the
116 abduction force of the index finger only and not to perform any voluntary movements when relaxing their
117 FDI. In this motor task, finger joint motion generally does not occur when participants perform muscle
118 contraction or relaxation because the lateral surfaces of their index and little finger are fixed on each hard
119 metal plate. Before any data was collected, participants practiced the task until they were able to perform it
120 correctly.

121 The experiment consisted of two sessions: with and without TMS pulses. The session with the
122 TMS pulses was 140 trials long and programmed in LabVIEW so that each TMS pulse was triggered
123 randomly between 30 ms before and 130 ms after the go signal (Figure 1). Additionally, the latter TMS
124 pulse timing was adjusted according to each subject's RT during the experiment. The session without TMS
125 pulses was conducted to analyze the offset of the electromyographic (EMG) signals and force curve data
126 without contamination by the TMS pulse. It consisted of three sessions of 10 trials each at the beginning,
127 middle, and end of the experiment.

128

129 *Measurements*

130 Surface EMGs in a belly-tendon montage were recorded from the right FDI using disposable

131 bipolar silver/silver chloride surface electrodes 10 mm in diameter. The raw signal was amplified and
132 filtered (band-pass 5–3000 Hz) using a bioelectric amplifier (Neuropack MEB-2200; Nihon Kohden Corp.,
133 Tokyo, Japan), digitized at 4000 Hz, and stored for offline analysis on a laboratory computer (Power Lab
134 system; AD Instruments Pty Ltd., New South Wales, Australia).

135 TMS was delivered using a Magstim 200 (Magstim Co., Dyfed, UK) stimulator attached to a
136 figure-of-eight-shaped coil with an internal wing diameter of 9 cm. The coil was placed with the handle
137 pointing backwards, laterally at 45° from the midline, and approximately perpendicular to the central
138 sulcus to evoke anteriorly directed current in the brain; it was optimally positioned to produce MEPs in the
139 contralateral FDI. Surface markings drawn on a swim cap placed on the scalp served as a reference for coil
140 positioning. The active motor threshold (aMT) was defined as the lowest stimulus intensity producing
141 MEPs greater than 200 μV in at least 5 of 10 successive trials during isometric contraction of the tested
142 muscle (Rossini et al. 1994). For experiments, the intensity of TMS was set to 1.2 × aMT.

143 We calculated the offline peak-to-peak amplitudes of all MEPs of the right FDI using Lab Chart
144 7 software (AD Instruments Pty Ltd). In addition, to assess the EMG activity of the FDI during the 20%
145 MVC periods when the TMS pulse was delivered, we calculated the root mean square (RMS) value of
146 background EMG activity for a 20-ms period before the TMS pulse. Auditory cue presentation and TMS
147 output were controlled using LabVIEW.

148

149 *Time Course Analysis*

150 For analyzing the time course of MEP variations, we adopted a time-zero reference point for the
151 onset of voluntary relaxation that we felt was more appropriate than in previous studies: the offset of
152 voluntary EMG. First, similarly to a previous study (Begum et al. 2005), we calculated the average RTs
153 from the go signal to the offset of EMG in control trials without TMS. Then, this time was added together
154 with the time of the go signal in each trial with TMS, defined as the average RT, and used as a reference
155 point in each trial with TMS (A in Figure 2). Because the beginning of the decline in an EMG signal is
156 difficult to estimate, we visually evaluated the time it took for an EMG signal to decrease to the baseline
157 level and set this as the offset of EMG, as in Buccolieri et al. (2004a).

158 Ideally, the reference point should be based on the offset of EMG measured in each trial.
159 However, because an EMG signal is contaminated with the MEPs elicited by TMS, the offset of EMG
160 cannot be detected. A plausible solution would have been to estimate the offset of EMG based on the EMG
161 signal of a non-target muscle in a bilateral and simultaneous relaxation task (Buccolieri et al. 2004a;
162 Sugawara et al. 2009). However, such a task would induce bilateral cortical activity and interaction
163 between the hemispheres, and our aim was to analyze purely unilateral cortical control.

164 Therefore, we attempted to define the reference point based on a force curve measured in each
165 TMS trial. Electro-mechanical delay can occur even with the use of a strain gauge or accelerometer, and
166 the decline of a force curve is difficult to estimate due to instability during sustained isometric contraction.
167 Thus, we examined the period immediately before the go signal in each trial in the 20% MVC condition.
168 We then calculated when the mean of a 200-ms period of force data decreased to 50% of the force curve

169 (i.e., the time point where force was reduced by half, hereafter referred to as force-curve halving; B in
170 Figure 2).
171 However, individual differences in the interval between the cessation of EMG activity and
172 force-curve halving are inevitable. Obviously, this difference will be affected by individual differences in
173 motor time (Weiss 1965) and the magnitude of the load against the metal plate. Moreover, it may also
174 depend on the form of the force decay curve, which differs between subjects.
175 Therefore, we calculated the average time from the cessation of EMG activity to force-curve
176 halving in control trials without TMS, and subtracted this time from the time of force curve halving in each
177 trial with TMS. We defined the time corrected in this way as a reference point: the estimated EMG offset
178 (the start of arrow C in Figure 2).
179
180 *Data Analysis and Statistics*
181 To analyze MEPs and the RMS background EMG statistically, these time course data were
182 binned into 20-ms intervals, and average MEPs and the RMS were calculated for each bin. The data
183 obtained within the 30 ms just after the go signal was excluded from analysis since it was assumed that this
184 section did not yet reflect changes related to the control of muscle relaxation. Consequently, the time
185 course data that was more than 100 ms before the estimated EMG offset was excluded from the statistical
186 analysis, because these data were difficult to collect because of the generally short RTs in each subject.
187 Additionally, based on the latency of MEPs (approximately 20 ms in this study), the data recorded less

188 than 20 ms before the estimated EMG offset was also excluded from our analysis. MEP amplitude had
189 already undergone a marked decrease by that time, indicating that the relaxation signal had already left the
190 cerebral cortex by then.

191 Accordingly, four consecutive 20-ms bins between 100 and 20 ms before the estimated EMG
192 offset (0 ms) were analyzed, and each bin was normalized to the average value prevailing before
193 presentation of the go signal. To analyze this single factor (time before offset), we used Mauchly's
194 sphericity test, one-way repeated-measures analysis of variance (ANOVA), and Bonferroni's post-hoc test
195 for multiple comparisons. All the statistical analyses were conducted using IBM SPSS statistics 20 for
196 Windows (SPSS Inc., Chicago, IL, USA). All statistical tests were two-sided, and statistical significance
197 was set at a value of $p < .05$.

198

199 **RESULTS**

200 Firstly, we expressed the timing of TMS pulses relative to the average RT calculated in control
201 trials without TMS. These time course data varied as a whole and decreased around 0 ms (A in Figure 3).
202 Secondly, we expressed the timing of TMS pulses relative to force-curve halving in each trial with TMS.
203 These time course data showed obviously greater MEP amplitudes concentrated in a particular localized
204 interval, but the time of peak amplitude was slightly different between subjects (B in Figure 3). In control
205 trials without TMS, the interval between the offset of EMG and force-curve halving also differed slightly
206 between subjects (mean, 103 ms; standard deviation, 17 ms). Thirdly, based on the estimated EMG offset

207 by correcting this difference, the MEP amplitude was largest between 80 and 60 ms before the estimated

208 EMG offset (C in Figure 3). Accordingly, these time course data were binned into 20-ms intervals between

209 100 and 20 ms before the estimated EMG offset (0 ms).

210 In a one-way repeated measures ANOVA, the assumption of sphericity was met (p = .057), and a

211 significant main effect in the average FDI MEPs was found for each bin ($F_{3,27}$ = 55.617, p < .001). These

212 multiple comparisons showed that the MEP amplitude from 80 to 60 ms before the estimated EMG offset

213 was significantly greater than that from 100 to 80 ms (p = .016), 60 to 40 ms (p = .001), and 40 to 20 ms (p

214 < .001). Moreover, MEP amplitude from 40 to 20 ms was significantly smaller than that from 100 to 80 ms

215 (p < .001) and 60 to 40 ms (p < .001, Figure 4). No significant difference in RMS background EMG was

216 found for any bin ($F_{3,27}$ = 2.079, p = .127).

217

218 **DISCUSSION**

219 We observed M1 excitability changes just before voluntary muscle relaxation from isometric

220 contraction by using a simple RT task. Our results agree with our hypothesis that M1 activity does not just

221 decrease prior to voluntary muscle relaxation. Instead, the cortical control system of relaxation seems to

222 originate not from inhibitory, but from excitatory, changes. An important suggestion of this study is that the

223 timing of TMS pulses should be expressed relative to the estimated EMG offset in each trial. If the timing

224 of the TMS pulse had been expressed relative to the average RT calculated in under control conditions

225 (Begum et al. 2005), we could not have drawn the conclusions we did.

226 In this study, we estimated the appropriate reference point based on the EMG offset calculated in
227 control trials without TMS. This was because it is difficult to determine the start of muscle relaxation (i.e.,
228 the point when the EMG began to decline), as indicated by a previous study (Buccolieri et al. 2004a). The
229 timing at which M1 is facilitated prior to muscle relaxation will be somewhat different depending on the
230 reference point used for comparison. However, M1 excitability increases immediately before a subsequent
231 rapid decrease. Additionally, our results show that active cortical processes for quick voluntary muscle
232 relaxation occur within a short time (approximately 20 ms) and are completed immediately afterwards.
233 Furthermore, after approximately 60 ms, EMG activity may return to resting levels (i.e., muscle
234 contractions terminate).

235 It is assumed that muscle relaxation involves an active cortical process similar to muscle
236 contraction, although these are opposite actions from a neurophysiological viewpoint (Rothwell et al.
237 1998; Terada et al. 1995; Toma et al. 1999). Our results support this hypothesis and show transient M1
238 excitability changes just prior to voluntary muscle relaxation. At 80-60 ms before the termination of
239 muscle contraction, there is no change in either EMG activity, force curve, or muscle contraction.
240 Therefore, it is assumed that the afferent input to the muscle does not change this early. Additionally, time
241 course studies on the H-reflex show that activation at the spinal level does not dynamically change before
242 voluntary muscle relaxation (Buccolieri et al. 2003; Schieppati and Crenna 1984; Schieppati et al. 1986;
243 Sugawara et al. 2009). Notably, Buccolieri et al. (2003) have suggested that control of distal arm muscle
244 relaxation is mainly related to reduction of motor cortical output. Therefore, it is possible that excitability

245 changes at a supraspinal level before muscle relaxation occurs.

246 We propose that increased M1 excitability prior to muscle relaxation reflects active motor
247 control necessary to relax the muscle during contraction. Because such cortical control is transient and M1
248 is markedly deactivated afterwards, it is possible that M1 triggers the withdrawal of ongoing excitatory
249 input during isometric contraction (Rothwell et al. 1998) or activates the cortical inhibitory pathways
250 thought to be important in muscle relaxation (Motawar et al. 2012).

251 Results shown here may be of particular relevance to the understanding aberrant relaxation and
252 impaired inhibitory control in movement disorders such as focal hand Dystonia (Stinear et al. 2009) or
253 motor dysfunction following stroke (Dewald et al. 1995; Kamper and Rymer 2001). Chae et al. (2002)
254 reported a delay in the termination of muscle contraction in the paretic arm of stroke survivors, which was
255 related to their degree of motor impairment and physical disability. Similarly, Buccolieri et al. (2004b)
256 reported a longer relaxation RT in patients with dystonia compared with normal controls. Such findings for
257 movement disorders might be explained in part by the impairment of an active cortical control system for
258 quickly relaxing the muscle.

259 This study investigated the time course of M1 excitability changes just prior to voluntary muscle
260 relaxation, which has not previously been analyzed in detail. Our results show that M1 is temporarily
261 activated 60–80 ms prior to quick voluntary muscle relaxation and is markedly deactivated thereafter.
262 Furthermore, we show that for detecting these changes in a time course study, it is necessary to express the
263 timing of the TMS pulse relative to the onset of voluntary relaxation in each trial. In muscle relaxation

264 studies using TMS, it is very difficult to determine the RT for relaxation. The more dramatic the M1

265 excitability changes, the more accurately must the relaxation RT be measured.

266 The changes in M1 excitability induced during voluntary muscle relaxation indicate that cortical

267 control of muscle relaxation is established through active processing. Cortical mechanisms underlying

268 muscle relaxation should be discussed in the light of such M1 excitability dynamics, particularly the

269 mechanisms for cortical inhibitory circuits. For example, we think that SICI will also dynamically change

270 prior to muscle relaxation, along with M1 excitability changes; indeed, this may explain the disparity in

271 previously reported results for SICI (Begum et al. 2005; Buccolieri et al. 2004a). In addition, the cortical

272 mechanisms involved may vary depending on the relaxation task (Pope et al. 2007; Rothwell et al. 1998).

273 To clarify the cortical mechanism underlying muscle relaxation *per se*, further studies should analyze the

274 differences in the time courses of excitability changes for different relaxation tasks and investigate the

275 changes in cortical inhibitory circuits. Necessarily, we should reconsider the use of EMG data or the way it

276 was used here, seeking more precision in determining the relaxation RT and greater resolution in

277 determining the MEP amplitude time course in the 100 ms prior to muscle relaxation.

278

279 **GRANTS**

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281

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337

338 **FIGURE LEGENDS**

339 ***Figure 1.*** The experimental set up for the measurement of isometric abduction of the right index finger at

340 20% maximal voluntary contraction (left) and the simple reaction time paradigm and the timing of the

341 TMS pulse (right). EMG, electromyography; TMS, transcranial magnetic stimulation.

342

343 ***Figure 2.*** TMS pulse relative to three different reference points. The top panel shows a control trial
344 without TMS and the bottom panel shows a trial with TMS. The force curve and EMG of the FDI are
345 shown for one trial. Dashed arrow X in the bottom panel shows the time of the go signal relative to the
346 TMS pulse, which randomly changes in each trial. A, B, and C; stimulation times calculated relative to
347 three reference points. Arrow A in the bottom panel is the TMS time relative to the average reaction time
348 (RT), the latter measured in control trials without TMS in each subject. Arrow B in the bottom panel is the
349 TMS time relative to the point at which the mean of the force data is halved after the go signal. Arrow C in
350 the bottom panel is the TMS time relative to the estimated EMG offset, the latter being the force halving
351 point minus the average motor delay as measured in control runs without TMS. Motor delay is interval
352 between the EMG offset and the force halving point.

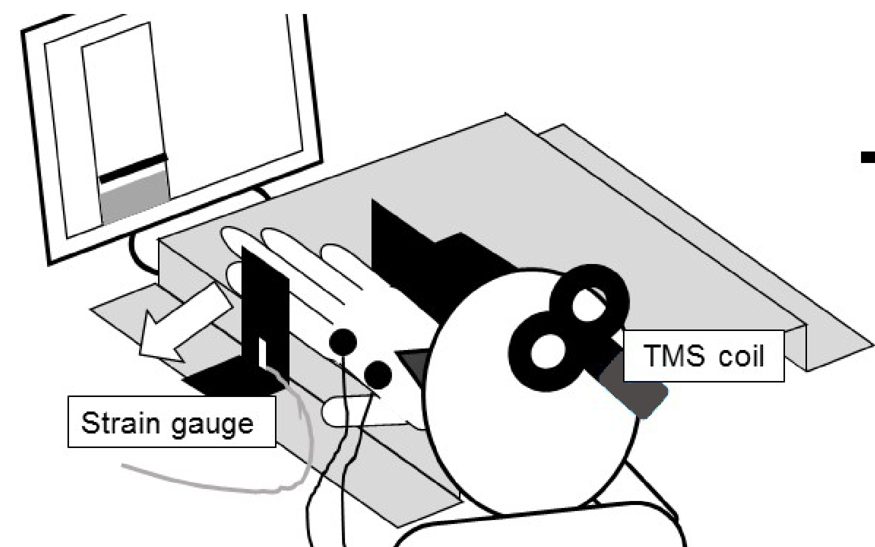
353

354 ***Figure 3.*** Motor evoked potentials (MEPs) as a function of the time of the TMS pulse relative to three
355 different reference points (A, average RT; B, force-curve halving; C, estimated EMG offset). The time
356 course data in three subjects is shown. The MEP amplitude is normalized to the mean MEP amplitudes
357 measured before the go signal. The differences among the three graphs are only in the choice of reference
358 point (0 ms); original data is common.

359

360 ***Figure 4.*** Mean MEP amplitudes for all subjects ($n = 10$) plotted against the time of the TMS pulse relative
361 to the estimated EMG offset (= 0 ms), binned in 20-ms intervals. The mean MEP amplitude in each bin is
362 normalized to the mean value before the go signal. The average number of observations per participant
363 within each 20-ms bin is shown beside each datum. The MEP amplitude from -80 to -60 ms is significantly
364 greater compared with that from -100 to -80 ms, -60 to -40 ms, and -40 to -20 ms. *, $p < .05$; **, $p < .01$.
365 Error bars represent SDs.

Fig. 1



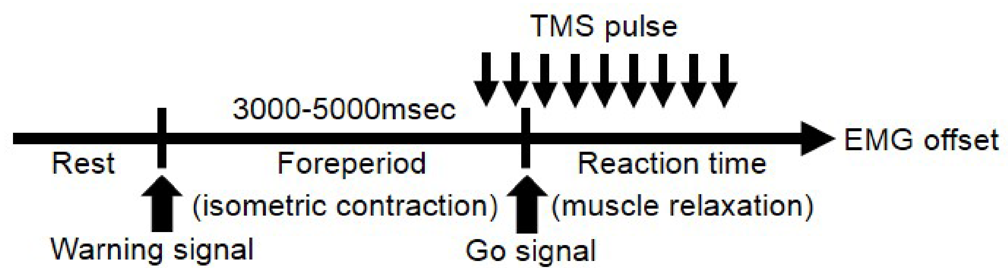


Fig. 2

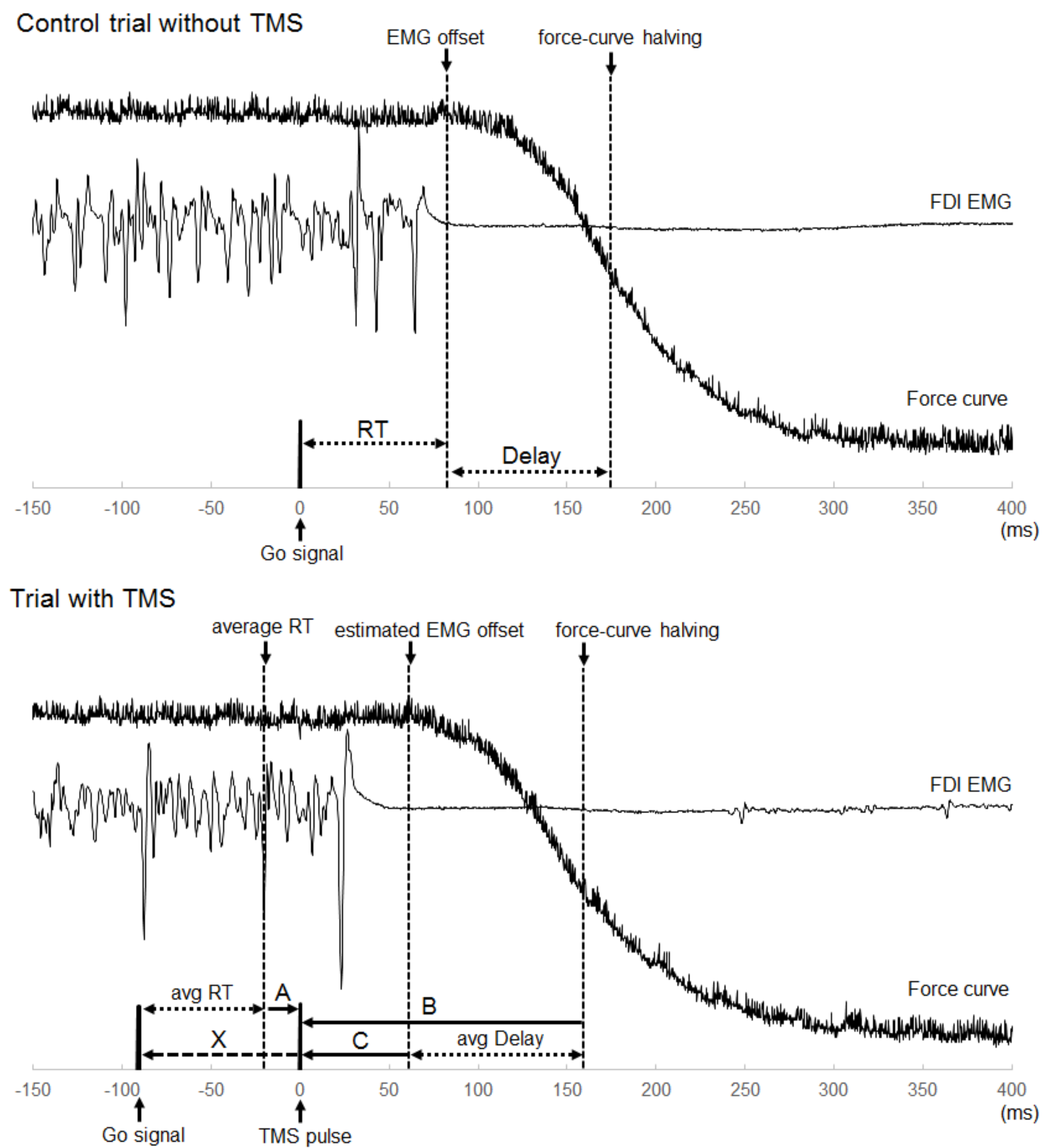


Fig. 3

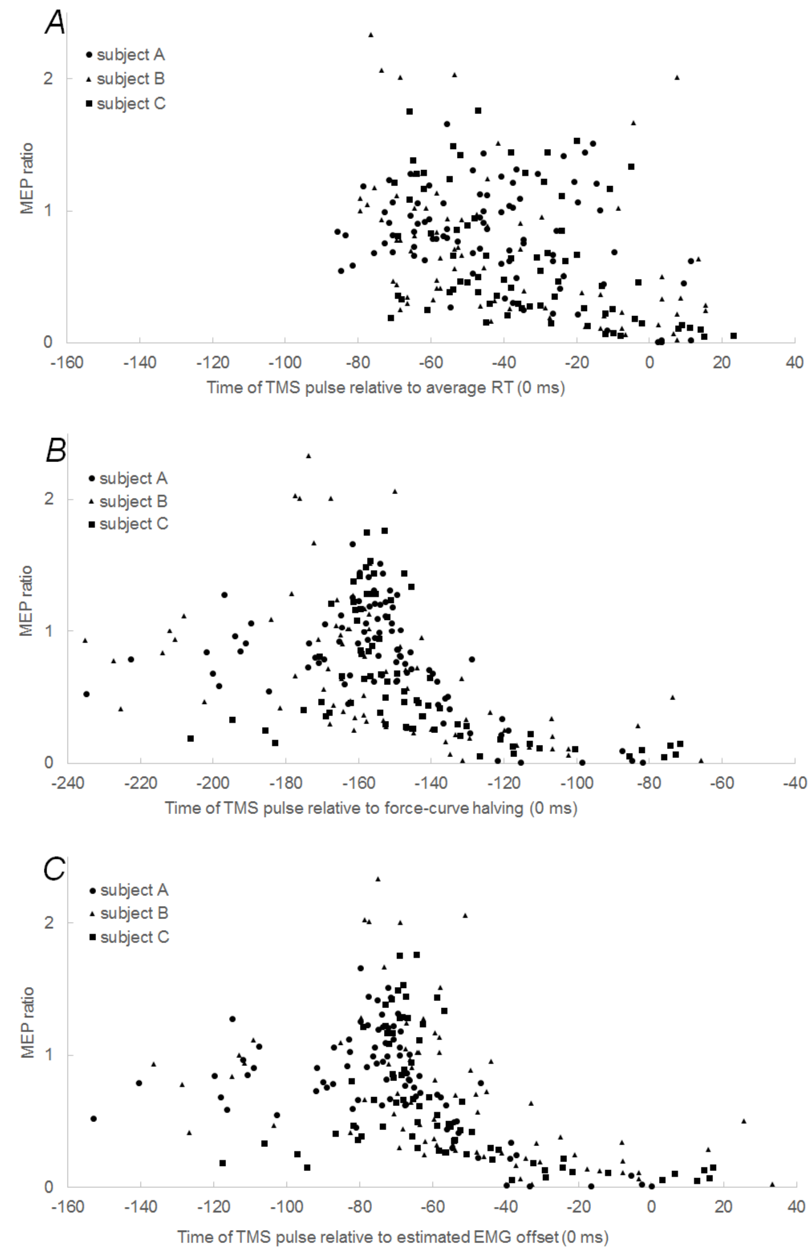


Fig. 4