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なお、このタイトルは研究の一部として発表されたものです。
Excitability changes in the primary motor cortex just prior to voluntary muscle relaxation

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Abstract

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

Keywords:

Transcranial magnetic stimulation, muscle relaxation, motor cortex, motor evoked potential, electromyography
INTRODUCTION

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

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

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

Additionally, an event-related functional magnetic resonance imaging (fMRI) study suggested that the primary motor cortex (M1) contralateral to the effector and bilateral supplementary motor areas
(SMA) are commonly activated in preparation and execution phases of both muscle relaxation and contraction (Toma et al. 1999). Thus, it is believed that, like muscle contraction, voluntary muscle relaxation is controlled by an active cortical process.

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

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

However, these time course studies analyzed long and different periods in the transition from
muscle contraction to relaxation. Hence, their findings are insufficient for clarifying phasic M1 excitability changes related to voluntary muscle relaxation.

Our focus in this study was motor control just prior to muscle relaxation. We previously reported that M1 was temporarily activated prior to muscle relaxation; however, we did not analyze M1 excitability changes in detail (i.e., with a narrow time window) (Sugawara et al. 2009). Additionally, we had often observed relatively large motor evoked potential (MEP) amplitudes when the TMS pulse had been delivered just prior to muscle relaxation (Sugawara K, unpublished observations).

From this viewpoint, we hypothesized that during muscle relaxation, M1 activity does not only gradually decrease to attenuate muscle contraction, but also dynamically changes, thus acting as an active cortical process that evokes the transition from contraction to relaxation. The discrepancy between previous studies may be due to the dynamic state just prior to muscle relaxation not having been sufficiently investigated. Therefore, this study analyzed the detailed time course of M1 excitability changes just prior to voluntary muscle relaxation to understand cortical control during this time.

METHODS

Participants

The participants were 10 students (five men and five women aged 20–23 years) from Kanagawa University of Human Services. All participants were right-handed according to their scores on a handedness questionnaire (Chapman and Chapman 1987). The mean score was 13.3 and the standard
deviation was 0.7. None of the participants had any history of neuromuscular or physical functional impairment that may have affected task performance. All participants gave their informed consent before the experiment. This study was conducted with the approval of the Research Ethics Committee of Kanagawa University of Human Services.

Experimental Paradigm

In this study, we used a simple reaction time (RT) paradigm for a voluntary muscle relaxation task. The participants sat comfortably on a chair with their right forearm pronated and digits extended on a table. The distal interphalangeal joint of the right index finger was positioned at the middle of a hard metal plate. A strain gauge (Kyowa Electronic Instruments Co., Tokyo, Japan) was mounted on the vertically bent portion of this plate (Figure 1). The analog signal was amplified (SA-250 STRAIN AMPLIFIER; TEAC, Tokyo, Japan), filtered, and digitized (NI USB-6229 BNC; National Instruments Corp., Austin, Texas, USA). These data were entered into a laboratory computer and presented as the cursor on a liquid crystal display monitor in front of the participant, using LabVIEW (LabVIEW2009; National Instruments Corp.). In short, the cursor was moved in real time by the in-progress force output against the strain gauge.

Before initiating the experiment, we measured the abduction force exerted against the plate by each subject’s maximal voluntary contraction (MVC) of the first dorsal interosseous muscle (FDI). At the onset of an acoustic warning signal, the participants were required to perform an isometric abduction of the right index finger at 20% of MVC, pressing steadily against the plate while self-controlling their
performance by observing the cursor and the target line (20% MVC for each individual). After an acoustic go signal was presented, the participants were required to terminate isometric contraction (that is, initiate muscle relaxation) as quickly as possible. The interval between the warning and go signals was 3000–5000 ms, which was randomized using LabVIEW. The participants were instructed to press the plate with the abduction force of the index finger only and not to perform any voluntary movements when relaxing their FDI. In this motor task, finger joint motion generally does not occur when participants perform muscle contraction or relaxation because the lateral surfaces of their index and little finger are fixed on each hard metal plate. Before any data was collected, participants practiced the task until they were able to perform it correctly.

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

Measurements

Surface EMGs in a belly-tendon montage were recorded from the right FDI using disposable
bipolar silver/silver chloride surface electrodes 10 mm in diameter. The raw signal was amplified and
derived (band-pass 5–3000 Hz) using a bioelectric amplifier (Neuropack MEB-2200; Nihon Kohden Corp.,
Tokyo, Japan), digitized at 4000 Hz, and stored for offline analysis on a laboratory computer (Power Lab
system; AD Instruments Pty Ltd., New South Wales, Australia).

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

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

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

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

Therefore, we attempted to define the reference point based on a force curve measured in each TMS trial. Electro-mechanical delay can occur even with the use of a strain gauge or accelerometer, and the decline of a force curve is difficult to estimate due to instability during sustained isometric contraction. Thus, we examined the period immediately before the go signal in each trial in the 20% MVC condition. We then calculated when the mean of a 200-ms period of force data decreased to 50% of the force curve.
(i.e., the time point where force was reduced by half, hereafter referred to as force-curve halving; B in Figure 2).

However, individual differences in the interval between the cessation of EMG activity and force-curve halving are inevitable. Obviously, this difference will be affected by individual differences in motor time (Weiss 1965) and the magnitude of the load against the metal plate. Moreover, it may also depend on the form of the force decay curve, which differs between subjects.

Therefore, we calculated the average time from the cessation of EMG activity to force-curve halving in control trials without TMS, and subtracted this time from the time of force curve halving in each trial with TMS. We defined the time corrected in this way as a reference point: the estimated EMG offset (the start of arrow C in Figure 2).

Data Analysis and Statistics

To analyze MEPs and the RMS background EMG statistically, these time course data were binned into 20-ms intervals, and average MEPs and the RMS were calculated for each bin. The data obtained within the 30 ms just after the go signal was excluded from analysis since it was assumed that this section did not yet reflect changes related to the control of muscle relaxation. Consequently, the time course data that was more than 100 ms before the estimated EMG offset was excluded from the statistical analysis, because these data were difficult to collect because of the generally short RTs in each subject.

Additionally, based on the latency of MEPs (approximately 20 ms in this study), the data recorded less
than 20 ms before the estimated EMG offset was also excluded from our analysis. MEP amplitude had already undergone a marked decrease by that time, indicating that the relaxation signal had already left the cerebral cortex by then.

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

RESULTS

Firstly, we expressed the timing of TMS pulses relative to the average RT calculated in control trials without TMS. These time course data varied as a whole and decreased around 0 ms (A in Figure 3). Secondly, we expressed the timing of TMS pulses relative to force-curve halving in each trial with TMS. These time course data showed obviously greater MEP amplitudes concentrated in a particular localized interval, but the time of peak amplitude was slightly different between subjects (B in Figure 3). In control trials without TMS, the interval between the offset of EMG and force-curve halving also differed slightly between subjects (mean, 103 ms; standard deviation, 17 ms). Thirdly, based on the estimated EMG offset
by correcting this difference, the MEP amplitude was largest between 80 and 60 ms before the estimated EMG offset (C in Figure 3). Accordingly, these time course data were binned into 20-ms intervals between 100 and 20 ms before the estimated EMG offset (0 ms).

In a one-way repeated measures ANOVA, the assumption of sphericity was met ($p = .057$), and a significant main effect in the average FDI MEPs was found for each bin ($F_{3,27} = 55.617$, $p < .001$). These multiple comparisons showed that the MEP amplitude from 80 to 60 ms before the estimated EMG offset was significantly greater than that from 100 to 80 ms ($p = .016$), 60 to 40 ms ($p = .001$), and 40 to 20 ms ($p < .001$). Moreover, MEP amplitude from 40 to 20 ms was significantly smaller than that from 100 to 80 ms ($p < .001$) and 60 to 40 ms ($p < .001$, Figure 4). No significant difference in RMS background EMG was found for any bin ($F_{3,27} = 2.079$, $p = .127$).

**DISCUSSION**

We observed M1 excitability changes just before voluntary muscle relaxation from isometric contraction by using a simple RT task. Our results agree with our hypothesis that M1 activity does not just decrease prior to voluntary muscle relaxation. Instead, the cortical control system of relaxation seems to originate not from inhibitory, but from excitatory, changes. An important suggestion of this study is that the timing of TMS pulses should be expressed relative to the estimated EMG offset in each trial. If the timing of the TMS pulse had been expressed relative to the average RT calculated in under control conditions (Begum et al. 2005), we could not have drawn the conclusions we did.
In this study, we estimated the appropriate reference point based on the EMG offset calculated in control trials without TMS. This was because it is difficult to determine the start of muscle relaxation (i.e., the point when the EMG began to decline), as indicated by a previous study (Buccolieri et al. 2004a). The timing at which M1 is facilitated prior to muscle relaxation will be somewhat different depending on the reference point used for comparison. However, M1 excitability increases immediately before a subsequent rapid decrease. Additionally, our results show that active cortical processes for quick voluntary muscle relaxation occur within a short time (approximately 20 ms) and are completed immediately afterwards. Furthermore, after approximately 60 ms, EMG activity may return to resting levels (i.e., muscle contractions terminate).

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

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

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

This study investigated the time course of M1 excitability changes just prior to voluntary muscle relaxation, which has not previously been analyzed in detail. Our results show that M1 is temporarily activated 60–80 ms prior to quick voluntary muscle relaxation and is markedly deactivated thereafter. Furthermore, we show that for detecting these changes in a time course study, it is necessary to express the timing of the TMS pulse relative to the onset of voluntary relaxation in each trial. In muscle relaxation
studies using TMS, it is very difficult to determine the RT for relaxation. The more dramatic the M1 excitability changes, the more accurately must the relaxation RT be measured.

The changes in M1 excitability induced during voluntary muscle relaxation indicate that cortical control of muscle relaxation is established through active processing. Cortical mechanisms underlying muscle relaxation should be discussed in the light of such M1 excitability dynamics, particularly the mechanisms for cortical inhibitory circuits. For example, we think that SICI will also dynamically change prior to muscle relaxation, along with M1 excitability changes; indeed, this may explain the disparity in previously reported results for SICI (Begum et al. 2005; Buccolieri et al. 2004a). In addition, the cortical mechanisms involved may vary depending on the relaxation task (Pope et al. 2007; Rothwell et al. 1998).

To clarify the cortical mechanism underlying muscle relaxation per se, further studies should analyze the differences in the time courses of excitability changes for different relaxation tasks and investigate the changes in cortical inhibitory circuits. Necessarily, we should reconsider the use of EMG data or the way it was used here, seeking more precision in determining the relaxation RT and greater resolution in determining the MEP amplitude time course in the 100 ms prior to muscle relaxation.

GRANTS

This work was supported by JSPS KAKENHI Grant Number 25750212.

REFERENCES


FIGURE LEGENDS

Figure 1. The experimental set up for the measurement of isometric abduction of the right index finger at 20% maximal voluntary contraction (left) and the simple reaction time paradigm and the timing of the
Figure 2. TMS pulse relative to three different reference points. The top panel shows a control trial without TMS and the bottom panel shows a trial with TMS. The force curve and EMG of the FDI are shown for one trial. Dashed arrow X in the bottom panel shows the time of the go signal relative to the TMS pulse, which randomly changes in each trial. A, B, and C; stimulation times calculated relative to three reference points. Arrow A in the bottom panel is the TMS time relative to the average reaction time (RT), the latter measured in control trials without TMS in each subject. Arrow B in the bottom panel is the TMS time relative to the point at which the mean of the force data is halved after the go signal. Arrow C in the bottom panel is the TMS time relative to the estimated EMG offset, the latter being the force halving point minus the average motor delay as measured in control runs without TMS. Motor delay is interval between the EMG offset and the force halving point.

Figure 3. Motor evoked potentials (MEPs) as a function of the time of the TMS pulse relative to three different reference points (A, average RT; B, force-curve halving; C, estimated EMG offset). The time course data in three subjects is shown. The MEP amplitude is normalized to the mean MEP amplitudes measured before the go signal. The differences among the three graphs are only in the choice of reference point (0 ms); original data is common.
Figure 4. Mean MEP amplitudes for all subjects (n = 10) plotted against the time of the TMS pulse relative to the estimated EMG offset (= 0 ms), binned in 20-ms intervals. The mean MEP amplitude in each bin is normalized to the mean value before the go signal. The average number of observations per participant within each 20-ms bin is shown beside each datum. The MEP amplitude from -80 to -60 ms is significantly greater compared with that from -100 to -80 ms, -60 to -40 ms, and -40 to -20 ms. *, p < .05; **, p < .01. Error bars represent SDs.
Fig. 2

Control trial without TMS

EMG offset  force-curve having

FDI EMG

Force curve

RT Delay

-150 -100 -50 0 50 100 150 200 250 300 350 400 (ms)

Go signal

Trial with TMS

average RT estimated EMG offset force-curve having

FDI EMG

Force curve

avg RT AB avg Delay

-150 -100 -50 0 50 100 150 200 250 300 350 400 (ms)

Go signal TMS pulse