Brain Stimulation 12 (2019) 1020–1026

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Supplementary motor area plays a causal role in automatic inhibition of motor responses

Yuichiro Shirota [a, *], Ristuko Hanajima [a, b], Shinya Ohminami [a], Ryosuke Tsutsumi [a], Yoshikazu Ugawa [c], Yasuo Terao [a, d]

[a] Department of Neurology, Division of Neuroscience, Graduate School of Medicine, The University of Tokyo. 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8655, Japan
[b] Division of Neurology, Department of Brain and Neurosciences, Faculty of Medicine, Tottori University 36-1 Nishi-cho, Yonago-shi, Tottori-ken, 683-8503, Japan
[c] Department of Neuro-Regeneration, Fukushima Medical University, 1 Hikariga-oka, Fukushima City, Fukushima, 960-1295, Japan
[d] Department of Cell Physiology, Kyorin University 6-20-2 Shinkawa, Mitaka-shi, Tokyo, 181-8611, Japan

ARTICLE INFO

Article history:
Received 11 August 2018
Received in revised form
1 March 2019
Accepted 3 March 2019
Available online 6 March 2019

Keywords:
Transcranial magnetic stimulation
Negative compatibility effect
Masked-priming
Response inhibition
Motor evoked potential

ABSTRACT

Background: The masked-priming paradigm is used to test unconscious inhibitory processes of the brain. A tendency towards responses that are incompatible with the prime, designated as negative compatibility effect (NCE), emerges when the perception of a priming visual stimulus is "masked" afterwards. This effect presumably stems from a subliminal inhibitory process against the masked-prime. Prior lesions as well as activation studies suggest a key role of SMA in this effect.
Objective: This study was conducted to elucidate a causal role of SMA in the subliminal response inhibition represented by the NCE.
Methods: Using a repeated-measures pre–post design with a group of healthy people, physiological measures (resting and active motor thresholds and motor evoked potential (MEP) amplitude) and behavioral ones (choice reaction time (CRT), positive compatibility effect (PCE) and NCE) were obtained before and after three quadripulse stimulation (QPS), namely sham, M1-QPS, and SMA-QPS, on different days. CRT and PCE served as indices for different aspects of motor execution.
Results: Motor thresholds were not altered after any QPS, although the M1-QPS increased MEP amplitude. Neither CRT nor PCE was altered significantly after QPS protocols. NCE was abolished after the SMA-QPS.
Conclusions: Abolished NCE after the SMA-QPS in the absence of MEP changes suggests that (1) SMA plays a cardinal role in the NCE, and (2) the network involved in NCE is different from that of MEP generation.

Introduction

Inhibition of an unwanted motor response is fundamentally important for everyday life. Neural mechanisms associated with that inhibition have been rigorously investigated using stop-signal tasks [1] and go – no-go tasks [2] in combination with brain stimulation. Under such experimental settings, signals requiring motor inhibition are explicitly indicated. By contrast, few reports have described studies examining mechanisms underlying unconscious motor inhibition of subliminally presented stimuli.

Masked-priming paradigm is a method to detect subliminal auto-inhibition of motor responses. Participants typically respond to left-oriented and right-oriented arrowheads ("targets") as quickly as possible by the left and right hands, respectively [3]. The target is preceded by a "prime" and a "mask" preventing overt perception of the prime. The prime has the same shape and either the same ("compatible" condition) or opposite ("incompatible" condition) direction as the target [3]. A natural assumption would be that the average reaction time (RT) is shorter when the prime and the target are in the same direction (i.e. in the compatible condition) than in an incompatible direction. Such is exactly the case in which the interval between the mask and the target is short, e.g. 50 ms, designated as the positive

* Corresponding author.
E-mail address: yshirota-tky@umin.ac.jp (Y. Shirota).

https://doi.org/10.1016/j.brs.2019.03.002
1935-861X/

compatibility effect (PCE). Quite interestingly, however, when the interval is around 100–150 ms, the average RT for the incompatible condition becomes shorter than that for the compatible one. This counter-intuitive RT difference is known as a negative compatibility effect (NCE) reproduced by subsequent studies [4–11]. This task is distinct from the so-called Posner task, describing inhibition of return [12], in that the current task "masked" the prime [13].

Earlier studies using lateralized readiness potential (LRP) have elucidated how NCE occurs. LRP is a physiological marker suggesting which side of the hemisphere is more prepared for action [14]. When a rightward prime is presented, for example, there would be a small activation in the left hemisphere, favoring a right-hand movement. This small activation is reversed if the prime is not consciously perceived because of the mask. Around 100–150 ms later, this reversal even favors an "incompatible" response (i.e. left-hand movement when the prime is rightward). When the prime is leftward, the same process facilitates a rightward response, resulting in NCE as well [3,4]. In contrast, when the mask-target interval is shorter, the reversal is not expected to favor the incompatible response.

Then, which brain areas are associated with the NCE? Because LRP is usually measured using electroencephalography (EEG) recorded from C3 and C4 of the 10–20 system, the primary sensorimotor area is a plausible candidate. Especially, the primary motor area (M1) is the final output region of movements. It seems reasonable to argue that intervention to the M1 should affect NCE. A recent study indicated relevance of the M1 in action inhibition [15]. The current evidence, however, supports involvement of the supplementary motor area (SMA) in the NCE. A study of post-stroke patients revealed that a small lesion in the SMA abolished the NCE, although different patients with a larger lesion in the frontal lobe sparing the SMA showed normal NCE [16]. Neuroimaging studies using magnetic resonance imaging (MRI) in a healthy population indicated that the compatibility effect is linked to the blood-oxygen level-dependent (BOLD) signal in the SMA [6,17], and that it is correlated with the concentration of gamma-aminobutyric acid (GABA) in the SMA [18].

For this study, we used transcranial magnetic stimulation (TMS) to investigate a causal role played by the SMA in the NCE. Repetitive TMS (rTMS) can induce long-lasting changes in the stimulated brain area [19]; such changes have something in common with long-term potentiation (LTP) or depression (LTD). On certain occasions, an rTMS protocol inducing LTP-like plasticity can suppress later task performance [20], resembling homeostatic plasticity. Here, we applied a protocol for which the canonical consequence is LTP-like plasticity [21,22] over the M1 and SMA to test how NCE were modulated, and whether rTMS-induced alteration of NCE, if any, was related to physiological markers of the motor system. Some results were presented at the Fifth International Conference on Non-invasive Brain Stimulation, Leipzig, 2013.

Methods

Participants

Nine right-handed healthy volunteers with normal or corrected-to-normal vision participated in this study. None had contraindication to TMS [23]. Written informed consent was obtained from all of them according to the study protocol approved by the local ethics committee of the Graduate School of Medicine, The University of Tokyo.

Electrophysiological recordings and single pulse TMS

Motor evoked potentials (MEPs) were recorded from right and left first dorsal interosseous (FDI) muscles with the belly tendon montage. Signals were filtered from 100 Hz to 3 kHz, digitized at 20 kHz, and stored in the computer for later off-line analyses. Stimulation was performed using a device (Magstim 200^2; The Magstim Co. Ltd., UK) and a hand-held 70-mm figure-of-eight coil (The Magstim Co. Ltd.). The coil was held to induce a current in the latero-posterior to medio-anterior direction in the brain, approximately 45° from the sagittal plane. First, the hand motor area was determined as the point where the largest MEP was constantly elicited. This point was marked with a felt pen for coil repositioning. Resting motor threshold (RMT), active motor threshold (AMT), and amplitude of MEP were used as indices of corticospinal excitability. RMT was defined as the lowest stimulus intensity that elicited at least 50 μV MEP in more than half of the trials in the resting FDI. Also, AMT was defined as the lowest stimulus intensity that elicited at least 100 μV MEP in more than half of the trials under slight voluntary contraction of FDI. To measure the MEP amplitude, stimulus intensity was set at the intensity which elicited 0.5 mV MEP on average before the intervention. The mean amplitude of 20 trials represented the MEP amplitude. RMT, AMT, and MEP amplitudes were measured four times (Fig. 1): at the beginning (Pre1), after the first task and before QPS (Pre2), immediately after QPS (Post1), and at the end after the second behavioral measurements (Post2).

In addition, MEPs from right tibialis anterior muscle were recorded initially to find the leg motor area, which was searched along with the midline with leftward-induced current [22,24]. The leg motor area was referred to when defining the rTMS target for SMA (*see* below).

Repetitive TMS

We used a modified version of quadripulse stimulation (QPS). The original protocol of QPS consists of 360 bursts with four subthreshold (typically 90% of the AMT for a hand muscle) pulses repeated every 5 s (30 min in total); variation in the intra-burst interval results in difference in the aftereffect, so that QPS-5 ms increases and QPS-50 ms decreases M1 excitability [21]. Reportedly,

Fig. 1. Timeline of the study. Each participant underwent three sessions: Sham, M1-QPS, and SMA-QPS. Before and after intervention, behavioral measures consisting of choice reaction time (CRT), positive compatibility effect paradigm (PCE), and negative compatibility effect paradigm (NCE) were obtained. They were preceded and followed by the physiological measurements: active motor threshold (AMT), resting motor threshold (RMT), and motor evoked potential (MEP).

QPS has metaplastic effects on human SMA when applied for 10 min with higher intensity [22]. Inspired by another study that found aftereffects of SMA rTMS with lower stimulus intensity [24], we used the same intensity, 110% AMT for the right FDI instead of 90% AMT for the TA, for QPS. We decided to apply 1000 bursts (instead of 1440 in the original) to try to reduce the time necessary for induction of aftereffects. Three conditions were tested on different days with a minimum interval of one week: QPS-5 ms over the SMA (SMA-QPS), QPS-5 ms over the left M1 (M1-QPS), and a sham condition. For SMA-QPS, the coil was placed 3 cm anterior to the leg motor area along the midline [22,24,25]. M1-QPS was conducted with the coil over the left hemisphere hand motor area for the right FDI. In the sham condition, a coil disconnected from the stimulator was positioned over the SMA. Another coil produced the same sound as the real QPS behind the participant's head. TMS pulses for QPS were delivered through a custom-made combinatory module that incorporates four Magstim 200^2 stimulators.

Task design and experimental procedure

Three behavioral measures were obtained to evaluate the influence of QPS: choice reaction time (CRT), positive compatibility effect (PCE), and NCE. Timing of visual stimuli was controlled using SuperLab Pro 2.0 (Cedrus Corp., San Pedro, CA, USA). In all tasks, visual stimuli were presented in the middle of a 17-inch monitor. The target was presented as "<<" or ">>" on a black-on-white background. In the CRT task, a fixation point "+" was followed by either of the targets 500 ms later. Participants responded to the target "«" with their left index finger and "»" with their right one as quickly and accurately as possible by clicking the left and right buttons of a mouse, respectively. Each target was presented 30 times.

PCE and NCE tasks were structured similarly, except for the presence of a prime and mask (Fig. 2). In both tasks, the prime was presented for 32 ms after the fixation cross, then a mask composed of a mosaic pattern for 50 ms. In the PCE task, the mask was followed immediately by the target, although there was a 100 ms blank in the NCE task. Next the target appeared, to which participants were asked to respond as rapidly and accurately as possible. Compatible and incompatible trials for left and right targets were presented 25 times each, amounting to 100 trials both for the PCE and NCE.

Experimental procedure

The timeline for a single session is presented in Fig. 1. After preparation, physiological measures (RMT, AMT, and MEP amplitude) were obtained before (pre1) and after (pre2) the task. The order of the three measurements was counter-balanced across sessions. Next, the leg motor area was defined as described above; then came QPS. The three conditions (SMA-QPS, M1-QPS, and sham) were investigated in the same participants in a counterbalanced order. Intervals between the conditions were at least one week. After QPS, the physiological and behavioral measures were obtained similarly. Here again, RMT, AMT, and MEP amplitudes were measured before (post1) and after (post2) the task to ascertain whether the motor task affected these physiological parameters.

Data analyses

Physiological measures, i.e., RMT, AMT, and MEP amplitudes, were compared using a three-way repeated-measures analysis of variance (rmANOVA) separately. Within-subject factors were CONDITION (Sham, M1-QPS, SMA-QPS), HAND (right and left), and TIME (pre1, pre2, post1, post2).

Behavioral measures consisting of CRT, PCE, and NCE were also compared using three-way rmANOVA. Here within-subject factors were CONDITION (sham, M1-QPS, SMA-QPS), HAND (right and left), and TIME (pre, post). CRT was analyzed using the raw mean reaction times in milliseconds; PCE and NCE were calculated as the mean reaction time difference between compatible and incompatible trials. Only correct responses were included in the reaction time analysis. Error rate was analyzed in the same manner for the PCE and NCE conditions separately. Error rate difference was calculated by subtracting the number of errors in the compatible condition from that in the incompatible condition, so that negative numbers indicate more errors in the compatible condition, which was expected in the NCE condition.

The rmANOVAs were conducted with Greenhouse–Geisser correction for sphericity when necessary (as suggested by the Mauchly's test). As a measure of effect size, we report partial eta-squared (η_p^2) for significant results. When a significant interaction was found, *post-hoc* analyses were conducted with the paired-*t* test corrected for multiple comparisons using the Bonferroni method.

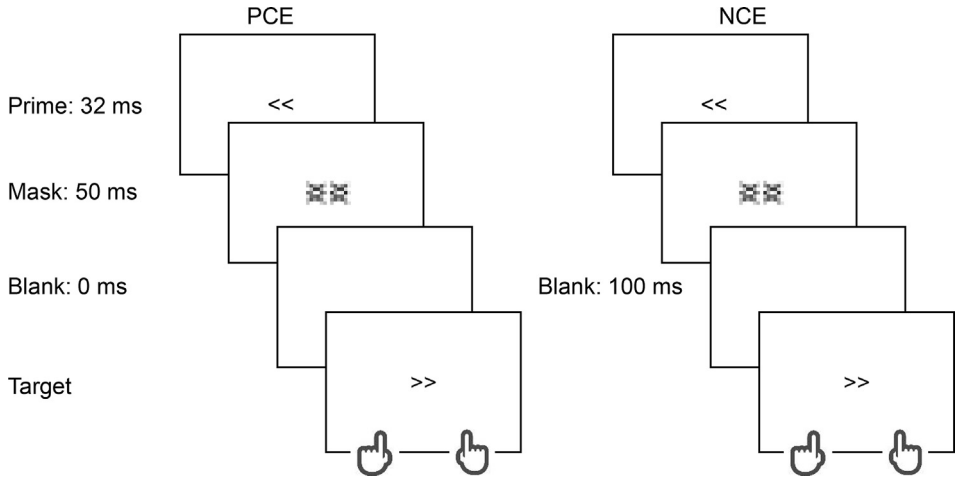

Fig. 2. Masked-priming paradigm. In the masked-priming paradigms, a mask was presented for 32 ms first, with leftward ("«") or rightward ("»") arrowheads. Next came a mask for 50 ms, followed by no (positive compatibility effect) or 100-ms (negative compatibility effect) blank. Finally, a target appeared, to which participants were asked to respond as quickly as possible with their index finger of the same side (left or right). The target can be in the same (compatible) or opposite (incompatible) direction to the prime. NCE, negative compatibility effect; PCE, positive compatibility effect.

Significance was inferred for $p = 0.05$. Values were reported as mean ± standard error of the mean (SEM).

Results

Physiological measures

Neither RMT nor AMT was altered significantly after any type of QPS (Table 1). The MEP amplitude was increased after the M1-QPS only with the right hand (Fig. 3). Statistical analysis applied to the MEP amplitude revealed a significant three-way interaction among CONDITION, HAND, and TIME ($F_{6,48} = 2.51$, $p = 0.034$, $\eta_p^2 = 0.24$) and an interaction between CONDITION and HAND ($F_{2,16} = 4.87$, $p = 0.022$, $\eta_p^2 = 0.38$), thereby confirming this observation. Post-hoc comparison of averaged MEP amplitude revealed significant difference between before and after M1-QPS (paired-*t* test, $p = 0.044$), too.

Behavioral measures

The results are presented in Fig. 4. CRT was not changed significantly by QPS. The rmANOVA showed a trend for interaction between HAND and TIME ($F_{1,8} = 3.6$, $p = 0.095$) suggesting a faster reaction time with the right hand, irrespective of QPS conditions.

PCE tended to be enhanced with the right hand after M1 and SMA QPS (Fig. 4), but the three-way interaction among QPS, HAND, and TIME remained not significant ($F_{1.1,9.2} = 3.51$, $p = 0.09$). Analysis of the error rates revealed a significant main effect of HAND ($F_{1,,8} = 2.37$, $p = 0.047$, $\eta_p^2 = 0.41$) and an interaction between CONDITION and TIME ($F_{2,,16} = 3.81$, $p = 0.044$, $\eta_p^2 = 0.32$). *Post-hoc* tests failed to show significant change before and after any kinds of QPS in neither hand.

NCE was abolished bilaterally after SMA QPS (Fig. 4). This result was confirmed by three-way rmANOVA results. We found a main effect of TIME ($F_{1,8} = 8.3$, $p = 0.021$, $\eta_p^2 = 0.51$) with a significant interaction between CONDITION and TIME ($F_{2,16} = 4.66$, $p = 0.025$, $\eta_p^2 = 0.37$). *Post-hoc* analysis revealed that NCE was significantly different before and after SMA-QPS for the right ($p = 0.005$), but we also found a trend for the left hand ($p = 0.074$). For all other comparisons, the *p*-values were greater than 0.1.

Table 1
Physiological parameters.

	Pre1	Pre2	Post1	Post2
Right FDI				
Sham				
RMT	47.8 ± 3.4	47.8 ± 3.6	48.6 ± 3.5	47.2 ± 3.6
AMT	33.2 ± 2.7	33.6 ± 2.5	33.6 ± 2.8	33.4 ± 2.8
M1-QPS				
RMT	48.3 ± 4.0	47.8 ± 3.6	48.1 ± 3.6	47.3 ± 3.3
AMT	33.3 ± 2.9	33.6 ± 3.0	33.0 ± 3.0	33.3 ± 3.3
SMA-QPS				
RMT	48.2 ± 3.4	49.6 ± 3.7	49.1 ± 3.7	48.8 ± 3.5
AMT	35.1 ± 2.7	35.7 ± 2.8	35.2 ± 2.7	34.7 ± 2.6
Left FDI				
Sham				
RMT	48.3 ± 3.6	49.2 ± 3.5	49.6 ± 3.4	49.1 ± 3.5
AMT	32.2 ± 1.9	32.7 ± 1.8	32.8 ± 1.7	32.8 ± 2.1
M1-QPS				
RMT	48.8 ± 3.5	50.0 ± 3.4	50.3 ± 3.3	50.4 ± 3.3
AMT	32.6 ± 2.1	33.4 ± 2.0	32.6 ± 2.3	32.6 ± 2.3
SMA-QPS				
RMT	47.3 ± 3.4	47.8 ± 3.1	48.9 ± 3.0	48.6 ± 3.5
AMT	32.8 ± 2.2	34.3 ± 2.2	33.7 ± 2.3	34.6 ± 2.3

Finally, we explored correlation between changes in physiological measures and behavioral measures. We found no significant ones among them, including MEP increase with M1-QPS or abolished NCE after SMA-QPS. Analysis of the error rates revealed a significant main effect of TIME ($F_{1,,8} = 15.1$, $p = 0.005$, $\eta_p^2 = 0.65$), suggesting less difference in the error counts after QPS, regardless of the type of it (see Fig. 5).

Discussion

We have demonstrated that the masked-priming effect, NCE, was abolished by SMA-QPS. Our results suggest a causal and specific role played by SMA for subliminal inhibition underlying the NCE. Results of the other QPS protocols and differences in other behavioral and physiological measures before and after QPS support such specificity. Our results are consistent with those of a lesion study [16] and neuroimaging studies [6,17,18], which have pointed to SMA as a key structure for NCE.

First, we argue that QPS functioned as expected. The M1-QPS with 5 ms intra-burst, inter-pulse interval increased the MEP amplitude of the right FDI similarly to the original study [21], although we used a slightly modified version of QPS. Given this result, SMA-QPS is likely to have had a similar influence to that reported from an earlier study [22]. Reportedly, SMA-QPS has a metaplastic effect, so that facilitatory QPS with 5 ms interval over the SMA let subsequent M1-QPS be less facilitatory or even inhibitory, probably by invoking a history of activation in the SMA. Because SMA has a facilitatory influence on M1 including a network for MEP generation [26–30], the activation would later work on it in a suppressive manner. Although not explicitly proven, the same history of activation could have disturbed normal functioning of SMA itself after the SMA-QPS, thereby resulting in decreased NCE.

Actually, SMA-QPS have specifically influenced inhibitory processes necessary for NCE while leaving other aspects such as action selection or motor execution unaffected. Results show that SMA-QPS did not change the CRT, which is associated with selective suppression of the M1 during preparation according to a recent study [31]. That study demonstrated the timing-dependent role of suppression of the corticospinal pathway. Similar suppression could have been provoked by our study, but neither SMA-QPS nor M1-QPS with the present protocol caused such suppression. The discrepancy between CRT and NCE supports a close association between normal functioning of SMA and NCE. Alternatively, it might have been the case in which strict timing-dependency would be required for (r)TMS to affect CRT. The results of PCE, however, might seem more difficult to explain given the NCE change. Positive or natural priming effects, however, can be realized by numerous mechanisms including one related to action selection under conflict [32], so that alteration of SMA function by the SMA-QPS could have been “bypassed” using them involving other part of the brain. An observation that PCE and NCE are not completely parallel in elderly people or patients with Parkinson disease [33] supports this idea.

Increased MEP after M1-QPS, together with a lack of behavioral effects of the same QPS protocol, suggests that a neural circuit generating MEP is distinct from mechanisms underlying CRT, PCE, or NCE. First, descending volleys leading to activation of the spinal motor neurons (then resulting in MEP) are primarily produced within the M1, although one report [34] presents an argument emphasizing afferent fibers to M1 from the premotor cortex. By stark contrast, reports of the literature point to the crucially important role of SMA for NCE [6,16–18]. Therefore, a simple view would be a contrast between M1 as a generator of MEP and SMA as a key brain area for NCE. Moreover, a recent discussion of the relevance of MEP change as a surrogate marker of human behavior is noteworthy [35]. Physiological markers can vary for different

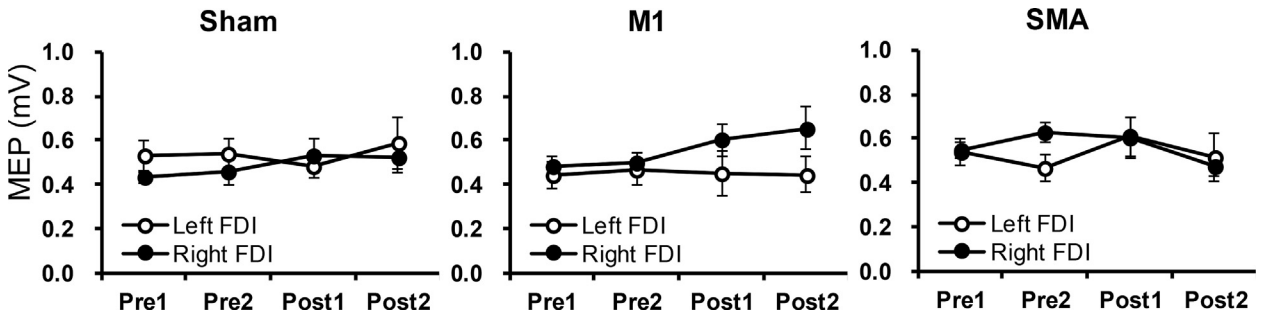

Fig. 3. Time course of MEP amplitude. Raw MEP amplitudes before (Pre1 and Pre2) and after (Post1 and Post2) are presented for Sham-QPS, M1-QPS, and SMA-QPS conditions. Circles denote MEP from the left FDI; dots denote MEP from the right. Error bars show standard errors of the mean. Asterisk (*) denotes significant difference before and after M1-QPS on the right side.

behaviors. What markers are suitable to characterize NCE remains to be elucidated.

Few reports of the relevant literature describe effects of TMS on NCE. One study tested hand NCE before and after rTMS. 1 Hz rTMS was applied over the M1, premotor cortex, and somatosensory cortex, none of which modulated NCE [11]. Our results were in line with those of that study because M1-rTMS did not change NCE. That earlier study also showed that the premotor cortex did not

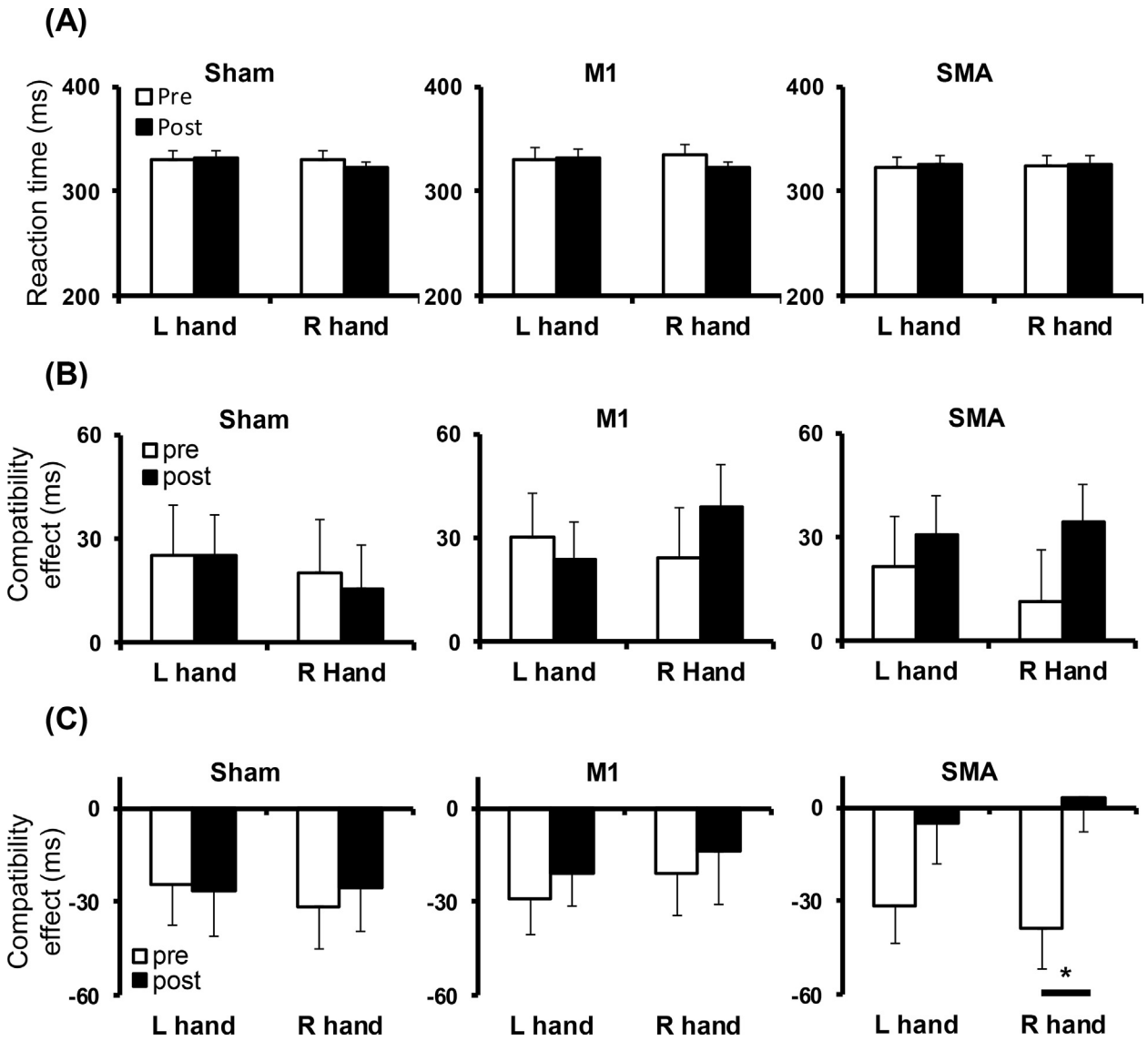

Fig. 4. Time course of behavioral tasks. Results of the behavioral tasks are presented for each stimulation (sham-QPS, M1-QPS, and SMA-QPS), hand (left and right), and timing (white for pre and black for post). (A) The choice reaction time (CRT) is not different across conditions. (B) Positive compatibility effect (PCE) tends to increase after M1-QPS and SMA-QPS, but not significantly. (C) Negative compatibility effect (NCE) is abolished bilaterally after SMA-QPS, with significance found for the right hand.

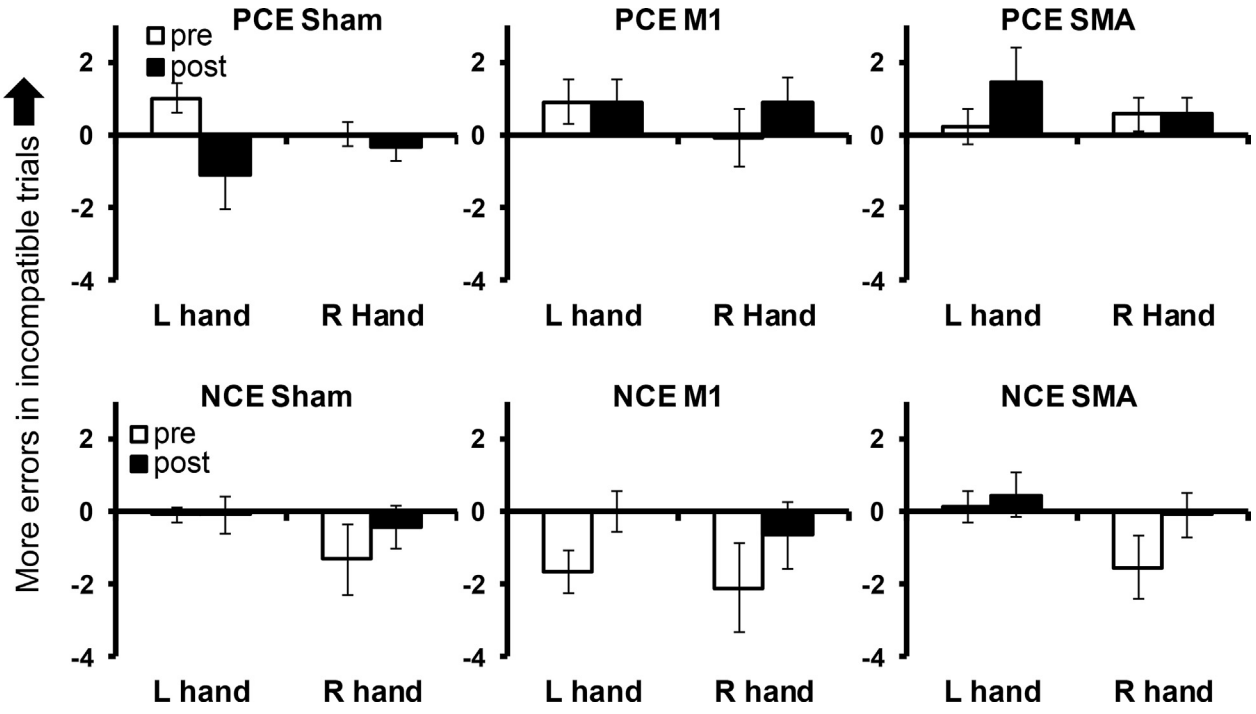

Fig. 5. Error rate. Results of the behavioral tasks are presented for each stimulation (sham-QPS, M1-QPS, and SMA-QPS), hand (left and right), and timing (white for pre and black for post). The upper row represents positive compatibility effect (PCE), and the lower negative compatibility effect (NCE). The Y axis indicate average difference in the number of error trials (compatible trials − incompatible trials). PCE tends to produce more errors in compatible trials, whereas NCE in incompatible trials.

affect NCE. These results support a specific role of SMA for NCE by excluding other brain areas as plausible candidates. By contrast with rTMS, which investigates the off-line influence of TMS, single-pulse or double-pulse TMS can test on-line effects with more emphasis on timing when a brain area is involved in the task in question. One study investigating saccade-NCE with double-pulse TMS over the supplementary eye field indicated that the supplementary eye field might influence an interaction between saccade preparation and sub-threshold activation caused during prime and mask, rather than an inhibitory process [36].

Response inhibition of movement is a complex process involving many brain areas. The NCE tested here is proposed to reflect subliminal inhibition of motor responses in the presence of unperceived prime. In contrast, other paradigms investigating motor inhibition are related to other brain areas with a considerable overlap across paradigms [37]. For instance, lateral prefrontal cortex is involved in the go − no-go task [38,39], although an earlier report [40] presents the possibility that influence of the prefrontal cortex is age-dependent. Pre-SMA and right inferior frontal gyrus are supposedly involved in the stop-signal task [41,42]. Such variation suggests that motor inhibition is encoded in multiple ways according to the context. Pursuing task-specific involvement of each brain area by conducting a factorial experiment in this regard presents an interesting future direction.

This study has several limitations. First, the small number of participants precluded robust and detailed exploration of correlation among measures, even though the η_p^2 values reported in Results suggest that substantial part of the variance be explained by the significant factors. Starting from current evidence indicating SMA-mediated subliminal motor inhibition, a more confirmatory research is warranted to elucidate a role of SMA in the context of response inhibition. For example, contrasting different function of SMA and pre-SMA between different response inhibition tasks would be a good instance. Secondly, rTMS is useful to reveal lasting changes caused by stimulation, but information about the timing of involvement of a brain area cannot be extracted. On-line TMS study for the hand NCE is expected to add further evidence. It is of note that Chiau et al. nicely revealed importance of timing of TMS in an on-line paradigm for eye movements [36]. They showed that TMS during the mask did not modify NCE but TMS after target onset did. Here, the meaning of timing can be analyzed from two distinct viewpoints. First is the one taken by Chiau, that is, timing of intervention such as TMS. This approach is possible only by on-line TMS experiments. The other is a timing of transition from PCE to NCE; the former occurs with a shorter interval between prime and target. We did not test such a factor in more detail primarily because of time limitation. It would be a nice future direction to explore the issue of timing by changing the interval between the prime and target (e.g. 200 ms or more in addition to the 50 and 150 ms in the current study). Finally, neural correlates of the observed change in NCE can be revealed by physiological measures such as LRP.

In conclusion, subliminal response inhibition indexed by NCE was diminished by rTMS over the SMA. The results constitute further evidence indicating that SMA plays a cardinal role in inhibitory processes of the brain.

Conflicts of interest

We declare no conflict of interest. Financial contributions are described in Acknowledgements just below.

Acknowledgments

Part of this work was supported by a subsidy from the Magnetic Health Science Foundation to Y.S. R.H. has been supported by JSPS KAKENHI grant numbers 23591270, 26461308, 17K09809, and 15H05881. Y.U. has been supported by JSPS KAKENHI grant numbers 22390181, 25293206, 15H05881, and 16H05322, and 18K10821, the Research Committee on Degenerative Ataxia from

the Ministry of Health. Y.T. was supported by Research Project Grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (16K09709, 16H01497).

References

[1] Cai W, George JS, Verbruggen F, Chambers CD, Aron AR. The role of the right presupplementary motor area in stopping action: two studies with event-related transcranial magnetic stimulation. J Neurophysiol 2012;108:380–9.
[2] Ficarella SC, Battelli L. The critical role of the dorsal fronto-median cortex in voluntary action inhibition: a TMS study. Brain Stimulat 2017;10:596–603.
[3] Eimer M, Schlaghecken F. Effects of masked stimuli on motor activation: behavioral and electrophysiological evidence. J Exp Psychol Hum Percept Perform 1998;24:1737–47.
[4] Aron AR, Schlaghecken F, Fletcher PC, et al. Inhibition of subliminally primed responses is mediated by the caudate and thalamus: evidence from functional MRI and Huntington's disease. Brain 2003;126:713–23.
[5] D'Ostilio K, Garraux G. Automatic stimulus-induced medial premotor cortex activation without perception or action. PLoS One 2011;6:e16613.
[6] D'Ostilio K, Collette F, Phillips C, Garraux G. Evidence for a role of a cortico-subcortical network for automatic and unconscious motor inhibition of manual responses. PLoS One 2012;7:e48007.
[7] Eimer M, Schlaghecken F. Response facilitation and inhibition in subliminal priming. Biol Psychol 2003;64:7–26.
[8] Klapp ST, Hinkley LB. The negative compatibility effect: unconscious inhibition influences reaction time and response selection. J Exp Psychol Gen 2002;131:255–69.
[9] Peinemann A, Reimer B, Loer C, et al. Long-lasting increase in corticospinal excitability after 1800 pulses of subthreshold 5 Hz repetitive TMS to the primary motor cortex. Clin Neurophysiol 2004;115:1519–26.
[10] Schlaghecken F, Eimer M. A central–peripheral asymmetry in masked priming. Percept Psychophys 2000;62:1367–82.
[11] Schlaghecken F, Munchau A, Bloem BR, Rothwell J, Eimer M. Slow frequency repetitive transcranial magnetic stimulation affects reaction times, but not priming effects, in a masked prime task. Clin Neurophysiol 2003;114:1272–7.
[12] Posner MI, Rafal RD, Choate LS, Vaughan J. Inhibition of return: neural basis and function. Cogn Neuropsychol 1985;2:211–28.
[13] Hilchey MD, Satel J, Ivanoff J, Klein RM. On the characteristics of the delayed "inhibitory" Cueing effects generated by uninformative arrows at fixation. Psychon Bull Rev 2013;20:593–600.
[14] Praamstra P, Torney L, Rawle CJ, Chris Miall R. Misconceptions about mirror-induced motor cortex activation. Cerebr Cortex 2011;21:1935–40.
[15] Liebrand M, Kristek J, Tzvi E, Krämer UM. Ready for change: oscillatory mechanisms of proactive motor control. PLoS One 2018;13:e0196855.
[16] Sumner P, Nachev P, Morris P, et al. Human medial frontal cortex mediates unconscious inhibition of voluntary action. Neuron 2007;54:697–711.
[17] Boy F, Husain M, Singh K, Sumner P. Supplementary motor area activations in unconscious inhibition of voluntary action. Exp Brain Res 2010;206:441–8.
[18] Boy F, Evans CJ, Edden RAE, Singh KD, Husain M, Sumner P. Individual differences in subconscious motor control predicted by GABA concentration in SMA. Curr Biol 2010;20:1779–85.
[19] Parkin BL, Ekhtiari H, Walsh VF. Non-invasive human brain stimulation in cognitive neuroscience: a primer. Neuron 2015;87:932–45.
[20] Jung P, Ziemann U. Homeostatic and nonhomeostatic modulation of learning in human motor cortex. J Neurosci 2009;29:5597–604.
[21] Hamada M, Terao Y, Hanajima R, et al. Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. J Physiol 2008;586:3927–47.
[22] Hamada M, Hanajima R, Terao Y, et al. Primary motor cortical metaplasticity induced by priming over the supplementary motor area. J Physiol 2009;587:4845–62.
[23] Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008–39.
[24] Matsunaga K, Maruyama A, Fujiwara T, Nakanishi R, Tsuji S, Rothwell JC. Increased corticospinal excitability after 5 Hz rTMS over the human supplementary motor area. J Physiol 2005;562:295–306.
[25] Shirota Y, Ohtsu H, Hamada M, Enomoto H, Ugawa Y. Supplementary motor area stimulation for Parkinson disease: a randomized controlled study. Neurology 2013;80:1400–5.
[26] Arai N, Lu M-K, Ugawa Y, Ziemann U. Effective connectivity between human supplementary motor area and primary motor cortex: a paired-coil TMS study. Exp Brain Res 2012;220:79–87.
[27] Ghosh S, Brinkman C, Porter R. A quantitative study of the distribution of neurons projecting to the precentral motor cortex in the monkey (*M. fascicularis*). J Comp Neurol 1987;259:424–44.
[28] Luppino G, Matelli M, Camarda R, Rizzolatti G. Corticocortical connections of area F3 (SMA-proper) and area F6 (pre-SMA) in the macaque monkey. J Comp Neurol 1993;338:114–40.
[29] Muakkassa KF, Strick PL. Frontal lobe inputs to primate motor cortex: evidence for four somatotopically organized 'premotor' areas. Brain Res 1979;177:176–82.
[30] Shirota Y, Hamada M, Terao Y, et al. Increased primary motor cortical excitability by a single-pulse transcranial magnetic stimulation over the supplementary motor area. Exp Brain Res 2012;219:339–49.
[31] Hannah R, Cavanagh SE, Tremblay S, Simeoni S, Rothwell JC. Selective suppression of local interneuron circuits in human motor cortex contributes to movement preparation. J Neurosci 2018;38:1264–76.
[32] Mars RB, Klein MC, Neubert F-X, et al. Short-latency influence of medial frontal cortex on primary motor cortex during action selection under conflict. J Neurosci 2009;29:6926–31.
[33] Seiss E, Praamstra P. Time-course of masked response priming and inhibition in Parkinson's disease. Neuropsychologia 2006;44:869–75.
[34] Shimazu H, Maier MA, Cerri G, Kirkwood PA, Lemon RN. Macaque ventral premotor cortex exerts powerful facilitation of motor cortex outputs to upper limb motoneurons. J Neurosci 2004;24:1200–11.
[35] Bestmann S, Krakauer J. The uses and interpretations of the motor-evoked potential for understanding behaviour. Exp Brain Res 2015;233:679–89.
[36] Chiau HY, Muggleton NG, Juan CH. Exploring the contributions of the supplementary eye field to subliminal inhibition using double-pulse transcranial magnetic stimulation. Hum Brain Mapp 2017;38:339–51.
[37] Dutra IC, Waller DA, Wessel JR. Perceptual surprise improves action stopping by nonselectively suppressing motor activity via a neural mechanism for motor inhibition. J Neurosci 2018;38:1482–92.
[38] Hege MA, Preissl H, Stingl KT. Magnetoencephalographic signatures of right prefrontal cortex involvement in response inhibition. Hum Brain Mapp 2014;35:5236–48.
[39] Menon V, Adleman NE, White CD, Glover GH, Reiss AL. Error-related brain activation during a Go/NoGo response inhibition task. Hum Brain Mapp 2001;12:131–43.
[40] Huang CC, Su TP, Shan IK, Wei IH. Effect of 5 Hz repetitive transcranial magnetic stimulation on cognition during a Go/NoGo task. J Psychiatr Res 2004;38:513–20.
[41] Juan C-H, Muggleton NG. Brain stimulation and inhibitory control. Brain Stimulat 2012;5:63–9.
[42] Watanabe T, Hanajima R, Shirota Y, et al. Effects of rTMS of pre-supplementary motor area on fronto basal ganglia network activity during stop-signal task. J Neurosci 2015;35:4813–23.