Video Article

Intracortical Inhibition Within the Primary Motor Cortex Can Be Modulated by Changing the Focus of Attention

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URL: https://www.jove.com/video/55771
DOI: doi:10.3791/55771

Keywords: Behavior, Issue 127, Attentional foci, cognitive manipulation, motor cortex, movement control, short-interval intracortical inhibition, time to task failure, transcranial magnetic stimulation

Date Published: 9/11/2017

Citation: Kuhn, Y.A., Keller, M., Ruffieux, J., Taube, W. Intracortical Inhibition Within the Primary Motor Cortex Can Be Modulated by Changing the Focus of Attention. *J. Vis. Exp.* (127), e55771, doi:10.3791/55771 (2017).

Abstract

It is well recognized that an external focus (EF) compared with an internal focus (IF) of attention improves motor learning and performance. Studies have indicated benefits in accuracy, balance, force production, jumping performance, movement speed, oxygen consumption, and fatiguing task. Although behavioral outcomes of using an EF strategy are well explored, the underlying neural mechanisms remain unknown. A recent TMS study compared the activity of the primary motor cortex (M1) between an EF and an IF. More precisely, this study showed that, when adopting an EF, the activity of intracortical inhibitory circuits is enhanced.

On the behavioral level, the present protocol tests the influence of attentional foci on the time to task failure (TTF) when performing submaximal contractions of the first dorsal interosseous (FDI). Additionally, the current paper describes two TMS protocols to assess the influence of attentional conditions on the activity of cortical inhibitory circuits within the M1. Thus, the present article describes how to use single-pulse TMS at intensities below the motor threshold (subTMS) and paired-pulse TMS, inducing short-interval intracortical inhibition (SICI) when applied to the M1. As these methods are assumed to reflect the responsiveness of GABAergic inhibitory neurons, without being affected by spinal reflex circuitries, they are well suited to measuring the activity of intracortical inhibitory circuits within the M1.

The results show that directing attention externally improves motor performance, as participants were able to prolong the time to task failure. Moreover, the results were accompanied by a larger subTMS-induced electromyography suppression and SICI when adopting an EF compared to an IF. As the level of cortical inhibition within the M1 was previously demonstrated to influence motor performance, the enhanced inhibition with an EF might contribute to the better movement efficiency observed in the behavioral task, indicated by a prolonged TTF with an EF.

Video Link

The video component of this article can be found at https://www.jove.com/video/55771/

Introduction

It is now generally accepted that adopting an EF compared to an IF or neutral focus of attention promotes motor performance and learning in numerous settings[1]. It has been shown, for example, that adopting an EF leads to benefits in accuracy[2,3], balance[4,5,6], force production[7,8], jumping performance[7,9,10,11], movement speed[12], oxygen consumption[13,14], and fatiguing tasks[15,16].

On the other side, since brain activation is the basis of all movements, several aspects of the neural control of movement have been investigated. For example, the level and the ability to modulate intracortical inhibition within the M1 has been shown to have a strong influence on motor function, such as interlimb coordination[17], postural control[18], and dexterity[19]. Furthermore, populations with poorer motor control abilities than young adults, such as elderly subjects or children (born preterm[20]), usually show less pronounced inhibitory control. Thus, although the role of inhibitory processes is not yet well understood, inhibitory processes nevertheless seem to be important to the quality of motor execution in general.

A possibility to investigate intracortical inhibitory circuitries is to use non-invasive transcranial magnetic stimulation (TMS). The most commonly used stimulation protocol applies paired-pulse TMS (ppTMS) to induce SICI. This protocol uses a conditioning stimulus below the motor threshold to reduce the amplitude of the suprathreshold control stimulus response elicited at an interstimulus interval of 1-5 ms[21,22,23,24]. Then, reported as the percentage of the control stimulus, the amplitudes of the motor-evoked potentials (MEPs) can be compared across conditions, giving information about cortical inhibitory activity and modulation within the M1.

Another stimulation protocol to assess the activity of intractortical inhibitory circuits applies single pulses, where all stimuli are delivered at intensities below the motor threshold (*i.e.,* subTMS). This protocol induces suppression in the ongoing EMG activity[18,25,26]. This so-called subTMS-induced EMG suppression can be compared in terms of amount and duration. Although this protocol is not so commonly used, it has

certain advantages compared to the standard SICI protocol. This protocol does not disturb motor execution, as it does not induce suprathreshold stimuli. Both methods test the responsiveness of intracortical gamma-aminobutyric acid (GABA) inhibitory interneurons[23,27].

Despite the well-known benefits of using an EF compared to an IF on motor performance[1], the underlying neural processes remain largely unknown. In a former fMRI study[28], it was shown that blood-oxygen level-dependent (BOLD) activation was enhanced in the M1, primary somatosensory, and insular cortices when subjects executed a finger sequence and adopted an EF compared to an IF. As excitatory and inhibitory activity cannot be differentiated by fMRI[29], another recent study[16] stipulated that the enhanced activity in the M1 associated with an EF could, in fact, be due to the enhanced activity of intracortical inhibitory circuits. More precisely, this study showed that the excitability of inhibitory GABAergic neurons can be modulated instantly by the type of attentional focus adopted in one and the same person.

The main aim of the present protocol is to show two possible ways to compare the immediate effects of cognitive manipulation (*i.e.,* focus of attention instructions) on the activity of intracortical inhibitory circuits within the M1. SubTMS and ppTMS are both used. In addition, this protocol shows one possible way to explore the influence of attentional foci on motor behavior in a very controlled way by investigating the TTF of submaximal isometric sustained contraction of the FDI.

Protocol

This protocol was approved by the local ethics committee, and the experiments are in accordance with the Declaration of Helsinki (1964).

1. Ethical Approval and Subject Instruction

1. Before starting the measurement, instruct all participants about the potential risk factors and the purpose of the study. Do not give information about the attentional foci, as this might affect the results. Ensure that the safety guidelines for the application of TMS in research settings [30] are followed.
   NOTE: When applying TMS, there are some medical risk factors, including implanted cranial electrodes and cochlear implants, personal history of syncope or seizure, epilepsy, cerebral lesion, drug/medication interactions, recent drug withdrawal, pregnancy, or illness. TMS should not be administered in children.
2. In the study, include healthy participants (n = 14) between 18 and 35 years old. Exclude subjects with any orthopedic and/or neurological/ mental diseases. Ensure that all participants are right-handed.

2. Experimental Design and Setup

1. Divide the group in two. Instruct one half of the group on IF instructions first, followed by EF instructions in the second experimental session (see section 4.2.2 for the verbal instructions). Instruct the other half in a counterbalanced order.
   NOTE: The experiment consists of a total of four laboratory sessions (see **Figure 1**) that must be separated by a minimum of 72 h. The first two sessions consist of measuring the maximal force (Fmax) and the TTF of submaximal sustained index finger abduction (see step 4). The third and fourth sessions consist of measuring the activity of inhibitory circuits within the M1 during the task by means of subTMS and ppTMS (see **Figure 1**).

3. Subject Preparation

1. Seat the participant in an adjustable and comfortable chair throughout the whole experiment. Place a monitor 1 m in front of the participant.
2. Place the left arm in a comfortable and relaxed position under the table, resting on the left leg. If needed, adjust the position of the arm with a pillow. Place the right arm of the subject in a custom-built splint in pronated position (see **Figure 2**).
   NOTE: Here, the splint is made of thermoplastic and fit all participants (for details, see[16]). In addition, the splint was conceived to restrict the degrees of freedom of the wrist joint (see **Figure 2B**). The only movements allowed were the abduction and adduction of the metacarpophalangeal joint of the index finger of the right hand.
3. Align the finger joint with the axis of rotation of the custom-made device. Once the optimal position is found, record manually and take a picture of the antero-posterior and medio-lateral positions of the splint to use comparable positions in sessions 2, 3, and 4.

4. Sessions 1 and 2: Behavioral Tests

1. **Maximal isometric contractions (see Figure 1A).**
   1. Align the axes of rotation of the goniometer and the metacarpophalangeal joint and fix the goniometer properly using screws (see **Figure 2**). Place the force transducer in a way that allows for maximum voluntary contractions (see **Figure 2B**).
   2. Connect the EMG cable (FDI muscle), the force transducer, and the goniometer cables to the appropriate amplifier and/or analog to digital (A-D) converter.
   3. Have the participant perform 3 maximal isometric abductions of the index finger, with a 30-s break between each contraction, and determine the Fmax.
      NOTE: The Fmax is determined as the highest peak in the force signal obtained from the force transducer. Explain to the participant that maximal contractions consist of a gradual increase in force from 0 N to the individual maximum. Importantly, instruct participants to perform an isometric contraction against the stationary force transducer. Participants should abduct the index finger at the metacarpophalangeal joint and push as hard as possible against the force transducer. A 3-s time span should be given per contraction, and participants should be instructed to sustain the maximal force for 2 s[16,25,26]. Between each contraction, give the participants a 30-s break.
   4. Have the subject push the lever against the force transducer, without giving any instruction about the focus of attention.

NOTE: The same task will be done at the beginning of session 2 to ensure that the Fmax and the position in the splint have not changed between sessions.

5. After maximal contractions, remove the force transducer, allowing the index finger to move freely in the transverse plane (abduction/adduction).
6. Calculate the Fmax from the maximal isometric abductions (step 4.1.3) using the raw data on the computer. Determine 30% (Fmax * 0.3; sessions 1 and 2) and 10% (Fmax * 0.1; sessions 3 and 4) of Fmax.
   NOTE: Consider the Fmax as the highest peak found in the force signal obtained from the force transducer. In the following sessions, the different contraction intensities (30% and 10%) will be calculated from the Fmax obtained at this stage of the experiment.
7. Fill a bottle of water to the amount representing the 30% of Fmax obtained from step 4.1.6. Attach the weight of Fmax to the rope of the device (see **Figure 2A**).
   NOTE: The volumetric mass density of water is 1 kg/L. Thus, if the 30% of Fmax of one participant represents 0.4 kg, adjust the weight of the bottle to the equivalent of 0.4 kg.

2. **Sustained contractions until TTF (see Figure 1A).**
   1. Instruct the participants about the task.
      NOTE: Participants must hold the finger in the target position by counteracting the weight (see **Figure 2**), performing an abduction of the index finger. The task must be performed until task failure. The task failure is determined as a deviation greater than 10 degrees from the target position. The deviation is measured by the goniometer and displayed on the monitor (see **Figure 2B**).
   2. Randomize the order of session (see step 2.1; EF or IF condition). Verbally instruct the participants on the adequate condition (IF or EF).
      1. For the EF condition, instruct as follows: "Concentrate on the position of the goniometer. Hold this position as long as possible. When the position of the goniometer changes, the thickness of the red line on the screen changes. Correct the position of the goniometer until the red line is thin again." Instruct the participant to "control and concentrate on the position of the goniometer" every 30 s.
      2. For the IF condition, instruct as follows: "Concentrate on the position of your finger. Hold this position as long as possible. When the position of your finger changes, the thickness of the red line on the screen changes. Correct the position of your finger until the red line is thin again." Instruct the participant to "contract and concentrate on his finger muscles" every 30 s.
   3. Have the participants hold the finger in the target position by counteracting the weight (see **Figure 2**), performing an abduction of the index finger. Have them perform the task until task failure.
   4. Press the "record" button on the recording software to start recording the goniometer signal and wait until task failure. Once task failure is reached, press the "stop recording" button on the recording software to stop the recording and save the goniometer signal on the computer. Remove the participant's hand from the orthopedic splint; the first session is now over.
   5. Respecting the minimum inter-session period (72 h), repeat steps 4.2.1-4.2.4. In addition, allow a minimum of a 72-h break between sessions 2 and 3 and sessions 3 and 4.

5. Sessions 3 and 4: Brain Stimulation

1. **Surface electromyography (sEMG) recordings.**
   1. Shave the hair on the skin over the right FDI muscle, if needed, and then slightly abrade the skin using abrading gel. Disinfect the abraded area with a solution containing 80% ethanol and 1% glycerin. Allow the ethanol to evaporate.
   2. Place the Ag/AgCl bipolar surface electrodes in a belly-tendon montage on the FDI, with 1-cm interelectrode distance. Place the reference electrode on the phalanx of the digitus medius.
   3. Connect the EMG cable (FDI muscle) and the goniometer cable to an EMG amplifier and to an A-D converter.
   4. Use Ag/AgCl bipolar surface electrodes to record and measure muscular activity and electrophysiological responses elicited by brain stimulation from the FDI muscle.
      NOTE: For the final analysis (subTMS-induced EMG suppression and peak-to-peak MEP amplitude), the EMG signal (from the FDI) must be adjusted as follows: amplification of x1000, Butterworth band-pass filtering of 10-1000 Hz, and sampling of 4 kHz. Store all EMG data on a computer for offline analysis.

2. Repeat steps 3.1 and 3.2.
3. **Transcranial magnetic stimulation**
   1. Fix the reflective markers on the participant's forehead with double-sided adhesive tape.
      NOTE: Reflective markers allow for the constantly delivery of TMS to the target area over M1 by using a neuronavigation system (see **Figure 2**). The advantage of the neuronavigation system is that the coil position can be recorded relative to the skull position in space and be checked at any time throughout the entire experiment.
   2. Use a 95-mm focal figure of eight coils attached to a TMS stimulator to deliver stimuli to the contralateral motor cortical hand area.
      NOTE: Check that the stimulator allows for paired-pulse stimulation paradigms (session 4). In addition, the induced current must be directed posterior to anterior and must be delivered in reverse mode. The wave form should be monophasic.
   3. Find the optimal position (hot spot) of the coil relative to the skull for eliciting motor evoked potentials (MEPs) in the FDI muscle by performing a classical mapping procedure.
      1. Start by placing the coil approximately 0.5 cm anterior to the vertex and over the midline, with the coil handle pointing at 45° towards the contralateral forehead.
         NOTE: This will ensure that the induced current flow is approximately perpendicular to the central sulcus[31].
      2. To get the participants used to the TMS stimuli, start at intensities below 25% of maximum stimulator output (MSO). Then, start to increase the stimulation intensity and move the coil in the medio-lateral and rostro-frontal direction to discover the hot spot.

4. Once the hot spot is found, record the optimal position with the neuronavigation system.Determine the active motor threshold (aMT) by adjusting the intensity of the stimulator output. Define the aMT as the minimum intensity required to evoke MEP peak-to-peak amplitudes in the EMG of the FDI larger than 0.1 mV in three out of five consecutive trials[21].

4. **Session 3: SubTMS-induced EMG suppression (see Figure 1B).**
    1. Prepare the weight representing the 10% of Fmax by filling the bottle of water (see step 4.1.7).
    NOTE: The 10% of Fmax are selected based on the Fmax (the best of 3 trials) performed in step 4.1.3. In the subthreshold TMS protocol, only 10% of the Fmax has to be selected, as it has previously been shown that fatigue has an influence on subTMS-induced EMG suppression[32,33]. For the same reason, the subTMS session must be carried out on a separate session. The volume of water used here is between 0.3 L (smallest 30% of Fmax) and 1.2 L (biggest 30% of Fmax).
    2. Instruct participants about the task; the motor task consists of holding the index finger in the target position by counteracting the light weight of 10% (abduction of the index finger; the same task as in sessions 1 and 2, but with less weight).
    3. As the participants remain relaxed in a comfortable position, find the optimal intensity for eliciting subTMS-EMG suppression, without giving any instruction about the focus of attention. To do this, successively diminish in steps of 2% MSO from the aMT determined previously.
    4. While they are still seated in the relaxed and comfortable position, have the participants perform two separate isometric index finger abductions at 10% of Fmax and record the EMG signal of the FDI. During this isometric index finger abduction, record (by pressing the "record" button on the recording software) 20 trials with and 20 trials without TMS, with a randomized interstimulus interval (ISIs) ranging from 0.8 to 1.1 s[16,25,26,33,34] in a 100-ms time window.
    NOTE: This interval ensures that participants do not have to perform the motor task for too long and therefore minimizes fatiguing effects. After each series, check the subTMS-induced EMG suppression.
        1. Apply a full-wave rectification by converting all negative amplitudes to positive amplitudes in the EMG signals. Average the EMG signals using time-normalized averaging[35].
        NOTE: The onset of subTMS-EMG suppression is defined as the moment when the difference between the trials with and those without TMS is negative for at least 4 ms in a time window from 20 to 50 ms after the TMS: $EMG_{Diff} = EMG_{Without} - EMG_{With}$.
    5. Repeat step 5.4.3 until the optimal stimulation intensity is found, indicated by the largest EMG suppression.
    NOTE: The optimal intensity is found at around 80% of aMT[16].
    6. Give the participant the adequate instructions (see step 4.2.2) regarding the condition (IF or EF). Repeat the instructions before each series (step 4.2.2).
    7. While they remain seated in the relaxed and comfortable position, have the participants perform four separate isometric index finger abductions (2 times with each focus: EF and IF) at 10% of Fmax and record the EMG signal of the FDI.
        1. During this isometric index finger abduction, record (by pressing the "record" button on the recording software) 40 trials with and 40 trials without TMS, with randomized ISIs for each condition (*i.e.,* IF and EF) in a counterbalanced order. Use the same intensity for each condition (determined in point 5.4.5).
    8. Between each series, allow a break of a minimum of 5 min to minimize any bias that may be induced by fatigue.

5. **Session 4: ppTMS (see Figure 1B).**
NOTE: The paired-pulse paradigm is composed of a conditioning stimulus at 0.8 aMT, followed by a suprathreshold control stimulus at 1.2 aMT.
    1. Repeat steps 5.1-5.4. In brief, place EMG electrodes over the FDI muscle, seat the participant in an adjustable and comfortable chair, and place the left arm in a comfortable and relaxed position under the table (*i.e.,* on the left leg). Find the hotspot for TMS over the M1.
    2. Set the intensity on the stimulator, the ISI at 2.5 ms[36], and the interval between paired and single-pulse TMS at 0.25 Hz.
    3. Give the participant adequate instructions (see step 4.2.2) regarding the condition (*i.e.* IF or EF). Repeat the instructions before each series.
    4. Have the participants perform four separate isometric index finger abductions (2 times with each focus: EF and IF) at 10% of Fmax and record the EMG signal of the FDI. During the isometric contraction, record 20 TMS stimuli for each condition (*i.e.,* IF and EF) in a counterbalanced order.
    NOTE: One set of 20 stimuli must be composed of 10 conditioned MEPs (paired-pulse at 0.8-1.2 aMT) and 10 control MEPs (single-pulse at 1.2 aMT). Use the same intensity for each condition (determined in step 5.5.2).
    5. Between each series, allow a break of a minimum of 5 min to minimize any bias that may be induced by fatigue.

6. Data Processing and Analysis

1. **SubTMS.**
    1. As explained above (step 5.1.1.3), rectify and average the EMG for analysis.
    2. Detect the onset of subTMS-EMG suppression (see **Figure 4**).
    NOTE: It is defined as the moment when the difference between the average of all trials with and those without TMS is negative for at least 4 ms in a time window from 20-50 ms after the TMS.
    3. To detect the end of subTMS-EMG suppression, define the moment after the onset of suppression (step 6.1.2) when the difference between the average of all trials with and those without TMS is positive again for at least 4 ms (see **Figure 4a**).
    4. Calculate the subTMS-induced EMG as follows:
    $EMG_{Diff} = EMG_{Without} – EMG_{With}$.
        1. Compute the cumulative trapezoidal numerical integration from the onset to the end of the suppression to quantify the amount of subTMS-induced EMG suppression.

2. **ppTMS.**

1. Use the following formula to express the magnitude of SICI as a percentage relating to the control MEP: 100 – (conditioned MEP/control MEP × 100).
    1. Use the results as percentage values for the final analysis.

2. Calculate peak-to-peak MEP amplitudes (in mV; in the EF and IF conditions) and compare the two conditions in the final analysis.

3. **EMG.**
    1. As background EMG has an influence on the magnitude of MEPs[37], determine the EMG activity by computing the root-mean-square value in a 100-ms window before the TMS.

Representative Results

The Influence of Attentional Foci on Motor Performance:

The behavioral tests in the current study were used to prove the feasibility of the motor task and to identify the subjects who reacted positively when applying an EF. In line with previous studies (see[1] for a review), our results show a prolonged TTF when the participants adopted an EF compared to an IF (see **Figure 3**). Thus, it seems that, during an isometric index finger abduction, the efficiency of the movement can be enhanced by an EF. McNevin and colleagues[38] posited the "constrained action hypothesis" to explain the effects of different foci of attention on motor performance and motor learning. The authors posited in their hypothesis: that using an EF ameliorates motor performance by promoting a greater automaticity in movement control. In contrast, the adoption of an IF is supposed to constrain the motor system, as a more conscious type of motor control is used. Nonetheless, despite the well-known benefits of using an EF compared to an IF on motor performance in general[1], the underlying neural processes remain poorly investigated. Therefore, the central question remains: determining how the enhanced movement efficiency associated with an EF compared to an IF is controlled from a motor cortical point of view.

Intracortical Inhibition and Motor Abilities:

Cortical activity is constituted of interactions between excitatory and inhibitory mechanisms within brain motor areas[24]. In addition, the modulation of these processes are essential for motor control[39]. For example, children[40,41,42] and elderly individuals[43] show reduced levels of intracortical inhibition—in contrast to healthy, young subjects—resulting in reduced coordinative abilities. In general, it seems that intracortical inhibitory processes and motor performance are closely interrelated when considering different populations. In addition, not only across age groups or different populations, but also within age groups, motor function seems to be strongly altered by corticospinal inhibitory processes, such as interlimb coordination[17] or dexterity[19]. Therefore, the level of intracortical inhibition within the M1 seems to affect the characteristics of motor control in general.

The Measure and Influence of Attentional Foci on Intracortical Inhibition:

In a previous fMRI study, Zentgraf and coworkers[28] started to investigate neural correlates associated with attentional foci (*i.e.,* EF versus IF). The results showed greater activation in different brain areas—the M1, the insular, and the primary somatosensory cortices—when subjects performed a keyboard finger sequence in an EF condition rather than an IF condition. Apart from the limitation that different subjects were investigated in the EF and IF tasks, making direct comparisons impossible, the fMRI technique is not able to differentiate between excitatory and inhibitory neural activity[29], as it uses intrinsic blood-tissue contrasts[44]. Therefore, the higher brain activation found in the M1 in the EF condition demonstrated in this previous fMRI study[28] may result from increased excitatory or inhibitory activity. Therefore, fMRI provides only an estimate about the overall neural activity[29]. In contrast and in complement to fMRI, TMS can give information about the nature of the enhanced activity, whether it results from excitatory or inhibitory activity. The reason for this is that TMS applied to the M1 at intensities below the active motor threshold inhibit motor cortical output, as the cortical inhibitory GABAergic interneurons have a lower threshold to TMS than the excitatory neurons[27,45,46,47,48]. In addition, it was shown that TMS under the motor threshold does not cause descending volleys and, therefore, does not activate spinal structures[23,27]. In this study, we used two TMS protocols to measure the cortical inhibition within M1. The first used a single-pulse subTMS protocol, which induces a suppression in the ongoing EMG activity. It has been proposed that the inhibition of the ongoing activity of fast-conducting corticospinal cells results in a subTMS-induced EMG suppression[49].

Thus, there is a relationship between the excitability of intracortical inhibitory circuits and the amount of subTMS-induced EMG suppression. In other words, an increase in cortical inhibition within the M1 results in more EMG suppression[18]. Although the subTMS protocol is not so widely used, it inherits many advantages compared to protocols using suprathresold stimuli: first, as the stimulation does not add but rather removes activity from the descending corticospinal volley, the effects can clearly be attributed to the primary motor cortex, as they are not affected by spinal circuitries[23,27]. Second, as subthreshold intensities are used, no muscle twitch in induced by stimulation, which may disturb motor performance. Using this technique, we demonstrated that the subTMS-induced EMG suppression was instantly enhanced when using an EF compared to an IF (see **Figure 4** for results and analysis). Specifically, our results showed that the activity of intracortical inhibitory circuits within the M1 is immediately modulated when different attentional foci are adopted.

Another more widespread possibility for measuring the activity of GABAergic motor interneurons is to apply a ppTMS paradigm with short interstimulus intervals over the contralateral M1. The paired-pulse stimulation induces a reduction in the MEP amplitude, which is called the SICI, and reflects the activity of inhibitory GABAergic neurons[21,45,50].

When adopting an EF, participants showed more SICI (see **Figure 5** for results and analysis). This is well in line with the subTMS results and suggests that GABAergic neurons, constituting the intracortical inhibitory circuits[51], are modulated differently within the M1 according to the type of attentional focus. This would be in line with former research showing that the M1 is sensitive to differential attentional situations[52]. In addition, as a positive correlation between the cerebral blood flow in the motor cortex and the amount of SICI has been revealed in a positron emission tomography study[53], our results might further support the enhanced cortical activity within the M1 that was found by Zentgraf and colleagues[28]. Finally, as the motor tasks and background EMG prior to stimulation were similar in both conditions, it has been deduced that verbal instructions stipulating the direction of attention indeed have a main modulatory influence on the activity of the intracortical inhibitory neurons projecting to the FDI.

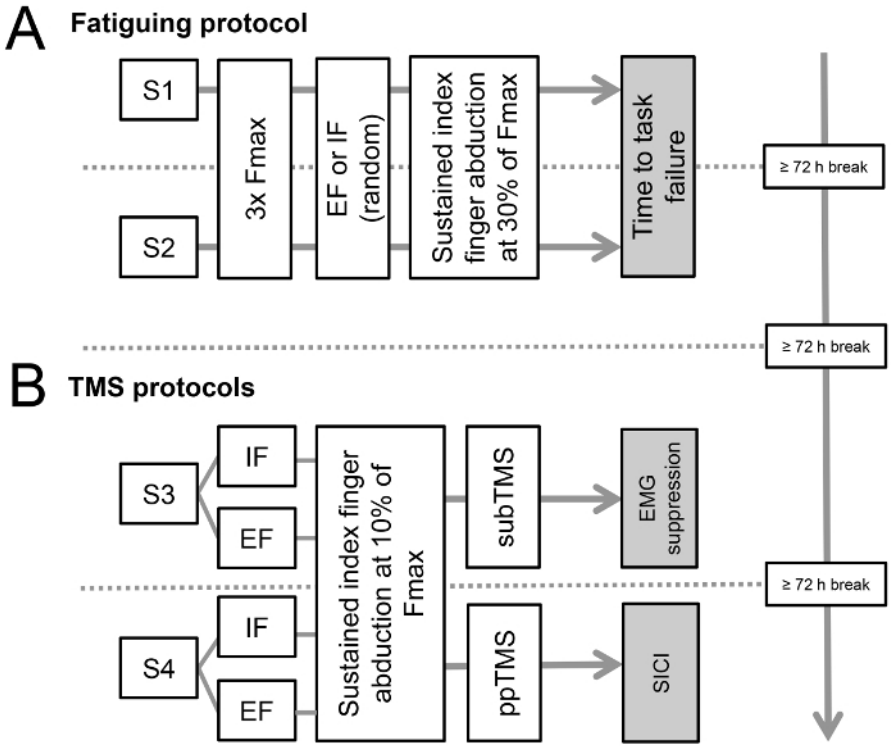

Figure 1. Time course of the four protocols. A. The aim of the first two sessions (S1 and S2) is to compare the time to task failure (TTF) of a submaximal sustained abduction of the right index finger at 30% of the Fmax between an external (EF) and an internal focus of attention (IF). During the EF session, the subjects are asked to concentrate on the goniometer angle (*i.e.,* the movement effect), while during the IF session, they are asked to concentrate on their index finger and muscle (*i.e.,* body movement). B. The third and fourth sessions (S3 and S4) aim to compare the cortical activity of intracortical inhibitory circuits within the M1 between an EF an IF. This can be achieved by comparing the amount and the duration of subthreshold TMS (subTMS) induced EMG suppression and by comparing the amount of short-interval intracortical inhibition (SICI) induced by paired-pulse TMS (ppTMS). This figure was adapted from Kuhn *et al*[16]. Please click here to view a larger version of this figure.

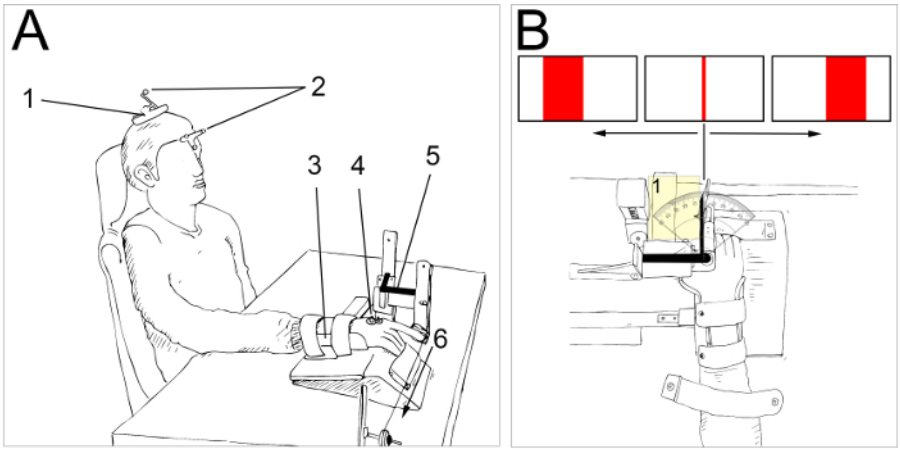

Figure 2. Experimental setup. A. 1. The TMS coil is placed over the contralateral M1 over the hand representation. 2. The participant's forhead and the TMS coil are mounted with reflecting markers to control the position of the TMS coil relative to the skull. 3. The orthopedic splint restricts movement of the wrist and only allows movements of the index finger. 4. EMG electrodes are placed in a tendon-belly montage over the FDI. 5. The goniometer calculates the angle of the metacarpophalangeal joint of the index finger. 6. The weight representing either 30% (S1 and S2) or the 10% (S3 and S4) of Fmax is attached to the rope. B. The movements of the metacarpophalangeal joint are displayed on a computer screen placed 1 m in front of the subject. When the angle is 90°, the red line displayed on the computer screen is the thinnest. As soon as the participant's finger moves to the left or right, the red line gets thicker in the corresponding direction. The aim of the motor task is to keep the red line as thin as possible. To measure the Fmax (S1 and S2), the force transducer is placed (1) such that the participants can push against it (*i.e.*, isometric contraction), keeping a constant angle of 90°. Please click here to view a larger version of this figure.

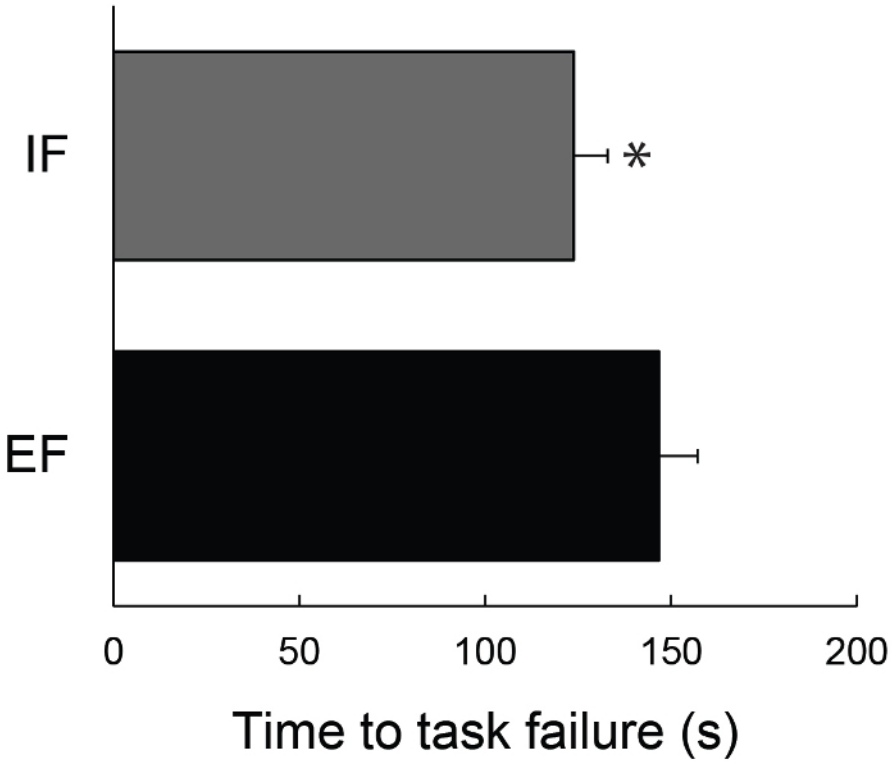

Figure 3. Time to task failure (TTF) of sustained contractions. The TTF was prolonged (approximately by +18%) when the participants (n = 14) adopted an external (EF) rather than an internal focus of attention (IF). * $p < 0.05$. The error bars represent the SEM. This figure was adapted from Kuhn *et al*[16]. Please click here to view a larger version of this figure.

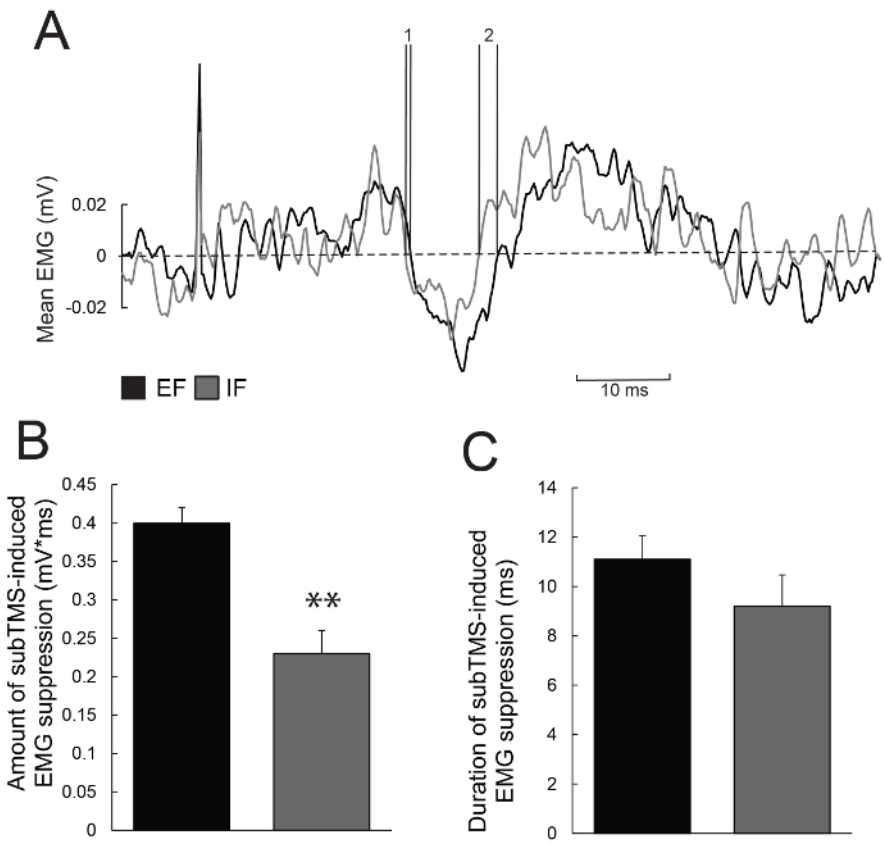

Figure 4. SubTMS-induced EMG suppression. A.To acquire the curves of the mean EMG activity during the sustained contraction of the right first dorsal interosseous (FDI) at 10% of Fmax, the rectified EMG (full-wave rectification) of the trials with subTMS is subtracted from that of the trials without stimulation. The vertical lines represent (1) the onset of subTMS-induced EMG suppression and (2) the end of subTMS-induced EMG suppression. B. Representative data (n = 10) of the amount of the subTMS-induced EMG suppression. The data are obtained by computing the cumulative trapezoidal numerical integration from the onset to the end of the suppression (*i.e.,* the negative area under each curve from 1 to 2 in A). The amount of subTMS-induced EMG suppression is enhanced when an external focus (EF) rather than an internal focus of attention (IF) is adopted. C. Representative data (n = 10) of the subTMS-induced EMG suppression duration from 1 to 2. No significant difference was found in the duration of the suppression, but it is longer with an EF. Thus, it is reasonable to assume that the effect size was too small to induce a significant difference in our relatively small sample size. ** $p < 0.01$. The error bars represent the SEM. This figure was adapted from Kuhn *et al*[16]. Please click here to view a larger version of this figure.

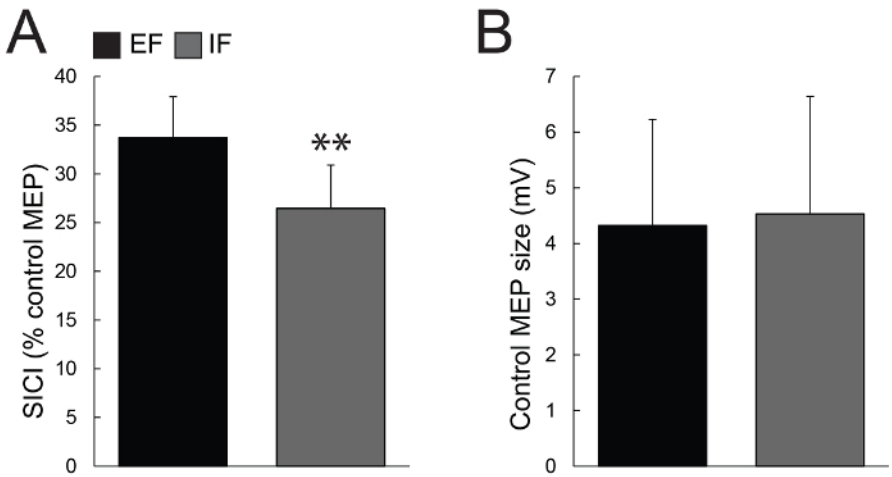

Figure 5. Short-interval intracortical inhibition (SICI). A. The SICI is expressed as the percentage of the control MEP in FDI by applying the following formula: 100 - (conditioned MEP / control MEP × 100). The SICI is enhanced when the participants adopt an EF compared to an IF. This reflects greater activation of intracortical inhibitory circuits. B. As the amplitude of the control MEP has an influence on the size of the conditioned MEP, the control MEPs at 1.2 aMT peak-to-peak amplitudes should be compared between the two conditions (*i.e.,* EF versus IF). ** $p < 0.01$. The error bars represent the SEM. This figure was adapted from Kuhn *et al*[16]. Please click here to view a larger version of this figure.

Discussion

This protocol shows two possible methods to investigate the activity of inhibitory circuits within the M1 using TMS. More precisely, these two protocols have been used in this study to investigate the impact of attentional foci on the activity of inhibitory circuits within the M1.

One limitation of the presented method is that it is not always possible to cause a subTMS-induced EMG suppression without a facilitation preceding it. In this study, for example, four subjects had to be removed from the final analysis, as they did not show any consistent subTMS-induced EMG suppression. Nonetheless, this non-invasive brain stimulation method is well accepted for measuring and quantifying the activity of intracortical inhibitory circuits within the M1[32,34]. Another limitation of this study is that it cannot be excluded that the differences between the foci of attention outlined by subTMS and ppTMS rely on brain areas upstream the M1. Despite the fact that both methods are assumed to test the responsiveness of intracortical GABA inhibitory interneurons[23,27], there is no correlation between the amount of subTMS-induced EMG suppression and the amount of SICI[16]; further investigations are needed.

In addition, it is important to use a light resistance (10% of Fmax) during the TMS protocols, to conduct the subTMS experiment in separate sessions (≥ 72-h break), and to randomize the conditions. The main reason is that fatigue can influence the magnitude of subTMS-induced EMG suppression[32] and the level of SICI[54], meaning that the main effect of attention might be biased by fatigue. During a fatiguing task, a number of peripheral, subcortical, and cortical mechanisms can also play a crucial role in performance. Moreover, it is important to use a neuronavigation system, as the TMS coil must be placed in the same spot before each trial. In addition, this system allows the experimenter to check the coil position at any time throughout the whole experiment.

The main finding of the present study is that cortical inhibition within the M1 can be affected instantly in the same subject according to the attentional focus adopted during the motor execution. As inhibitory processes seem to be closely related to the quality of motor execution in general, our results might explain on a neural level the enhanced efficiency of an EF compared to an IF. It can be speculated that the increased level of inhibition during EF avoids unnecessary co-activity and leads to a more focal activation, resulting in a more efficient motor execution. In this way, our results might constitute one of the underlying mechanisms of the "constrained action hypothesis." In addition, this protocol is the first to show how to apply subTMS and ppTMS to the same participants using a repeated-measures design. Moreover, despite the fact that a large number of studies show that adopting an EF compared to an IF promotes motor performance and learning in numerous settings[1], only very few investigate the underlying neural mechanisms when different attentional situations stipulated through verbal instruction are adopted[16,28,55].

Disclosures

The authors have nothing to disclose.

Acknowledgements

The authors have no acknowledgements.

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