Video Article

Non-invasive Assessment of Changes in Corticomotoneuronal Transmission in Humans

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Abstract

The corticospinal pathway is the major pathway connecting the brain with the muscles and is therefore highly relevant for movement control and motor learning. There exists a number of noninvasive electrophysiological methods investigating the excitability and plasticity of this pathway. However, most methods are based on quantification of compound potentials and neglect that the corticospinal pathway consists of many different connections that are more or less direct. Here, we present a method that allows testing excitability of different fractions of the corticospinal transmission. This so called H-reflex conditioning technique allows one to assess excitability of the fastest (monosynaptic) and also polysynaptic corticospinal pathways. Furthermore, by using two different stimulation sites, the motor cortex and the cervicomedullary junction, it allows not only differentiation between cortical and spinal effects but also assessment of transmission at the corticomotoneural synapse. In this manuscript, we describe how this method can be used to assess corticomotoneural transmission after low-frequency repetitive transcranial magnetic stimulation, a method that was previously shown to reduce excitability of cortical cells. Here we demonstrate that not only cortical cells are affected by this repetitive stimulation but also transmission at the corticomotoneuronal synapse at the spinal level. This finding is important for the understanding of basic mechanisms and sites of neuroplasticity. Besides investigation of basic mechanisms, the H-reflex conditioning technique may be applied to test changes in corticospinal transmission following behavioral (*e.g.*, training) or therapeutic interventions, pathology or aging and therefore allows a better understanding of neural processes that underlie movement control and motor learning.

Video Link

The video component of this article can be found at https://www.jove.com/video/52663/

Introduction

In primates, the corticospinal tract constitutes the major descending pathway controlling voluntary actions[1]. The corticospinal pathway connects motor cortical areas to spinal α-motoneurons via direct monosynaptic corticomotoneuronal connections and via indirect oligo- and polysynaptic connections[2,3]. Although the motor cortex can easily be excited non-invasively by Transcranial Magnetic Stimulation (TMS), the evoked electromyographic response to this stimulation is often difficult to interpret. The reason for this is that the compound Motor Evoked Potential (MEP) can be influenced by changes in the excitability of intracortical and corticospinal neurons, spinal interneurons and spinal α-motoneurons[4,5,6,7]. Several noninvasive electrophysiological techniques and stimulation protocols aim at determining whether changes in corticospinal excitability and transmission are caused by changes at the cortical or spinal level. Commonly, changes in the amplitude of the electrically evoked H-reflex are used as 'indicative' of alterations of excitability at the motoneuron pool. However, it was previously shown that the H-reflex depends not only on the excitability of the motoneuron pool but is also modulated by other factors such as presynaptic inhibition[8,9] or homosynaptic post-activation depression[5,10]. Another limitation when comparing MEPs and H-reflexes is the disability to detect excitability changes at the interneuronal level[11,12]. In addition to these drawbacks, the motoneurons might be differently activated by peripheral nerve stimulation than with TMS so that changes in the motoneuronal excitability would affect these responses in a different kind of way compared to responses mediated via the corticospinal pathway[13,14,15].

Another method used to separate spinal from cortical effects represents Transcranial Electrical Stimulation (TES) of the motor cortex[16]. Applied at low stimulation intensities, TES was argued to be unaffected by changes in cortical excitability. As both TES and TMS activate the α-motoneurons via the corticospinal pathway, the comparison of magnetically and electrically evoked MEPs provides a more attractive method to draw conclusions on the cortical nature of changes in the size of the MEPs than the comparison between H-reflexes and MEPs. However, when stimulation intensity is increased, TES-evoked MEPs are also influenced by changes in cortical excitability[17,18]. This problem can be circumvented when electrical stimulation is not applied to the motor cortex but at the cervicomedullary junction. However, although electrical stimulation can evoke cervicomedullary motor evoked potentials (cMEPs) in upper limb and lower limb muscles, most subjects perceive

electrical stimulation at the brainstem (and cortex) as extremely unpleasant and painful. A less painful alternative is to activate the corticospinal pathway at the cervicomedullary junction by use of magnetic stimulation at the inion[19]. It is generally accepted that Cervicomedullary Magnetic Stimulation (CMS) activates many of the same descending fibers as motor cortical TMS and that changes in cortical excitability can be detected by comparing MEPs with cMEPs[19]. Increases in the excitability of intracortical cells and corticomotoneuronal cells are thought to facilitate the cortically evoked MEP without a concurrent change in the cervicomedullary evoked MEP.

However, in most subjects it is impossible to obtain magnetically evoked cMEPs in the lower extremity at rest[20,21]. One approach to overcome this problem is to elevate the excitability of spinal motoneurons by voluntary precontracting the target muscle. However, it is well known that slight changes in contraction strength influence the size of the cMEP. Thus, it is difficult to compare different tasks. In addition, changes in the motoneuronal excitability due to pre-contraction will influence MEPs and cMEPs but not necessarily to the same extent. Finally, by comparing compound MEPs with compound cMEPs some information contained in the descending volleys is lost. This has been revealed by studies involving conditioning of the H-reflex of soleus, tibialis anterior, and carpi radialis muscles by magnetic motor cortical stimulation[12,22]. By combining peripheral nerve stimulation and TMS over the motor cortex with specific interstimulus intervals (ISI), it is possible to study facilitatory and inhibitory effects of the different descending volleys on the H-reflex. This technique is greatly inspired by the spatial facilitation technique used to determine transmission in neural pathways in animal experiments and may be seen as a non-invasive, indirect version of that technique[23]. While the H-reflex is not only important to differentiate between different fractions of the corticospinal pathway (fast versus slower corticospinal projections) it is also essential to elevate spinal excitability in a controlled and comparable way. Thus, at rest and during activity, this combination of stimulation techniques allows assessment of changes in different fractions of the corticospinal pathway with a high temporal resolution, *i.e.* in the fastest, presumably monosynaptic corticomotoneuronal connections and in slower oligo- and polysynaptic pathways[12,22,24,25]. Recently, this technique was extended by not only conditioning the H-reflex with TMS over the motor cortex (M1-conditioning) but also by additional conditioning stimulation at the cervicomedullary junction (CMS-conditioning)[26]. By comparing effects between M1- and CMS-conditioning, this technique allows pathway specific differentiation with a high temporal resolution and it allows interpretations to be made on cortical versus spinal mechanisms. Furthermore and most importantly with respect to the current study, this technique allows assessment of transmission at the corticomotoneural synapse when considering the early facilitation. The early facilitation of the H-reflex is in all likelihood caused by activation of direct, monosynaptic corticomotoneural projections to the spinal motoneurons[12,26]. To test the fastest corticospinal pathways and thus, the early facilitation, the H-reflex has to be elicited 2 to 4 ms before the TMS. The reason for this is the slightly shorter latency of the MEP (around 32 ms; see[27]) compared to the H-reflex (around 34 ms; see[25]). Eliciting the H-reflex shortly before applying TMS, leads to convergence of the ascending and fastest descending excitations at the level of the spinal motoneurons. When TMS is applied over the cervicomedullary junction, the descending volley will arrive around 3 - 4 ms earlier at the spinal motoneuron pool than after stimulation over M1. For CMS-conditioning, peripheral nerve stimulation should therefore be evoked 6 - 8 ms before the magnetic pulse. A change of the early facilitation after CMS-conditioning indicates differential transmission at the synapse between the corticospinal tract and the α-motoneuron[28]. In the current study, this recently developed technique was used to differentiate spinal from cortical effects following low frequency repetitive TMS (rTMS). More specifically, we hypothesized that if the early facilitation with M1-conditioning is reduced following the rTMS intervention but the early facilitation following CMS-conditioning is not, the effect should be purely cortical in origin. In contrast, if the early facilitation with CMS-conditioning also changes, this alteration should be related to mechanisms taking place at the spinal level. More specifically, as the early facilitation of the H-reflex is thought to be caused by activation of direct, corticomotoneuronal projections to the spinal motoneurones[12,29], a change of the CMS-and M1-conditioned H-reflex at the time of the early facilitation should indicate an altered corticomotoneuronal transmission *i.e.* synaptic efficacy[28].

Protocol

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

1. Subject Preparation

NOTE: Subject instructions - Before starting with the experiment, instruct each subject about the purpose of the study and potential risk factors. For transcranial magnetic stimulation (TMS), medical risks include any **history** of epileptic seizure, mental implants in eyes and/or head, any diseases of the cardiovascular system, and pregnancy. Exclude all subjects affirming to one of these risk factors. Furthermore, in the experiment testing healthy individuals, exclude all subjects with neurological and/or orthopedic disease.

1. **Subject Placement**
    1. Place the subject in a chair that supports legs, trunk, and head in place. Ensure that the legs are outstretched so that the knees are extended and the peripheral nerve is closer to the skin making the nerve easier and more reliably excitable by electrical stimulation.
    2. Make sure that the subject's head is flexed, resting on a stable support surface such as a table and is secured with cushions. Ensure that the neck and the atlanto-occipital are flexed to allow stimulation of the corticospinal pathway.
    3. Position the double cone magnetic coil so that its central portion is placed on or near the inion and the first derivative of the induced current is cranially directed [19,26]. Use elastic straps at the head and the trunk to ensure that this position is maintained throughout the experiment.

2. Using surface electrodes measure the electrophysiological responses by Peripheral Nerve Stimulation (PNS) and TMS.
    1. Prepare the skin over the muscle belly of soleus by shaving, disinfection with propanol, and light abrasion.
        1. Place self-adhesive EMG electrodes on the skin over the muscle belly of m. soleus. Place a reference electrode on the skin over the bone, *e.g.,* on the patella or the medial malleolus.
        2. Connect all electrodes to an EMG-amplifier and finally an analogue-digital converter. Amplify EMG signals (×1000), bandpass-filter (10 - 1000 Hz) and sample at 4 kHz.

    2. **PNS**

1. For H-reflex conditioning, record H-reflexes in the soleus muscle by stimulating the posterior tibial nerve in the popliteal fossa.Apply stimulation with square-wave pulses lasting 1 ms. For stimulation, fixate an anode of 5 x 5 cm with tape on the anterior aspect of the knee just underneath the patella.
   NOTE: Stable H-reflex amplitude is a prerequisite for successful H-reflex conditioning and the least variability of all muscles can be found when recording from the soleus muscle.
2. Move the cathode in the popliteal fossa until the best position for stimulation is found.
   NOTE: The best position refers to recording H-reflexes in the soleus muscle with minimum stimulation intensity, without a visible M-wave in the EMG recordings at these low stimulation intensities, and without receiving any response in the antagonist m. tibialis.
3. Avoid responses in m. tibialis muscle as those may affect results by reciprocal inhibition from Ia afferents of n. peroneus communis to spinal motoneurons of the soleus muscle. After finding the optimal location, place a self-adhesive electrode on the skin and fix the electrode with tape to ensure consistent stimulation conditions.

3. **TMS**
   1. Stimulate the motor cortical area of the contralateral hemisphere with TMS using a figure eight coil to elicit motor evoked potentials (MEPs) in the electromyographic recordings of the soleus muscle.
   2. In order to find the optimal stimulation spot, place the coil first over the vertex and 1 cm frontal. The handle of the coil should point backwards, evoking a posterior to anterior flux of the induced current in the center of the coil.
   3. Start stimulation with low intensities of around 20 - 30% of the maximal stimulator output so that subjects get accustomed to the magnetic stimulus. Choose the pause between successive stimuli to be 4 s.
   4. After a few trials, increase stimulation intensity to around 40 - 60% of the maximal stimulator output and move the coil in the frontal-rostral and medio-lateral direction in order to find the hotspot of m. soleus. The hotspot is defined as the position where MEPs in the m. soleus can be evoked with minimum stimulation intensity.
   5. After finding the soleus hotspot, determine the resting motor threshold (1.0 MT) as the minimum intensity required to evoke MEP peak-to-peak amplitudes in the EMG larger than 50 µV in six out of ten consecutive trials[30]. In subjects in whom the background EMG is already around 50 µV, use 100 µV as threshold.

4. **Fixation of the Coil**
   1. Place the subject's head on a table (see "Subject placement") and use rigid foam to prevent head movements in all directions. Fixate the coil to a stand and the subject's head to the chair.
   2. Fixate the coil with Velcro strips to the head and use an image-guided TMS navigational system for monitoring coil and head position throughout the experiment. Avoid even small movements of the coil relative to the subject's head as this changes the recruitment of neurons by TMS.

5. **Magnetic Stimulation at the Cervicomedullary Junction**
   1. Use a double-cone magnetic coil placed at the cervicomedullary junction to excite axons of the corticospinal tract.
   2. Position the coil so that the first derivative of the induced current is cranially directed and that its central portion is on or near the inion. Apply stimulation with maximum stimulator output (100%).
      NOTE: Even with this high stimulation intensity, the stimulus is too weak to sufficiently recruit spinal motoneurons and activate the muscles of the lower leg (*i.e.* m. soleus and m. tibialis anterior) in most subjects. Thus, with cervicomedullary stimulation, there is no compound potential in the surface EMG of lower leg muscles. Therefore, combine cervicomedullary simulation with the H-reflex (see "3.1) to raise the excitability of the spinal motoneurons.

2. Premeasurement

1. Adjust the size of the H-reflex (peripheral nerve stimulation)
   1. For H-reflex conditioning, adjust the size of the H-reflex to 20% of the maximum M-wave (Mmax) [31] by changing the stimulation intensity of the electrical stimulator. To obtain Mmax, record an H-reflex recruitment curve. For this purpose, apply stimuli with varying stimulation intensities. The pause between successive trials is 4 s.
   2. Calculate H-reflexes and M-waves as peak-to-peak amplitudes in the EMG (in mV) online in the recording software. Take care that the size of the control H-reflex stays constant at 20% of Mmax throughout the experiment and check its size in each trial. When detecting a systematic deviation of the H-reflex size (control H-reflex is always smaller or larger as the target size), adjust the stimulation intensity just prior to the consecutive trial.

2. Adjust the stimulation intensity of TMS prior to the experiment.
   1. For H-reflex conditioning at rest, set the stimulation intensity for TMS over the motor cortex to 90 - 100% of MT.Ensure that no MEP is seen in trials without PNS.
      NOTE: The simulation intensity should be close to 100% of MT in order to ensure large effects on the conditioned H-reflex at rest so that the early facilitation can easily be detected.
   2. Adjust cervicomedullary stimulation intensity prior to the experiment. Unlike cortical stimulation, always adjust stimulation intensity for cervicomedullary stimulation to 100% of the maximum stimulator output.

3. Condition the H-reflex with magnetic stimulation over the motor cortex.
   1. Apply TMS and PNS by varying the timing between the two stimuli (H-reflex conditioning) to allow assessment of changes in corticomotoneuronal transmission. To detect the early facilitation, start the conditioning protocol with an interstimulus interval (ISI) of -5 ms and alter ISIs in steps of milliseconds, from -5 - +1 ms (**Figure 1B**).
      NOTE: Negative ISIs indicate that PNS is elicited before TMS, positive ISIs indicate the opposite.

2. Vary the ISI between TMS and PNS randomly from stimulation trial to stimulation trial so that no bias due to a certain order of stimuli may arise.
   NOTE: The "early facilitation" should occur around ISIs -4 ms to -2 ms when applying TMS over the motor cortex. This means that the fastest (monosynaptic corticospinal pathways) collide with the afferent volley by PNS at the spinal motoneurons at this time (see 5.2 for detecting the early facilitation).
3. Set the pause between successive stimulation trials to 4 seconds.

4. Condition the H-reflex with magnetic stimulation over the cervicomedullary junction.
   NOTE: Using cervicomedullary stimulation for conditioning, excitation of the corticospinal pathways is spatially closer to the spinal motoneurons than with stimulation of the motor cortex. Therefore, the ISI corresponding to the early facilitation is shifted by approximately 3 - 4 ms. As an example, the early facilitation with TMS over the primary motor cortex at -4 ms would correspond to an ISI between -7 - -8 ms with cervicomedullary stimulation.
   1. Use ISIs between ISI -9 - -3 ms in steps of 1 ms for cervicomedullary conditioning. Apply ISIs for TMS over the motor cortex and TMS over the cervicomedullary junction always together in one trial and record a control H-reflex and a control MEP in this trial, too. Use the control H-reflex as a reference for the conditioned H-reflexes and the control MEP to ensure comparable stimulation conditions. Record (at least) ten trials in the pre-measurement.

5. **Alternating Stimulation over the Motor Cortex and Cervicomedullary Junction**
   1. Apply conditioning of the SOL H-reflex by magnetic stimulation of the motor cortex (M1-conditioning; see 2.1) and by magnetic cervicomedullary stimulation (CMS-conditioning; see 2.2) in a random order during the same trial.
      NOTE: It is recommended to alternately apply M1- and CMS-conditioning in one and the same trial in order to refer the conditioned H-reflexes to the same sample of control H-reflexes (see **Figure 1**).

3. Intervention - Slow Repetitive TMS

1. Set the stimulation intensity to 1.2 MT, which induces a long-lasting [32,33] suppression of corticospinal excitability required as H-reflex conditioning takes several minutes to accomplish. During the rTMS intervention, apply TMS over the primary motor cortex at 1 Hz for 20 min.

4. Postmeasurement

1. Directly after the intervention, apply H-reflex conditioning with the same ISIs as used in the premeasurement.
2. Use the same stimulation intensities for magnetic stimulation over M1 and the cervicomedullary junction than in the pre-measurement.
3. Ensure that the control H-reflex has the same size as in the pre-measurement. If a systematic deviation is detected, adjust the stimulation intensity.

5. Data Processing

1. Calculate all physiological responses such as H-reflexes, MEPs, and conditioned H-reflexes as peak-to-peak amplitudes of the unrectified EMG.
   1. For each ISI, average ten conditioned H-reflexes for a) cortical and b) cervicomedullary stimulation. Additionally, average ten control (*i.e.* unconditioned) H-reflexes that serve as a reference (*i.e.* 100%) for the conditioned H-reflexes.
   2. Consequently, express the mean amplitude of the conditioned H-reflexes for each ISI as a percentage of the mean amplitude of the control H-reflex in both the pre- and post-measurement. Take care when determining the early facilitation as this is of critical significance:
      NOTE: As there is inter-individual variability in the occurrence of the onset of the early facilitation, determine the early facilitation in the premeasurement for each subject separately.

2. Use nonparametric Wilcoxon tests to determine the first rise of the conditioned H-reflex. For CMS-conditioning, start the tests at ISI -9 ms, for M1-conditioning search the early facilitation beginning at ISI -5 ms. Compare the amplitude of this early facilitation obtained in the pre-measurement with the amplitude of the early facilitation obtained in the post-measurement using the same ISI.
3. Additionally, verify the early facilitation by visual inspection.
   NOTE: After M1-conditioning, the early facilitation is most likely to occur around ISI -3 ms. Shortly after the first rise in the conditioned H-reflex, *i.e.* 1 to 2 ms later, there is a decline in the conditioned H-reflex before it rises again. After CMS-conditioning, the early facilitation is likely to occur around ISI -7 ms, thus, around 4 ms earlier than after M1-conditioning.

Representative Results

Occurrence of the early facilitation after M1- and CMS-conditioning

H-reflex conditioning with TMS over M1 resulted in an early facilitation that occurred around ISI -3 & -4 ms. The early facilitation after CMS-conditioning occurred around 3 ms earlier (ISI -6 & -7 ms, respectively). Exemplary ISI-curves of one subject are displayed in **Figure 1**. In the present study, the early facilitation was assessed within the first ms of its occurrence with both M1- and CMS-conditioning (see **Figure 1C**, **D**). Thus, it is reasonable to assume that this early facilitation reflects activity of direct, monosynaptic corticospinal pathways[12,22,24,29,34]. The subsequent results therefore concentrate on this early facilitation in order to give an indication of how processing is altered in direct, monosynaptic corticospinal pathways after rTMS.

rTMS-induced changes in the amplitude of the early facilitation

After 20 min of rTMS, there was a decrease in both, the early facilitation with M1-conditioning and the early facilitation with CMS-conditioning. In contrast, the control H-reflex remained at a constant level. In **Figure 2A, B, C** an example of a representative subject is displayed. In **Figure 2D, E, F** the mean of two subjects is provided. It can be seen that although the reduction is not as prominent after CMS-conditioning than after M1-conditioning it is nevertheless clearly visible. The data set of the entire sample can be seen in[28].

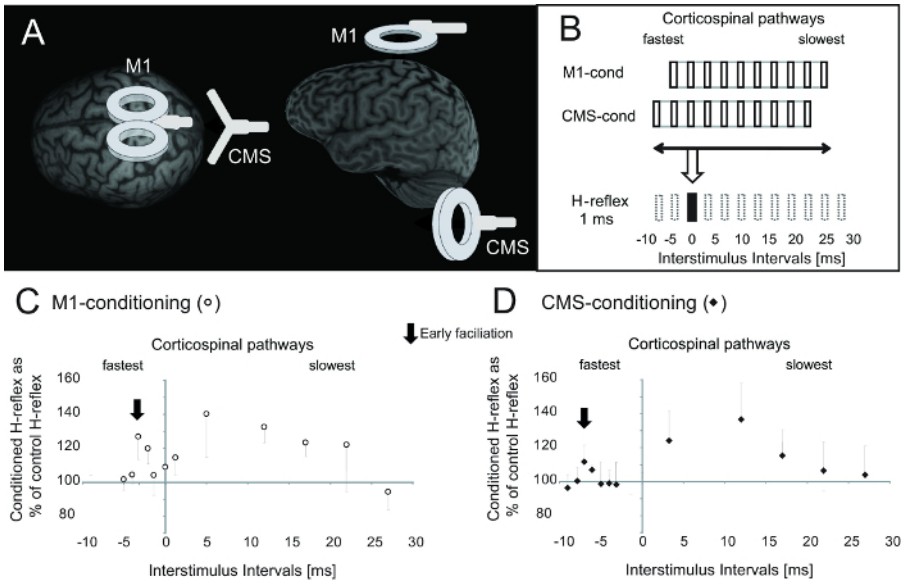

Figure 1: Procedure of M1- and CMS-conditioning.
This modified figure from one of our previous publications[28] displays a schematic drawing of the M1- and CMS-conditioning procedure. **(A)** It can be seen that one coil is placed over the primary motor cortex (indicated as M1) and the other over the cervicomedullary junction (indicated as CMS). (**B)** as the descending volleys after magnetic stimulation of the primary motor cortex (M1-cond) and the cervicomedullary junction (CMS-cond) are dispersed for some ms but peripheral nerve stimulation (H-reflex) produces only a short effect, the H-reflex can be shifted forward in relation to the descending volley so that it collides with the fast(est) fraction(s) of the descending corticospinal volley (early facilitation) or it can be shifted backwards so that slower corticospinal pathways can be tested (late facilitation). In **C**, an H-reflex conditioning curve after M1-conditioning is displayed. In **D**, the H-reflex conditioning curve after CMS-conditioning is illustrated. (Figure modified from[28] with permission from Oxford University Press). Please click here to view a larger version of this figure.

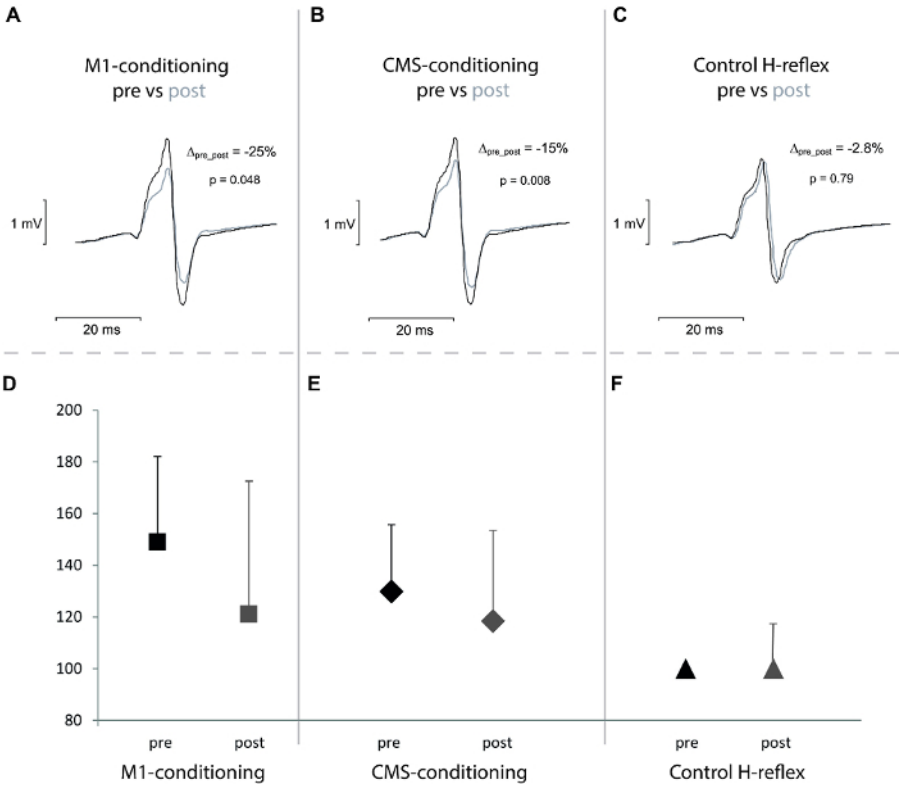

Figure 2: Effects of Low Frequency rTMS on the Early Facilitation after M1- and CMS-conditioning.
In **A, B,** & **C** data (averages of 10 traces) of one representative subject before and after the rTMS intervention are displayed. It can be seen that the conditioned H-reflexes representing the early facilitations are reduced after both, M1- (A) and CMS-conditioning (B) whereas the control H-reflexes remain unchanged (C). In **D, E,** & **F**, the mean of two subjects is displayed showing the same pattern: reduction in both M1- and CMS-conditioned H-reflexes without any change in the control H-reflex. The reduction after CMS-conditioning indicates altered transmission at the corticomotoneuronal synapses. However, it can be seen that the suppression after rTMS is larger after M1-conditioning. Thus, profound changes at the motor cortical level can be presumed, too. P-values in the first row refer to the data of the single subject. (Figure modified from [28] with permission from Oxford University Press). Please click here to view a larger version of this figure.

Discussion

The H-reflex conditioning procedure described here has been specifically addressed to assess acute changes in transmission over the corticomotoneuronal synapse following repetitive activation of the corticospinal pathway[28]. In this respect, H-reflex conditioning has highlighted that rTMS does not only affect excitability of cortical structures but also has an effect on the corticomotoneural transmission at the corticomotoneural synapse. However, this method may indeed have broader application as changes in corticospinal transmission occur during motor development and aging, motor learning, exercise and training, fatigue, inactivity, recovery from injury, neurophysiological and therapeutical interventions, pathology etc. Furthermore, the methods may be applied in able-bodied subjects or in patients as long as the TMS safety precautions are followed.

The introduced method may be applied to study within-session effects as in the present context or longitudinal effects across a longer time span. The M1-conditioning technique has previously been demonstrated to allow reliable assessment of effects following for instance 8 weeks of immobilization[35], 4 weeks of balance training[36,37] and four weeks of ballistic strength training[36]. In all of these studies, no changes in the conditioned H-reflexes were observed in the control groups, who were not subjected to a behavioral intervention. Considering the CMS-conditioning technique to our knowledge no study has so far been published on long-term effects.

A conditioning protocol including approximately 12 - 14 ISIs lasts approximately 15 minutes. This means that this stimulation protocol is not suitable for assessing shorter-lasting neural plasticity. It is however often possible to restrict post-intervention testing procedures to target specifically *e.g.,* the early facilitation assessed in the pre-measurement and thereby shorting the duration of the procedure markedly to a few minutes. In this case it is important to determine the early facilitation for each subject individually. This was made in the baseline measurement and later on compared with the early facilitation obtained in the post-measurement using the same ISI(s).

The advantage of conditioning the H-reflex by TMS applied over M1 or the cervicomedullary junction rather than monitoring the compound potentials is twofold. Firstly, it is possible to measure selectively transmission of distinct corticospinal projections, for instance to assess changes in the early facilitation reflecting activity of fast and direct corticomotoneuronal projections. This is a major advantage compared to analysis of compound potential amplitudes as these latter responses are influenced by a multitude of direct and indirect effects. Secondly, it is often not possible to elicit compound potentials (CMEPs) by cervicomedullary magnetic stimulation alone particularly for the lower extremity muscles and during measurements at rest (Ugawa et al. 1994, Oya et al. 2008). Using H-reflex conditioning, the H-reflex increases the susceptibility of spinal motor neurons to corticospinal transmission. However, it is important to keep the size of the SOL control H-reflex constant throughout the

experiment at around 20 - 25% of M_{max} as it was previously demonstrated that the sensitivity of the H-reflex to facilitatory or inhibitory inputs depends crucially on its size[31].

In order to assess whether changes in corticospinal excitability or transmission is caused by changes at a cortical or spinal level a number of studies have compared responses elicited by TMS over M1 with responses after TES of M1[16]. TMS and TES differ with respect to the way how they elicit descending corticospinal volleys. With TMS, a large proportion of the compound response is brought about by transsynaptic excitation of corticospinal cells[38,39]. In contrast, TES depolarizes a greater proportion of corticospinal neurons in a direct manner, probably at an axonal site distant to the axon hillock, resulting in a so-called 'direct', or D-wave [38,39,40]. Changes in the excitability of the motor cortex therefore more strongly influence responses after TMS than those after TES - at least at low stimulation intensities[17,18]. In the present context TES was not applied because a) this kind of stimulation is associated with considerable pain and b) we wanted to ensure the exclusion of cortical influences. Therefore, we compared responses elicited with TMS over M1 with responses elicited by TMS at the cervicomedullary junction. In order to allow stimulation of the corticospinal pathway at the cervicomedullary level it is necessary to place the subject in a position where the neck and the atlanto-occipital joint is flexed in order to allow positioning of the coil so that its central portion is placed on or near the inion resulting in a cranially directed current[19,26]. Changes in the responses of this CMS-conditioning procedure can therefore clearly attributed to changes at the spinal level. Furthermore, as the early facilitation of the conditioned H-reflex is thought to be caused by activation of direct, corticomotoneuronal projections to the spinal motoneurones[12,29], a change of the CMS-conditioned H-reflex at the time of the early facilitation indicates an altered corticomotoneuronal transmission *i.e.* synaptic efficacy[28].

Although it is indeed a relevant perspective that the described methods may also be applied in order to obtain measurements for the upper extremities through stimulation of peripheral nerves in the arm and recordings from arm or hand muscles, this technique is limited to muscles in which it is possible to elicit a stable H-reflex. Furthermore, due to the unpleasant character of CMS-conditioning, subjects may tense in anticipation of the stimulus. Thus, it is important to randomize M1- and CMS-conditioning in order to avoid a systematic bias. For the very same reason, certain experiments involving mental simulation or reaction time tasks may even not be possible. For instance, we asked subjects to imagine certain postural tasks (cf.[41]) but subjects could not concentrate on the mental simulation when anticipating CMS-conditioning. Another limitation is the usage of this method during more dynamic tasks as it is a) very difficult to fixate the coil over the cervicomedullary junction and b) to keep the head in a flexed position. Finally, the method is very time consuming, further limiting its application in a broad sense.

Disclosures

The authors have nothing to disclose.

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