New evidence of cortico-spinal network modulation induced by motor imagery

Sidney Grosprêtre*1,2, Florent Lebon1,2, Charalambos Papaxanthis1,2, Alain Martin1,2

1INSERM U1093, Faculté des sciences du sport, BP 27 877, Dijon F-21078, France
2Univ. Bourgogne Franche-Comté, Besançon, France

*Address for Correspondence:
Sidney GROSPRETRE
INSERM U1093,
Faculté des sciences du sport,
BP 27 877,
Université de Bourgogne,
Dijon F-21078, France
sidney.grospretre@univ-fcomte.fr

Copyright © 2015 by the American Physiological Society.
Abstract

Motor imagery (MI) is the mental simulation of movement, without the corresponding muscle contraction. While the activation of cortical motor areas during MI is established, the involvement of spinal structures is still under debate. We used original and complementary techniques to probe the influence of MI on spinal structures. Amplitude of motor-evoked potentials (MEP), cervico-medullary-evoked potentials (CMEP), and Hoffmann (H)-reflexes of the flexor carpi radialis (FCR) muscle and H-reflexes of the triceps surae (TS) muscles were measured in young healthy subjects at rest and during MI. Participants were asked to imagine maximal voluntary contraction of the wrist and of the ankle, while the targeted limb was fixed (static condition). We confirmed previous studies with an increase of FCR MEPs during MI in comparison to rest. Interestingly CMEPs, but not H-reflexes, also increased during MI, revealing a possible activation of subcortical structures. Then, to investigate the effect of MI on spinal network, we used two techniques: i) passive lengthening of the targeted muscle via an isokinetic dynamometer and ii) conditioning of H-reflexes with stimulation of the antagonistic nerve. Both techniques activate spinal inhibitory presynaptic circuitry, reducing the H-reflex amplitude at rest. In contrast, no reduction of H-reflex amplitude was observed during MI. These findings suggest that MI has modulatory effects on spinal neuronal network. Specifically, the activation of low-threshold spinal structures during specific conditions (lengthening and H-reflex conditioning) highlights the possible generation of subliminal cortical output during MI.

Key words: H-reflex, motor-evoked potential, cervico-medullar-evoked potential, transcranial magnetic stimulation, muscle lengthening, presynaptic inhibition.
Introduction

During the past three decades, evidence has emerged suggesting that mental training can improve motor performance (e.g., Lebon et al., 2010), thus promoting its interest for neuro-rehabilitation programs (for review, see Malouin et al., 2013). Mental training with motor imagery involves the internal simulation of movements without corresponding motor output (Jeannerod, 2001). Numerous investigations have revealed that similar cortical areas, particularly motor areas, were activated during actual and imagined movements (Decety et al., 1994; Roth et al., 1996; Lotze et al., 1999). Transcranial magnetic stimulation (TMS) studies provided evidence of cortico-spinal modulations during MI (for review, see Grosprêtre et al., 2015). This activation was classically expressed in the literature by an increase in amplitude of motor-evoked potentials (MEP) recorded in the muscle involved in the imagined action (e.g., Stinear and Byblow, 2003a; Liang et al., 2008; Rozand et al., 2014).

However, findings from previous studies have not provided evidences on whether the MI-induced modulation occurs at cortical, subcortical and/or spinal levels. Some authors found a facilitation of the Hoffmann (H) reflex amplitude, an index of spinal excitability, elicited by peripheral nerve stimulation (PNS) during MI in comparison to rest (Bonnet et al., 1997; Cowley et al., 2008). On the contrary, others showed a decrease of H-reflex (Oishi et al., 1994) or did not find any effect (Yahagi et al., 1996). These inconsistencies may arise from different methodologies employed to assess reflex excitability, different imagined tasks or particular sensitivity of spinal structures (interneurons, motoneurons, etc.). For example, the different modulation of H-reflex observed during MI might be related to various parameters, such as the intensity of PNS, the intensity of the mental simulation, or the normalization of H-reflex (see Supplementary Material).
Today, the question of the release of a potential cortical output elaborated during MI remains open (Guillot et al., 2012). Does this motor output reach the spinal level, therefore implying cortical and spinal interactions, or is it the product of supraspinal processes solely? In particular, the neural simulation theory of Jeannerod (1994, 2001) supports the first assumption, stipulating that during MI the inhibition of the motor output would be incomplete and would activate subliminally the descending tracks. In this case, such activation may reach the spinal network and may potentially modulate the activity of the most excitable neural structures, such as interneurons.

This study aimed to bring new insights on the interaction between cortical and spinal networks during MI, by applying stimulations at different levels of the cortico-spinal tract. TMS was used to probe cortico-spinal excitability. Cervico-medullar stimulation and PNS were used to investigate spinal excitability. Cervico-medullar-evoked potentials (CMEP) and H-reflexes can provide different insights on spinal excitability, as they are not influenced by the same circuitry (Taylor, 2006). Indeed, cortico-motoneuronal synapse is not subjected to presynaptic inhibition, contrary to Ia-alpha synapse, which means that the amplitude of CMEP can be modulated without changes of the H-reflex, and conversely. The comparison of muscle responses to these non-invasive stimulations provided the means to examine the functioning of the cortico-spinal pathway at the segmental level during MI (Taylor & Gandevia, 2004). We also used original techniques to assess the activity of spinal structures with lower excitability threshold than motoneurons during MI. We investigated the interaction between motor responses (MEPs and H-reflexes) and sensory feedbacks induced by passive muscle lengthening (such as used by Pinniger et al., 2001; Duclay et al., 2011 during actual contraction) and by a conditioning maneuver (method used by Achache et al., 2010) when participants imagined muscle contractions. We hypothesized that MI may elicit a subliminal cortical output that would reach the spinal network and modulate the excitability of low-
threshold spinal structures, such as presynaptic interneurons. Due to the methodological constraints, we measured motor responses in both upper and lower limbs (flexor carpi radialis – FCR – and triceps surae muscles – TS, respectively).
Materials and Methods

Participants

Twelve young healthy right-handed males volunteered to participate in this study (age: 23.8 ± 1.08 year-old, height: 1.76 ± 0.02 m, weight: 71.13 ± 2.93 kg). None of them reported neurological or physical disorders. They gave informed consent, after being fully informed about the investigation and the possible related risks and discomfort. All experimental procedures were performed in accordance with the Declaration of Helsinki and approved by the Regional Ethics Committee.

The tests were carried out in four experimental sessions, separated by at least 3 days. All participants (n=12) took part in FCR experiments in session 1 and 3. Among them, four performed the session 2 and ten performed the experiments with TS recordings in session 3 and 4. Table 1 depicts a simplified overview of the whole protocol (number of subjects, recorded parameters, number of stimulations per parameter, etc.). Stimulation and motor imagery techniques are depicted in figure 1, and detailed in the following sections.

Briefly, this study included four experimental sessions, involving different stimulation techniques and interventions. The first session was designed to probe the cortico-spinal excitability during imagined wrist contractions, by measuring FCR MEPs in the right forearm that was kept in a static position, i.e., without any active or passive movement. Four subjects took part in the second session during which CMEPs were measured in FCR muscle in the same conditions as in session 1. In session 3, we measured MEPs and H-reflexes in the upper limb (FCR muscle) and H-reflexes in the lower limb (TS muscles) when participants’ hand and foot was passively lengthened and shortened by an ergometer at rest and during MI. The fourth session was conducted to further understand spinal mechanisms underlying MI by conditioning TS H-reflexes. We chose to investigate presynaptic inhibition in TS muscles as it
provides constant and reliable results at rest (Aymard et al., 2000; for further information, see below).

General experimental procedures

Participants sat comfortably in an isokinetic dynamometer chair (Biodex Shirley, NY). To test FCR responses in the right hand, the rotation axis of the dynamometer was aligned with the styloid process of the ulna. Participants’ right hand was firmly strapped in neutral position to a home-made accessory adapted for wrist movement recordings. The upper-arm was vertical along the trunk (shoulder abduction and elevation angles at 0°) and the forearm semi-pronated and flexed at 90°. To test TS responses in the right leg, the rotation axis of the dynamometer was aligned with the external malleolus. Participants’ right leg was placed with hip, knee, and ankle joints at 90° (0° = full extension), foot being firmly strapped to the dynamometer pedal. During all conditions, particular care was taken in avoiding trunk and head rotations to maintain constant cortico-vestibular influences on the excitability of the motor pool (Schieppati, 1987). The trunk was stabilized by two crossover shoulder harnesses and movements of the head were reduced by a collar fastened to the headrest of the seat.

For all experiments and all types of stimulation, participants were asked to stay at rest or to imagine maximal voluntary efforts. In the flexion mode (muscle shortening), participants were instructed to imagine pressing maximally on the hand pad (for wrist MI) or on the pedal (for ankle MI) and to feel the corresponding muscle contractions (kinesthetic motor imagery). In the extension mode (muscle lengthening), they were asked to imagine pressing on the pad with the palm side of the hand or on the pedal with the sole of the foot and to feel the kinesthetic sensation normally elicited by this contraction. The participants were instructed
not to use visual motor imagery but to feel the intensity of muscle contraction normally elicited during actual performance. A kinesthetic strategy was shown to maximally modulate cortico-spinal excitability (Stinear et al., 2006). Before imagined contractions, participants were allowed to perform actual movements in order to have a correct representation of the level of muscle contraction. If actual contractions were performed, a rest period of at least two minutes was observed to allow the EMG responses to return at their baseline level before beginning the tests (for instance in Duclay & Martin 2005). Each imagined trial was preceded by two oral signals given by the experimenter: "get ready" and "go". The 3 types of stimulation (cortical, cervico-medullar and peripheral) were triggered between 2 and 3 seconds after the go signal. Participants were instructed to stop imagining few seconds after the stimulation. The numbers of stimuli are depicted in Table 1. The trials, separated by at least 10 seconds, were randomly assigned and each series of experiments also contained 4 trials without stimulation, in order to avoid stimulation habituation or expectation.

[Please insert table 1 about here]

For passive movements induced in session 3, the dynamometer provided instantaneous recording of joint torque and position at constant angular velocity (30°.s⁻¹). The range of wrist motion was 60°, from -30° (wrist extension) to +30° (wrist flexion), and the range of ankle motion was 30°, from -15° (dorsi-flexion) to +15° (plantar flexion). During passive lengthening and shortening, we automatically triggered the stimulation using the position of the dynamometer (0° angle between the wrist and the hand and 90° angle between the leg and the sole of the foot), to avoid alteration of H-reflex amplitude induced by changes in muscle length (Gerilovsky et al., 1989). At rest, the mean amplitude of the responses was matched between each condition to avoid size effect on the modulation analyzed (static, lengthening
and shortening). Indeed, passive muscle lengthening reduces cortico-spinal and spinal excitability (Chye et al., 2010; Howatson et al., 2011), mainly due to spinal presynaptic inhibitory mechanisms (Duclay et al., 2011). The stimulation intensity for shortening and static conditions was adjusted to match response amplitude recorded in lengthening condition. To reduce antidromic influences, we ensured in all conditions that the intensity of the H-reflex stimulation did not elicit responses beyond maximal H-reflex, Hmax (Grosprêtre and Martin, 2012), thus always lying in the ascending part of the recruitment curve (range of H/M\textsubscript{max} ratio: 0.11-0.40).

**Electromyographic (EMG) activity**

EMG was recorded from two muscles of the right forearm (FCR and Extensor Carpi Radialis, ECR) and from four muscles of the right leg (soleus, SOL; medial gastrocnemius, GM; lateral gastrocnemius, LG; and tibialis anterior, TA) in experiments measuring upper and lower limb activity, respectively. After shaving and dry-cleaning the skin with alcohol to keep low impedance (< 5 kΩ), EMG signals were obtained by using two silver-chloride surface electrodes (8 mm diameter) placed over the belly muscle with an inter-electrode center-to-center distance of 2 cm. The common reference electrode was placed in a central position between stimulation and recording sites, over the medial epicondyle of the humerus for upper limb measurements and between upper gastrocnemii insertions for lower limb measurements.

To record responses from arm muscles, electrodes were positioned over the muscle belly at 1/3 of the distance from the medial epicondyle and the radial styloid for FCR muscle and at 1/3 of the distance from the lateral epicondyle and the radial styloid for ECR muscle (Bartko and Carpenter, 1976). For SOL, MG and TA muscles, the electrodes were positioned 2 cm below the insertions of the gastrocnemii over the Achilles tendon, over the mid belly of
the muscle, and at 1/3 of the distance on the line between the fibula and the tip of the medial malleolus, respectively (Hermens et al., 2000).

EMG signals were amplified with a bandwidth frequency ranging from 15 to 1 kHz (gain = 1000). EMG and mechanical signals were digitized on-line (sampling frequency: 5 kHz) and stored for analysis with Tida software (Heka Elektronik, Lambrecht/Pfalz, Germany).

Transcranial magnetic stimulation (session 1)

TMS was used to elicit MEPs in FCR muscle of the right forearm. A figure-of-eight shaped coil was positioned over the left motor cortex and connected to a stimulator (Magstim 200, Magstim Company Ltd, Great Britain). The handle was oriented toward the back of the head and laterally at a 45° angle, assumed to be approximately perpendicular to the central sulcus.

First, the optimal spot for eliciting the largest MEP in the right FCR muscle was identified and this position was marked on the bathing cap worn by the participant. We then determined the resting motor threshold (rMT), defined as the minimal TMS intensity required to evoke MEP peak-to-peak amplitudes of ~50 µV in the targeted muscle on 5 out of 10 consecutive trials (Kobayashi and Pascual-Leone, 2003). During the main phase of the experiment, the TMS intensity was set to 120% of the participant’s rMT (range from 50 to 80 % of maximum stimulator output across participants, mean = 67.6 ± 2.51 %). The coil was secured by using a home-made tripod with a lockable articulated arm distributed by Otello Factory (T&O brand, France). The experimenter checked visually throughout the experiment that the coil was positioned identically for all conditions, and checked MEP amplitude during rest trials. During the session 1, PNS was also used to elicit maximal M waves in order to
normalize MEPs (see below). Each type of stimulation was randomly elicited across rest and MI trials.

**Cervico-medullary stimulation (session 2)**

In order to assess whether a subliminal motor output reaches spinal structures during MI of wrist contractions, CMEPs were recorded in the FCR muscle at rest and during MI for four subjects in session 2. Cervico-medullary stimulations (CMS) was shown to provide a direct measurement of motoneurons excitability by eliciting a single volley in descending axons at the pyramidal decussation (Taylor, 2006). CMEPs were measured in upper limb muscles, since it is easier to obtain responses in this body part (Taylor, 2006). However, the number of subjects recruited was limited and the number of stimulations restricted (see Table 2), since CMEPs can be difficult to record in some participants due to the discomfort induced by the electrical stimulation (Taylor et al., 2002). CMS were initially tested in 8 participants. They performed a familiarization protocol and were asked to fully relax with the EMG signal being monitored during all trials to ensure stable muscle activity. We discarded four participants who presented noise in EMG signal due to stimulation apprehension. We also elicited PNS with optimal intensity to elicit maximal M wave (M\text{max}). Trials with and without stimulation were randomly distributed.

CMS was given by placing two silver-chloride (2-cm diameter) electrodes over mastoid processes on either side, cathode placed on the left. We used a Digitimer DS7 AH (Hertfordshire, UK) to elicit 200-µs width pulses. The rMT was individually determined as the minimal intensity to evoke CMEP peak-to-peak amplitude of ~50 µV in the targeted muscle. The intensity was set to 120% of the participant’s rMT (mean intensity used for 120% rMT: 187.5 ± 65 mA; range: 100 – 250 mA).
Peripheral nerve stimulation (sessions 3 and 4)

To elicit H-reflex in the targeted muscles, single rectangular pulses (1 ms width) were delivered by two electrodes connected to a Digitimer stimulator (model DS7, Hertfordshire, UK). To evoke FCR H-reflex, two silver-chloride surface electrodes (8-mm diameter) were positioned in line with the median nerve up to the cubital fossa, below biceps’ muscle belly, with the cathode 2.5 cm proximal to the anode. ECR EMG activity was measured to ensure that the antagonist muscle was not activated by the stimulation. To evoke TS H-reflex, the posterior tibial nerve was stimulated via a self-adhesive cathode (8 mm diameter, Ag-AgCl) placed in the popliteal fossa and with the anode (5 x 10 cm, Medicompex SA, Ecublens, Switzerland) placed over the patellar tendon. TA EMG activity was measured to ensure that the common peroneal nerve was not activated. The best stimulation site to obtain the greatest H-reflex was first located by a hand-held cathode ball electrode (0.5-cm diameter). Then, the stimulation electrode was firmly fixed with straps. The intensity of stimulation was then increased from H-reflex threshold to maximal M-wave ($M_{\text{max}}$), with 1 mA increment. Maximal M waves were measured and used in each experimental session and condition to normalize each evoked response.

In session 3, FCR maximal H-reflexes ($H_{\text{max}}$) were first measured, as it provided a sufficient associated M-wave ($M_{\text{ath}}$) to ensure similar stimulation conditions throughout the experiment (Grosprêtre and Martin, 2012). Mean intensity was 7.1 ± 1.86 mA for FCR $H_{\text{max}}$ and 15.66 ± 2.83 mA for FCR $M_{\text{max}}$ recordings. Maximal H-reflexes with no associated M wave, called $H_{\text{free}}$ (Maffiuletti et al., 2001) or H1 (Katz and Pierrot-Deseilligny, 1998) were measured in SOL (intensity range: 10-40 mA). At this intensity, PNS minimizes the risk of an antidromic collision between alpha motoneuron axon and reflex volley that could interfere with presynaptic conditioning maneuverer. This allows avoiding misinterpretations regarding changes in spinal excitability due to presynaptic modulation (Grosprêtre and Martin, 2012).
We optimized the stimulation intensity for the SOL muscle, MG and LG H-reflexes being often associated with a small M wave at this intensity ($M_{\text{atH}}$). However, variations of MG and LG $M_{\text{atH}}$ are useful to identify any changes in PNS parameters relative to methodological issues (Grosprêtre et al., 2012).

In session 4, TS H-reflexes were conditioned by PNS of the antagonist nerve, i.e. the common peroneal nerve, in order to induce D1 presynaptic inhibition of Ia afferences onto alpha motoneurons (see figure 1). The decrease of H-reflex with such conditioning technique is attributed to an increased excitability of the primary afferent depolarization interneurons, thus reflecting an increased presynaptic inhibition of SOL Ia afference (Mizuno et al., 1971).

Here, the conditioning stimulus was applied to the branch of the common peroneal nerve with two silver-chloride surface electrodes (8 mm diameter) placed at the upper part of the anterolateral side of the leg, distal to the caput fibulae (Forget et al., 1989). To avoid monosynaptic facilitation in soleus motoneurons by Ia afferences of peroneal muscle (Meunier et al., 1993), we carefully checked visually and by tendon palpation that the stimulation did not induce any contraction of this muscle. The conditioning stimulus was a triple pulse over the common peroneal nerve at 300 Hz (1 ms duration), with the intensity set at 120 % of TA motor threshold (Achache et al., 2010). Conditioning-test interval was determined for each participant by testing several intervals between 15 and 25 ms, with 1 ms-steps. The interval used to obtain the greatest SOL H-reflex depression was $21 \pm 1$ ms, confirming previous findings of Faist et al. (1996). Conditioned and unconditioned H-reflexes were randomly elicited at rest and during imagined contractions of the TS muscles with ankle kept in a constant neutral position (angle: $90^\circ$).

Data analysis
For each session and for each muscle, the peak-to-peak amplitude average of MEP, CMEP, \( M_{\text{atH}} \), H-reflex, and \( M_{\text{max}} \) responses was calculated. Responses were normalized to \( M_{\text{max}} \) evoked in the same condition. Thus, MEP/\( M_{\text{max}} \), CMEP/\( M_{\text{max}} \), H/\( M_{\text{max}} \) and \( M_{\text{atH}}/M_{\text{max}} \) were considered as dependent variables and compared between MI and rest.

In all conditions, EMG activity was quantified with root mean square (RMS) values of the raw signal over a 500 ms period prior to the stimulation. EMG\(_{\text{RMS}}\) analyses ensured that no contraction was present during MI. For FCR and TS muscles, EMG\(_{\text{RMS}}\) activity was normalized by the corresponding \( M_{\text{max}} \) (RMS/\( M_{\text{max}} \)). As \( M_{\text{max}} \) was not recorded in ECR and TA muscles, RMS was not normalized in these muscles. The analysis of EMG signals also ensured that no background EMG activity was induced by the passive solicitations (e.g. shortening and lengthening).

**Statistical analysis**

First, the normality of the data was verified by the Shapiro-Wilk test (p>0.05) in order to ensure the use of classical analysis of variance (ANOVA) for parametric values.

Differences between MI and rest for RMS/\( M_{\text{max}} \), H/\( M_{\text{max}} \), \( M_{\text{atH}}/M_{\text{max}} \), \( M_{\text{max}} \) and MEP/\( M_{\text{max}} \) in static conditions of session 1 and 3 were analyzed with Student’s two-tailed paired \( t \) tests. A non-parametric Wilcoxon test was performed for CMEP/\( M_{\text{max}} \) of session 2, due to the low sample (four subjects).

For session 3 with passive movements (FCR and TS muscles), a one-way repeated measures ANOVA (rmANOVA), with the factor *muscle action mode* (shortening, lengthening and static), was first performed to ensure that H-reflexes and MEPs at rest were similar among the conditions. Then, a two-way rmANOVA was conducted with within-subjects factors *muscle action mode* (passive lengthening, static and passive shortening) and *task* (rest
and MI) for each variable (RMS/M\textsubscript{max}, H/M\textsubscript{max}, M\textsubscript{atlH}/M\textsubscript{max}, and MEP/M\textsubscript{max} for FCR and TS muscles, and RMS for ECR and TA muscles).

For session 4, H/M\textsubscript{max} was submitted to a two-way rmANOVA for each calf muscle (SOL, MG and LG), with presynaptic conditioning (with or without conditioning) and task (rest and MI) as within-subjects factors.

When a main effect or an interaction was found, a post-hoc analysis was made, using an HSD (honest significant difference) Tukey’s test. Statistical analysis was performed using STATISTICA (8.0 version, Statsoft, Tulsa, Oklahoma, USA). Level of significance was accepted at p < 0.05. The effect size was calculated by the eta-squared method (Levine and Hullett 2002). All data are expressed by their mean ± standard deviation.
Results

EMG activity

Mean values of all the recorded parameters are presented in Table 2. For each session and muscle, RMS/M_max and M wave amplitudes (M_max and M_ail/M_max) were not significantly affected by task or muscle action mode factors (all p>0.40). For instance, no effect of the factor task was found on M_max amplitude (FCR, F_1,11=0.67, p=0.43; SOL, F_1,9=0.29, p=0.75; MG, F_1,9=0.73, p=0.41; LG, F_1,9=0.33, p=0.72), neither on RMS/M_max (FCR, F_1,11=0.002, p=0.96; SOL, F_1,9=0.49, p=0.50; MG, F_1,9=0.41, p=0.54; LG, F_1,9=0.08, p=0.78). These results ensured that the possible modulation of motor responses (MEP, CMEP and H-reflex) would not be attributed to muscle contractions or stimulation conditions.

Cortico-spinal, cervico-medullar and spinal excitability in static position

In session 1 and 2, when the right hand was in static position, MI increased FCR MEP/M_max (t_1,11=3.27, p=0.007) and FCR CMEP/M_max (Z=2.52, p=0.012; range: +34.9 / +206.3 %) in comparison to rest (Figure 2).

In contrast, no change was observed between rest and MI regarding H/M_max in static position, in session 3 (FCR, p=0.62) and session 4 (MG, p=0.88; LG: p=0.62; SOL, p=0.84,
This lack of modulation was not dependent of the initial rest H-reflex amplitude. In fact, during static conditions, no changes between rest and MI H/M\textsubscript{max} were either found when stimulus intensity was reduced (to match lengthening H) in session 3 (FCR, p=0.99; SOL, p=0.95; MG, p=0.94; LG: p=0.50).

Thus, to elicit H-reflexes in each muscle, two stimulus intensities were used among the several experiments, corresponding to several points on the recruitment curve. As an example, static rest H-reflex was 3.01 ± 0.52 mV for SOL muscle (corresponding to H/M\textsubscript{max} ratio of 0.41 ± 0.05; see table 2, STATIC CONDITION unmatched) and it was 1.47 ± 0.38 mV when re-adjusted to match amplitude in lengthening condition (corresponding to H/M\textsubscript{max} of 0.23 ± 0.04; see table 2, STATIC CONDITION rest matched with lengthening). To summarize, no effects of MI was found on H/M\textsubscript{max} whatever the intensity of stimulation.

Cortico-spinal and spinal excitability with passive movements

Before performing any MI trials, we modulated the stimulus intensity to provide similar amplitudes of MEPs and H-reflexes at rest between the muscle action modes (passive lengthening, passive shortening and static), as varying muscle length affects supraspinal and spinal responses. Thus, no effect of the factor muscle action mode was found on the responses at rest for all tested muscles (MEPs: FCR, F\textsubscript{2,22}=0.42, P=0.66; H-reflexes: FCR, F\textsubscript{2,22}=1.26, p=0.30; SOL, F\textsubscript{2,18}=0.07, p=0.93; MG, F\textsubscript{2,18}=0.77, p=0.48; LG, F\textsubscript{2,18}=0.51, p=0.61).

With matched responses at rest, we found that MI increased FCR MEP amplitude in comparison to rest (main effect of task, F\textsubscript{1,11}=12.61, p=0.004, \eta\textsuperscript{2}=0.53), independently of muscle action modes (F\textsubscript{2,22}=1.53, p=0.24). As depicted in figure 3A, MEP/M\textsubscript{max} were facilitated by 71.3 ± 27.4 % during passive lengthening (p=0.006), by 59.8 ± 11.9 % during
passive shortening (p=0.009), and by 56.6 ± 8.3 % in static position (p=0.007). These results confirm ones observed in session 1 with unmatched responses.

Interestingly, H-reflex amplitude was modulated depending on the muscle solicitation. The rmANOVA revealed an interaction between the factors task and muscle action mode for both FCR (F_{2,22}=10.11, p<0.001, \eta^2_p=0.48) and TS muscles (SOL: F_{2,18}=15.19, p<0.001, \eta^2_p=0.70; MG: F_{2,18}=15.7, p<0.001, \eta^2_p=0.70; LG: F_{2,18}=8.96, p<0.001, \eta^2_p=0.532). During muscle lengthening, H/M_{max} while imagining were greater than those without MI (increase of: 101.33 ± 77.63 % for FCR, p=0.003; 91.81 ± 34.52 % for SOL, p<0.001; 103.76 ± 27.1 % for MG, p<0.001; and 77.96 ± 20.18 % for LG, p<0.001). During muscle shortening and static conditions, H/M_{max} while imagining were not different than those without MI (all p >0.83, Figure 3). Thus, MI modulated H-reflex amplitude when the targeted muscle was stretched, i.e. under specific neural solicitation of the spinal network. In addition, when looking at the FCR response increase during MI with muscle lengthening, the greater the H-reflex, the greater the MEP (r^2=0.64, P<0.05, see figure 3B). It can also be noticed that the slope of this relation, pictured in figure 3B, is closed to the identity line (y=x).

Presynaptic inhibition

We used a conditioning maneuver to identify the spinal neural mechanisms underlying MI. We compared unconditioned and conditioned H-reflex amplitude at rest and during MI. The rmANOVA revealed an interaction between presynaptic conditioning and task factors for TS muscles (SOL: F_{1,9}=15.87, p=0.003, \eta^2_p=0.64; MG: F_{1,9}=17.75, p=0.002, \eta^2_p=0.66 and LG: F_{1,9}=31.54, p=0.003, \eta^2_p=0.42). Unconditioned H-reflexes at rest and during MI were similar (all muscles, p >0.81, figure 4), confirming the results of session 3 (static condition).
At rest, we reduced H-reflex amplitude with the conditioning maneuver (-16.54 ± 2.53 % for SOL muscle, p<0.001; -25.44 ± 4.62 % for MG muscle, p<0.001; and -19.66 ± 4.39 % for LG muscle, p=0.018). Interestingly, during MI conditioned H/M\textsubscript{max} was similar to unconditioned H/M\textsubscript{max} (SOL: p=0.92, MG: p=0.92; LG: p=0.53, figure 4), revealing the removal of inhibitory effects induced by the conditioning maneuver. This results showed a possible effect of MI onto spinal interneurons network mediating presynaptic inhibition.

[Please insert figure 4 about here]
Discussion

The main result of the present study demonstrates the significant effect of MI on cortico-spinal and spinal structures. In static conditions, cortico-spinal (MEP) and cervico-medullary excitabilities (CMEP), but not Ia-alpha synapse excitability (H-reflex), were facilitated during MI, suggesting a subliminal activation of the cortico-spinal track insufficient to recruit alpha-motoneurons. When MI was associated to a passive muscle lengthening, both MEP and H-reflex increased. In addition to CMEP increase in static condition, these results emphasize the presence of descending volleys during MI, activating specific spinal structures. Finally, the decrease of presynaptic inhibition during MI, with the limb static, confirms that a subliminal output activates low-threshold interneurons mediating presynaptic inhibition, in all participants. These findings establish, for the first time, a strong statement about the influence of MI on the cortico-spinal and spinal excitability.

Cortico-spinal modulations with MI in static condition

First, an increase of MEPs with MI compared to rest was observed, when the targeted muscle was kept in a static position. This increase, in accordance with previous reports (e.g., Stinear and Byblow, 2003b; Li et al., 2004; Rozand et al., 2014), suggests that MI represents an intermediate level of activation of cortical structures between rest and voluntary contraction (Li, 2007). However, this sole motor response does not provide enough information to dissociate cortical from spinal activation. In the present study, we used additional techniques (sessions 2 to 4) to further identify neural mechanisms underlying MI, especially at the spinal level.
When the targeted muscle was kept in a static position, no modulation of H-reflex amplitude was observed during MI. Similar results were observed at several points of the recruitment curve (ascending part), since we tested two different intensities of H-reflex (at $H_{\text{max}}$ in session 3 and at sub-maximal H-reflex in session 4). Therefore, the absence of reflex modulation during MI may not originate from spinal saturation phenomenon (Grosprêtre and Martin, 2012).

CMS provided further clues about spinal level modulations during MI. CMEP responses are thought to represent a direct measure of the motoneuronal pool excitability, by activating descending axons at the spinal decussation (Taylor, 2006). In the present study, greater FCR CMEPs during MI in comparison to rest were observed for each of the four subjects tested. Even considering the low number of subjects tested for CMS stimulations, this original result suggests that the cortical activity generated by MI (Porro et al., 1996; Ehrsson et al., 2003) is sufficient to create a cortical output addressed to the spinal level. The two sites of stimulation activating different neural structures may explain the contrasting results between CMEP and H-reflex modulation. CMEP is the result of stimulation at theinion level and involves the synapse between descending tracks and motoneurons (Taylor, 2006). H-reflex induced by PNS reflects the transmission efficiency of Ia-alpha motoneuron synapse (Pierrot-Deseilligny & Burke 2005). Therefore, H-reflex is more likely subjected to presynaptic inhibitory mechanisms than CMEP (Nielsen and Petersen, 1994). CMEP enhancement during MI can reflect an increase in the excitability of the whole descending track, while unchanged H-reflex during MI rules out a potential increase of motoneuronal excitability or any post-synaptic activation. Nevertheless, the difficulty to evoke CMS in the present study, mainly attributed to stimulus discomfort, reduced the number of subjects tested. Further experiments are needed to generalize CMEP modulations during MI.
To further investigate the potential impact of MI on spinal structures, the participants were instructed to imagine a contraction while the targeted muscle was passively shortened or lengthened. First, we found that MI also increased MEP amplitudes when the targeted muscle was passively shortened and lengthened, indicating that concomitant passive movement does not alter MI effect on cortico-spinal modulations.

The model of muscle lengthening is especially known to reduce H-reflex amplitude through the activation of presynaptic inhibition mechanism of Ia afferences, while muscle shortening does not (Romanò and Schieppati, 1987; Pinniger et al., 2001; Duclay and Martin, 2005). For the first time, a facilitation of spinal excitability was found during MI with passive muscle lengthening, in upper and lower limb muscles, but not during passive shortening. In addition, the amount of facilitation observed on FCR H-reflexes was linearly correlated to the facilitation of FCR cortico-spinal responses (MEP) in the same condition (muscle lengthening). Aoyama and Kaneko (2011) already observed an increase of the stretch reflex during MI but no changes in H-reflex amplitude in static condition in comparison to rest, suggesting a selective effect of MI over spinal mechanisms. These changes may originate from several mechanisms, such as i) a change in neuromuscular fusimotor neurons excitability, i.e., gamma motoneurons that regulate the Ia discharge when changing muscle length, ii) a reduction of homosynaptic post-activation depression (HPAD), i.e. a depletion of neurotransmitters in the Ia-alpha synapse occurring after muscle lengthening or iii) a decrease of presynaptic inhibition induced by primary afferent depolarization interneurons. Regarding the first hypothesis, microneurographic recordings of muscle spindles activity showed no effect of MI on gamma motoneurons activity (Gandevia et al., 1997). Therefore, the modulation of fusimotor activity may not explain the increase of H-reflex during lengthening.
observed in the present study. Secondly, the absence of H-reflex modulation during MI
associated to passive shortening excludes the hypothesis of HPAD mechanisms, observed
during both shortening and lengthening at rest (Nordlund et al., 2004). We tested the third
hypothesis about the influence of MI on presynaptic inhibitory interneurons, with a
conditioning maneuver.

Effects of MI on spinal presynaptic inhibition

We conditioned the H-reflex of triceps surae muscles with a stimulation of the
antagonist nerve to induce D1 presynaptic inhibition and to assess the involvement of primary
afferent depolarization interneurons during MI (Meunier & Pierrot-Deseilligny, 1998). In the
present study, TS H-reflexes decreased by about 20 % at rest with the leg static. Interestingly,
during MI no significant decrease of H-reflexes was observed, suggesting a removal of
presynaptic inhibition. These results – with the targeted limb in static position – emphasize
those obtained with the passive lengthening model, suggesting specific modulation of
presynaptic inhibitory mechanism during MI.

Animal studies showed that corticospinal projections onto primary afferent
depolarization interneurons predominantly result in a decrease of presynaptic inhibition
(Lundberg and Vyklicky, 1963). Thus, a regulation pathway of this mechanism was suggested
(Figure 5), which is preponderant during muscle lengthening. It was previously suggested that
cortical projections could modulate interneurons activity through a specific spinal mechanism
(Pierrot-Deseilligny & Burke, 2005). In parallel to the mechanism involved in the reduction of
Ia inhibition by a sub-threshold stimulation of the motor cortex (Rothwell et al., 1984;
Grospître et al., 2014), MI may produce a subliminal cortical output that would reach
interneuronal network at spinal level, and more precisely presynaptic inhibitory interneurons.
Indeed, we recently found that subthreshold TMS increased H-reflex amplitude during passive muscle lengthening but not during passive muscle shortening or in static condition (Grosprêtre et al., 2014). Even though MI and TMS do not functionally engage neural mechanisms similarly, the subliminal motor outputs elicited by endogenous and exogenous cortical activation, respectively, are likely to reach the same spinal structures. For example, spinal interneurons, which are subjected to descending pathways (Chen et al., 2001), play several roles onto the modulation of primary afferents discharge, like presynaptic inhibitory or excitatory effects (Jankowska, 2001). In addition, animal studies have shown that GABA-ergic interneurons of the spinal cord have low electrical threshold (Daniele and MacDermott, 2009). This would explain why a sub-threshold cortical output and a subliminal motor command induced by TMS and MI, respectively, may activate these low-threshold structures and may be insufficient to activate the motoneuronal pool that have a higher threshold.

Conclusion

Neural mechanisms of MI are mainly attributed to cortical activations, without involvement of subcortical and spinal structures. Nonetheless, the neural simulation theory of Jeannerod (1994, 2001) considers the existence of a subliminal motor output generated during MI. The present study demonstrates the presence of such a response that activates low-threshold spinal structures, such as pre-synaptic interneurons, without modifying the excitability of alpha-motoneurons. The increase of the CMEP amplitude during MI gave a first hint of descending volleys along the corticospinal tract. The modulation of the H-reflex amplitude during MI, both when passively lengthening the muscle and when conditioning the
spinal response, supported the activation of pre-synaptic spinal interneurons when imagining. We suggest that MI activates both cortical and sub-cortical structures and that its impact on spinal networks depends at least on the excitability thresholds of the involved neuronal structures (interneurons and motoneurons).

These findings bring new insights into MI-induced neural adaptations. The enhancement of motor performance (strength gain or error decrease in motor sequence) after mental practice with MI has been mainly attributed to cortical changes (e.g., Pascual-Leone et al., 1995; Ranganathan et al., 2004). The results of the present study bring new evidence for a complementary hypothesis: in addition to neural plasticity at the cortical level, the reinforcement of synapse conductivity at the spinal level might also participate to the benefits of MI practice.
Additional information

No conflicts of interest, financial or otherwise, are declared by the author(s). This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM).

References


Figures and tables captions

Table 1. Overview of the experimental design with number of stimulations per measure.

In the table are reported the number of evoked responses by condition in each subject. The number of subjects by session is indicated in the third column (which title is “n”). MI: motor imagery. FCR: flexor carpi radialis. SOL: soleus. MG: medial gastrocnemius. LG: Lateral gastrocnemius. CMEP: cervico-medullary-evoked potential. MEP: motor-evoked potential. Mmax: maximal M wave. Unmatched responses in the first column represent maximal responses amplitudes recorded at rest in static conditions. Matched responses represent the recordings with the adjusted stimulation intensity to match similar amplitude at rest between conditions (lengthening, shortening and static).

Table 2. Raw electrophysiological data at rest and during MI. *: significant difference with rest condition (p<0.001). MI: motor imagery. FCR: Flexor Carpi Radialis. ECR: Extensor Carpi Radialis. SOL: soleus. MG: medial gastrocnemius. LG: lateral gastrocnemius. TA: Tibialis anterior. RMS: root mean square of electromyographic activity. MEP: motor-evoked potential. CMEP: cervico-medullary-evoked potential. Unmatched responses in the first column represent maximal responses amplitudes recorded at rest in static conditions. Matched responses represent the recordings with the adjusted stimulation intensity to match similar amplitude at rest between conditions (lengthening, shortening and static).

Figure 1. Graphical overview of experimental setup. The several sites of stimulations and recordings are depicted for upper limb (A) and lower limb (B) measurements. The lens provide further details about the circuit activated by the conditioning stimulation applied on common peroneal nerve (MTN = motoneurons). TMS: Transcranial Magnetic Stimulation; CMS: Cervico-Medullary Stimulation; MNS: Median Nerve Stimulation; FCR: Flexor Carpi Radialis; ECR: Extensor Carpi Radialis; PNS: Posterior tibial Nerve Stimulation; CPS: Common Peroneal Nerve Stimulation; SOL: Soleus; TA: Tibialis Anterior; LG: Lateral Gastrocnemius; MG: Medial Gastrocnemius.

Figure 2. FCR evoked responses during rest and motor imagery. On the left panels are depicted mean (± standard deviation) FCR MEP/Mmax, CMEP/Mmax and Hmax/Mmax for rest (white bars) and motor imagery (black bars) conditions. Results of each subject are depicted over bars by empty grey circles. On the right panels typical responses are shown for one representative subject (same subject depicted for MEP, CMEP and H panels). Twelve responses are superimposed for MEPs and H-reflexes and eight for CMEP responses. Dashed lines represent the mean peak-to-peak amplitude at rest. *: p < 0.05 between rest and motor imagery.

Figure 3. Evoked responses during passive muscle solicitations. Responses at rest and during motor imagery are pictured with white and black bars, respectively, during passive lengthening, passive shortening and static conditions. Each response at rest in static and shortening condition (MEP, H-reflexes) are elicited to match lengthening value. Normalized MEPs and H-reflexes of FCR muscle are presented in panels A and C, respectively (exp. 1, n=12). Panel B represents the correlation between H-reflex and MEP increase during MI in comparison to rest in FCR muscle during passive lengthening. Normalized H-reflexes of SOL, MG and LG muscles are presented in panels D, E and F, respectively (exp. 2, n=10). FCR = flexor carpi radialis, SOL = soleus, MG = medial gastrocnemius, LG = lateral gastrocnemius *: p<0.05 ***: p < 0.001.
Figure 4. Impact of motor imagery on Ia presynaptic inhibition. Conditioned and unconditioned H/M_{max} are depicted for soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles, at rest (without MI, white bars), and during motor imagery (black bars). *, **, ***: significant differences with P<0.05, P<0.01 and P<0.001 respectively.

Figure 5. Simplified diagram of the hypothetical spinal neurons network involved during motor imagery. The different levels of the nervous system (cortical, spinal and peripheral) are delimited by the dotted lines. The several stimulation techniques employed in the present study are indicated by empty flashes: transcranial magnetic stimulation (TMS), cervico medullar stimulation (CMS) and nerve stimulation (NS). Classically, voluntary motor command activates directly the motoneuronal pool through the pyramidal tract (1+2), to initiate muscle contraction. Motor imagery might generate a subliminal impulse that does not induce a discharge of α-motoneurons (2). In the same time, it might activate a network of interneurons— IN (5+6), with lower threshold of activation, through a parallel path. Presynaptic interneurons receive input from agonist Ia afferences (3) as from antagonist Ia afferences (3'). Thus, during MI cortical output may reduce the presynaptic inhibition induced by muscle lengthening (3+4) or antagonist Ia stimulation (3'+4).
<table>
<thead>
<tr>
<th>Muscle</th>
<th>n</th>
<th>parameter</th>
<th>Session 1</th>
<th>Session 2</th>
<th>Session 3</th>
<th>Session 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>STATIC CONDITION</td>
<td>PASSIVE LENGTHENING</td>
<td>STATIC CONDITION</td>
<td>PASSIVE SHORTENING</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>unmatched</td>
<td>rest matched with lengthening</td>
<td>rest matched with lengthening</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>REST</td>
<td>MI</td>
<td>REST</td>
<td>MI</td>
</tr>
<tr>
<td>FCR</td>
<td>12</td>
<td>MEP</td>
<td>12</td>
<td>12</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$M_{\text{max}}$</td>
<td>4</td>
<td>4</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>FCR</td>
<td>4</td>
<td>CMEP</td>
<td>8</td>
<td>8</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$M_{\text{max}}$</td>
<td>4</td>
<td>4</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>FCR</td>
<td>12</td>
<td>MEP</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H-reflex</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$M_{\text{max}}$</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>SOL</td>
<td>10</td>
<td>H-reflex</td>
<td>---</td>
<td>---</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$M_{\text{max}}$</td>
<td>---</td>
<td>---</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>SOL</td>
<td>10</td>
<td>H-reflex</td>
<td>12</td>
<td>12</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$M_{\text{max}}$</td>
<td>4</td>
<td>4</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>SOL</td>
<td>10</td>
<td>Conditioned H</td>
<td>12</td>
<td>12</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$M_{\text{max}}$</td>
<td>4</td>
<td>4</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>STATIC CONDITION</td>
<td>PASSIVE LENGTHENING</td>
<td>STATIC CONDITION</td>
<td>PASSIVE SHORTENING</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>------------------</td>
<td>---------------------</td>
<td>------------------</td>
<td>-------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unmatched</td>
<td>rest matched with lengthening</td>
<td>rest matched with lengthening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>REST</td>
<td>MI</td>
<td>REST</td>
<td>MI</td>
<td>REST</td>
<td>MI</td>
</tr>
<tr>
<td>FCR 12</td>
<td>RMS (µV)</td>
<td>5.4 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>5.4 ± 0.3</td>
<td>5.5 ± 0.4</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>MEP (mV)</td>
<td>0.14 ± 0.04</td>
<td>0.26 ± 0.05 *</td>
<td>0.11 ± 0.03</td>
<td>0.21 ± 0.04 *</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>$H_{max}$ (mV)</td>
<td>0.81 ± 0.28</td>
<td>0.74 ± 0.24</td>
<td>0.20 ± 0.06</td>
<td>0.40 ± 0.14 *</td>
<td>0.19 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>$M_{sth}$ (mV)</td>
<td>0.93 ± 0.31</td>
<td>0.96 ± 0.30</td>
<td>0.97 ± 0.30</td>
<td>0.83 ± 0.22</td>
<td>0.93 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>$M_{max}$ (mV)</td>
<td>3.75 ± 1.50</td>
<td>3.61 ± 1.47</td>
<td>3.55 ± 1.26</td>
<td>3.59 ± 1.32</td>
<td>3.73 ± 1.47</td>
</tr>
<tr>
<td>ECR 12</td>
<td>RMS (µV)</td>
<td>5.2 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>5.2 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>FCR 4</td>
<td>RMS (µV)</td>
<td>5.3 ± 0.4</td>
<td>5.4 ± 0.03</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>CMEP (mV)</td>
<td>0.25 ± 0.04</td>
<td>0.35 ± 0.07 *</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>SOL 10</td>
<td>RMS (µV)</td>
<td>5.3 ± 0.3</td>
<td>5.5 ± 0.3</td>
<td>5.4 ± 0.3</td>
<td>5.4 ± 0.3</td>
<td>5.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>$H_{free}$ (mV)</td>
<td>3.01 ± 0.52</td>
<td>3.09 ± 0.63</td>
<td>1.51 ± 0.46</td>
<td>2.23 ± 0.54 *</td>
<td>1.47 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>$M_{max}$ (mV)</td>
<td>6.91 ± 0.66</td>
<td>6.87 ± 0.28</td>
<td>5.59 ± 0.90</td>
<td>5.60 ± 0.90</td>
<td>5.68 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>Conditioned H (mV)</td>
<td>2.53 ± 0.45</td>
<td>3.03 ± 0.55 *</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>MG 10</td>
<td>RMS (µV)</td>
<td>5.4 ± 0.3</td>
<td>5.5 ± 0.3</td>
<td>5.7 ± 0.4</td>
<td>5.4 ± 0.3</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>$H$ (mV)</td>
<td>0.79 ± 0.09</td>
<td>0.76 ± 0.06</td>
<td>1.64 ± 0.63</td>
<td>2.33 ± 0.74 *</td>
<td>1.63 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>$M_{sth}$ (mV)</td>
<td>1.50 ± 0.50</td>
<td>1.22 ± 0.38</td>
<td>3.11 ± 0.72</td>
<td>3.16 ± 0.67</td>
<td>3.31 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>$M_{max}$ (mV)</td>
<td>4.08 ± 0.49</td>
<td>3.98 ± 0.60</td>
<td>5.40 ± 0.69</td>
<td>5.16 ± 0.64</td>
<td>5.16 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Conditioned H (mV)</td>
<td>0.59 ± 0.08</td>
<td>0.74 ± 0.06 *</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>LG 10</td>
<td>RMS (µV)</td>
<td>5.5 ± 0.3</td>
<td>5.4 ± 0.3</td>
<td>5.5 ± 0.3</td>
<td>5.5 ± 0.3</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>$H$ (mV)</td>
<td>0.59 ± 0.08</td>
<td>0.63 ± 0.09</td>
<td>1.08 ± 0.45</td>
<td>1.77 ± 0.67 *</td>
<td>1.07 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>$M_{sth}$ (mV)</td>
<td>1.54 ± 0.46</td>
<td>1.51 ± 0.29</td>
<td>3.45 ± 1.11</td>
<td>3.49 ± 1.17</td>
<td>3.48 ± 1.12</td>
</tr>
<tr>
<td></td>
<td>$M_{max}$ (mV)</td>
<td>3.91 ± 0.48</td>
<td>4.01 ± 0.71</td>
<td>5.72 ± 1.23</td>
<td>5.69 ± 1.22</td>
<td>5.74 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>Conditioned H (mV)</td>
<td>0.48 ± 0.07</td>
<td>0.61 ± 0.08 *</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>TA 10</td>
<td>RMS (µV)</td>
<td>5.5 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>5.5 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>5.5 ± 0.3</td>
</tr>
</tbody>
</table>