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Repetitive Activation of the Corticospinal Pathway by Means of rTMS may Reduce the Efficiency of Corticomotoneuronal Synapses

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Low-frequency rTMS applied to the primary motor cortex (M1) may produce depression of motor-evoked potentials (MEPs). This depression is commonly assumed to reflect changes in cortical circuits. However, little is known about rTMS-induced effects on subcortical circuits. Therefore, the present study aimed to clarify whether rTMS influences corticospinal transmission by altering the efficiency of corticomotoneuronal (CM) synapses. The corticospinal transmission to soleus α-motoneurons was evaluated through conditioning of the soleus H-reflex by magnetic stimulation of either M1 (M1-conditioning) or the cervicomedullary junction (CMS-conditioning). The first facilitation of the H-reflex (early facilitation) was determined after M1- and CMS-conditioning. Comparison of the early facilitation before and after 20-min low-frequency (1 Hz) rTMS revealed suppression with M1- (−17 ± 4%; $P = 0.001$) and CMS-conditioning (−6 ± 2%; $P = 0.04$). The same rTMS protocol caused a significant depression of compound MEPs, whereas amplitudes of H-reflex and M-wave remained unaffected, indicating a steady level of motoneuronal excitability. Thus, the effects of rTMS are likely to occur at a premotoneuronal site—either at M1 and/or the CM synapse. As the early facilitation reflects activation of direct CM projections, the most likely site of action is the synapse of the CM neurons onto spinal motoneurons.

Keywords: corticospinal tract, H-reflex conditioning, synaptic plasticity

Introduction

The strength of synapses and synaptic transmission has been shown to be modifiable, and changes in synaptic efficiency contribute to an essential property of our central nervous system (CNS): its plasticity. This capacity for neural reorganization is fundamental for the formation of memory and learning as well as neural repair. During recent years, different noninvasive electrophysiological methods have been developed, which allow induction of plastic changes such as long-term potentiation or inhibition within the CNS in isolation or in combination with motor learning or rehabilitation paradigms. These methods encompass repetitive transcranial magnetic stimulation (rTMS), repetitive peripheral nerve stimulation (Ridding et al. 2000), transcranial direct current stimulation (Nitsche and Paulus 2000), and paired associative stimulation (PAS) at the cortical (Stefan et al. 2000) and spinal levels (Taylor and Martin 2009). rTMS has proven to be a powerful instrument to either reduce or enhance cortical/corticospinal excitability depending on the stimulation protocol: low-frequency rTMS (stimulus rates of, e.g., 1 Hz or less) induces a longer lasting decrease in corticospinal excitability (Chen et al. 1997) whereas conventional high-frequency rTMS (5 Hz or more) produces an increase in corticospinal excitability and a reduction in intracortical inhibition (Fitzgerald et al. 2006). In general, the aftereffects of rTMS are ascribed to changes in cortical circuits. For instance, Tsuji and Rothwell (2002) demonstrated that the long-latency responses of the transcortical stretch reflex but not the spinal short-latency components were affected by rTMS indicating intracortical processes at work. In an elegant study involving direct recordings from the high cervical epidural space, Di Lazzaro et al. (2008) strengthened this hypothesis by showing that 1 Hz rTMS decreased the amplitude of descending later I-waves that depend on the intrinsic circuitry of M1. Although evidence of the underlying cellular processes is scarce, currently favored mechanisms are activity-dependent changes in the efficiency of synaptic connections between cortical neurons (Hallett 2007; Funke and Benali 2011).

However, it is important to emphasize that the finding that low-frequency rTMS induces effects at a cortical level does not by any means preclude concomitant changes at a subcortical and/or spinal level. These spinal plastic changes not only may involve up- or down-regulation of membrane channels and receptors in motoneurons and interneurons leading to changes in their excitability (Heckman et al. 2009), but may also involve plastic changes in the efficiency of the corticospinal synapses. Given the central role of the synapse for neuronal plasticity, there is, however, surprisingly little research on the modulation and plasticity of the corticospinal synapses. It has been demonstrated in human (Nielsen and Petersen 1994) and animal experiments (Rudomin et al. 1975) that corticospinal synapses are not influenced by presynaptic GABAergic inhibition evoked by large diameter afferents, but this does not rule out the possibility that the efficiency of the synapses is controlled by other intrinsic and extrinsic mechanisms. Indeed, it has been known for some time that corticomotoneuronal (CM) synapses show activity-dependent short-term plastic changes (Porter and Lemon 1993). Furthermore, changes in the efficacy of CM synapses were proposed to be—at least partly—responsible for altered transmission from M1 neurons to α-motoneurons (Davidson et al. 2007). In humans, Taylor and Martin (2009) and Leukel et al. (2012) demonstrated that repeated pairs of presynaptic (produced by cortical stimulation) and antidromic postsynaptic volleys (produced by peripheral nerve stimulation), delivered to the corticospinal–motoneuronal synapses, altered corticospinal transmission. It was suggested that these changes could partly be explained by changes in the efficiency of the CM synapse. Furthermore, there is indirect evidence that strong voluntary contractions reduce the transmission across, that is, efficacy of corticospinal

synapses (Petersen et al. 2003). Apart from these few findings, there is, to our knowledge, no evidence of plastic changes in the efficiency of the CM synapse.

It was, therefore, the purpose of the present study to investigate whether rTMS can induce a change in the efficiency of the CM synapse, and thus whether part of the depression of motor-evoked potential (MEP) amplitudes observed following rTMS (see, e.g., Lundbye-Jensen et al. 2011) may be explained by a change in CM synaptic transmission. In the first step (Experiment I), the effect of 20-min low-frequency (1 Hz) suprathreshold rTMS on the compound MEP, and the H-reflex was tested in order to assess changes in corticospinal and motoneuronal excitability, respectively. To test for changes in motoneuronal excitability, H-reflex recruitment curves were recorded in order to evaluate the whole range of the motoneuron pool. In the next step (Experiment II), an H-reflex conditioning technique was applied in order to assess alterations in the fastest, direct corticospinal pathways (Nielsen et al. 1993). During classical H-reflex conditioning (called M1-conditioning herein), cortical stimulation is timed so that the descending volley coincides with the excitation generated by the Ia afferent volleys after peripheral nerve stimulation (Nielsen et al. 1993). Owing to the high temporal resolution of this technique, excitability in different fractions of the corticospinal pathway, that is, in the fastest, presumably monosynaptic, CM connections and in indirect oligo- and polysynaptic pathways, can be probed and quantitatively assessed (Nielsen et al. 1993; Nielsen and Petersen 1995a; Petersen et al. 1998; Taube et al. 2006). Furthermore, the H-reflex conditioning technique allows an evaluation of the efficiency of the corticospinal activation of the motoneurons that is not influenced by the excitability of the motoneurons themselves (Nielsen et al. 1993, 1995). Therefore, the size of the earliest facilitation produced by corticospinal stimulation reflects the size of the descending volley evoked by the stimulation and the efficiency of the CM synapse. For TMS over M1, the descending volley(s) may be changed by prior rTMS due to changes in cortical excitability, but this is not the case for the descending volley evoked by stimulation of the corticospinal tract at the cervicomedullary junction (CMS) (Taube et al. 2011). Consequently, M1-conditioning can indicate whether rTMS influences the fastest direct corticospinal projections (the early facilitation) while H-reflex conditioning with stimulation at the cervicomedullary junction (CMS-conditioning) further specifies whether transmission at the corticomotoneural synapse is modified. In the present experiment, we therefore compared the effect of 20-min low-frequency (1 Hz) suprathreshold rTMS on the early facilitation of the soleus H-reflex evoked by either M1- or CMS-conditioning.

Materials and Methods

Study Participants

Twenty-two subjects (age 25 ± 4 years) without neurological or orthopedic disorder participated in the present study. Nine subjects participated in Experiment I and 13 in Experiment II. In Experiment I, H-reflex recruitment curves and MEP recruitment curves (see "Peripheral nerve stimulation: H/M recruitment curves" and "Transcranial magnetic stimulation: MEP recruitment curves," respectively) were obtained (see "rTMS Intervention"). In Experiment II, the effects of rTMS on corticospinal transmission were investigated using H-reflex conditioning by means of magnetic stimulation of the motor cortex and the cervicomedullary junction (see "SOL H-reflex conditioning in pre- and postmeasurement"). Written informed consent was obtained from all participants prior to their participation in the study. The experiments (KF 01-131/03) were approved by the local ethics committee of the Copenhagen Capital Region of Denmark (De Videnskabsetiske Komiteer for Region Hovedstaden) and followed the regulations expressed in the Declaration of Helsinki (1964).

Electromyography (EMG)

EMG recordings were obtained from the soleus (SOL) and tibialis anterior muscle (TA) of the right leg. After preparation, bipolar surface electrodes (Blue sensor N, Ambu®, Ballerup, Denmark) were attached to the skin longitudinally above the muscle belly (2-cm interelectrode distance). The reference electrode was placed on the tibia plateau. EMG signals were amplified (×1000), bandpass-filtered (10–1000 Hz), and sampled at 4 kHz. The EMG was stored for offline analysis using custom-built software (LabView® based, National Instruments®, Austin, TX, USA).

Peripheral Nerve Stimulation: H/M Recruitment Curves

H-reflexes in the SOL were elicited with an electrical stimulator (Digitimer DS7A, Hertfordshire, UK) by stimulating the posterior tibial nerve in the popliteal fossa. Stimuli consisted of square-wave pulses of 1 ms duration. The anode, a rubber pad of 5 × 5 cm, was fixed on the anterior aspect of the knee just underneath the patella. The cathode (2 cm in diameter) was placed in the popliteal fossa and moved stepwise until the best position for eliciting the H-reflex was found. It was ensured that stimulation evoked no response in the TA muscle. After finding the optimal position, the cathode was fixed with tape. In Experiment I, an H-reflex and M-wave recruitment curve was obtained at 4 different time points while subjects were seated at rest: 1) before the rTMS intervention (baseline), 2) immediately after rTMS, and 3) 10, and 4) 20 min after rTMS. Prior to the generation of recruitment curves, the M-wave threshold was defined as the minimum intensity required to elicit an M-wave that was visible in the online soleus EMG. During baseline measurements, stimuli were applied with intensities ranging from 0.5 to 3 × M-wave threshold (e.g., 10–100 mA) in steps of 0.1. Stimuli were elicited with a 4-s interstimulus interval, and the stimulation intensity was varied in a randomized order. For each intensity, 5 responses were elicited. Once the full recruitment curve was obtained, the stimulation intensity was increased until the maximal M wave (M_{max}) was obtained. When the M wave ceased to increase and a plateau was reached, the stimulation intensity was further markedly increased in order to ensure that the maximal M-wave was indeed obtained.

Recruitment curves following rTMS were generated in an identical procedure but with stimulation intensities in steps of 0.2 MT due to the short time in which we expected the neural adaptations in response to rTMS to be active. For each stimulus, the peak-to-peak amplitude of the M-wave and the H-reflex was measured. The responses evoked at a specific stimulation intensity were averaged across the 5 trials. Responses were normalized and expressed relative to the corresponding M_{max}.

Transcranial Magnetic Stimulation: MEP Recruitment Curves

MEPs were elicited in SOL by applying TMS to the contralateral motor cortical leg area using a Magstim Rapid^2 TMS stimulator and a 90-mm figure-of-eight coil (SP16097, Magstim Company Ltd., Whitland, UK; Fig. 2). The optimal position of the coil for eliciting MEPs in the SOL muscle was established through a mini-mapping procedure of M1 and the coil was placed on the scalp over the hot spot of the SOL representation with the handle of the coil pointing horizontally backward. After positioning of the coil, the resting motor threshold (1.0 MT) was determined as the minimum intensity required to evoke MEP amplitudes larger than 50 μV in 3 of 5 consecutive trials. Responses were normalized and expressed relative to the corresponding M_{max}. TMS was applied with an interstimulus interval of 4 s. To ensure a constant position of the coil throughout the experiment, the head of the subjects and the coil were mechanically fixed. The head was laid down on a

table and was secured by means of rigid foam preventing head movements in all directions. The handle of the coil was fixed to a stand (Manfrotto®, Italy) and secured with Velcro® straps to the subject's head. During all experiments involving TMS, a Brainsight™ image-guided TMS navigational system (Brainsight 2, Rouge Research, Montreal, Canada) was used for online monitoring of the coil position and orientation relative to the head and the identified stimulation hot spot.

In the control experiment (Experiment I), MEP recruitment curves were obtained in the same 9 subjects in whom H/M recruitment were investigated before and after rTMS. Magnetic stimuli were applied over M1 with different stimulation intensities in a random sequence ranging from 0.8 to 1.5 MT in steps of 0.1 with a 4-s interstimulus interval. At each stimulation intensity, 5 stimuli were recorded and the mean MEP was obtained as the average peak-to-peak amplitude of 5 trials. MEP recruitment curves were obtained 1) before the rTMS intervention, 2) immediately after rTMS, and 3) 10, and 4) 20 min after rTMS.

Cervicomedullary Stimulation by TMS

In the main experiment (Experiment II), cervicomedullary TMS was applied with maximum stimulator output using a Magstim® rapid magnetic stimulator (Magstim, Whitland, UK) with a double-cone coil (in line with Taube et al. 2011 and Leukel et al. 2012; Fig. 2). A limitation of this technique is that, in most subjects, it is impossible to obtain magnetically evoked cMEPs in the leg muscles at rest (Ugawa et al. 1994; Oya et al. 2008). One approach to overcome this problem is to voluntary precontract the muscle. However, as changes in the contraction strength may influence the size of the cMEP, the comparison of different tasks is difficult and measurements at rest are impossible. Therefore, we collided the cervicomedullary volley with the H-reflex (see "SOL H-reflex conditioning in pre- and postmeasurement") and used a Magstim Rapid stimulator with a biphasic pulse, because it was previously shown that for a given amplitude of initial current, biphasic stimulation was more effective than monophasic stimulation (Kammer et al. 2001; Sommer et al. 2006). The coil was positioned so that the first derivative of the induced current was cranially directed, and that its central portion was placed on or near the inion (Taylor 2006; Taube et al. 2011). During the measurements, the subjects were seated in a custom-built chair that fixed their legs and trunk in place, and were asked to bend their back and head forward. The head rested on a custom-built table and was secured with cushions. This position was maintained throughout all pre- and postmeasurements and was only changed during the rTMS intervention. In all 13 subjects, stimulation with the maximal stimulator output (100%) was still subthreshold and therefore did not elicit detectable responses in the surface EMG of the SOL muscle. Thus, the stimulus intensity of the magnetic stimulator remained constant at its maximal output (100%) throughout the experiment. The time interval between successive stimuli was 5 s.

H-Reflex as a Test (Control) Reflex

The size of the test H-reflex was measured as the peak-to-peak amplitude and was expressed as a percentage of M_{max}. It has been demonstrated that the susceptibility of the H-reflex to conditioning depends on the size of the control reflex (Crone et al. 1990). Therefore, it was ensured that the test reflex always had the same size of ~20% of the maximal M-response, and that it was on the ascending portion of the H-reflex recruitment curve. Accordingly, the susceptibility of the test H-reflex for facilitation or inhibition induced by a constant conditioning stimulus should be the same for cervicomedullary and cortical stimuli and in pre- and postmeasurements. It is important to note that the H-reflex is not only dependent on the motoneuron excitability, but also affected by presynaptic factors such as presynaptic inhibition of Ia afferents. Therefore, it has to be emphasized that, in the current study, we have tried to control for confounding factors. First, the control H-reflex had the same size in pre and postmeasurements (Fig. 4*C*,*F*). Second, the measurements were performed at rest so that both movement-related efferent and afferent activity is unlikely to exert any influence. Third, we assessed control ISIs to ensure that we observe a pathway-specific effect (Fig. 3). Lastly, the position of the subjects in pre- and postmeasurement was identical so that for instance changes in muscle length did not bias the H-reflex response.

SOL H-Reflex Conditioning in Pre- and Postmeasurement

The SOL H-reflex was conditioned by magnetic stimulation of the motor cortex (M1-conditioning) and by magnetic cervicomedullary stimulation (CMS-conditioning) in a random order during the same measurement (Fig. 2). The H-reflex conditioning was in accordance with previous studies using M1- (Nielsen et al. 1993; Petersen et al. 1998; Taube et al. 2006, 2007; Schubert et al. 2008) or CMS-conditioning (Taube et al. 2011; Leukel et al. 2012). However, compared with the initial study introducing M1-conditioning (Nielsen et al. 1993), the stimulation intensity of the magnetic stimulus was higher (0.9 MT) so that the early facilitation (explained later on in detail) could be evoked in all subjects at rest.

Cervicomedullary and cortical stimuli were applied with different interstimulus intervals (ISIs in ms). In order to detect the early facilitation, the ISIs for CMS-conditioning were −9, −8, −7, −6, −5, −4, and −3 ms whereas M1-conditioning involved the following ISIs: −5, −4, −3, −2, −1, 0, and +1 ms. Negative ISIs indicate that the peripheral nerve stimulation was applied before TMS (or cervicomedullary stimulation).

Ten trials were recorded at each ISI as well as for the control (unconditioned) H-reflex in a randomized order (the ISIs were randomized as well as the type of conditioning stimulation: TMS over M1 or cervicomedullary stimulation). Peripheral nerve stimulation was applied with an intensity to evoke SOL H-reflexes of ~20% of M_{max}. The intensity of the TMS pulses was subthreshold (0.9 MT). Cervicomedullary stimulation was also subthreshold but the exact level of stimulation intensity relative to MT could not be identified as 100% of the maximal stimulator output was not sufficient to elicit a response in the relaxed soleus muscle. Thus, the stimulator output was chosen to be constant at its maximal intensity (100%) throughout the experiment.

The conditioned H-reflexes were expressed as the percentage of the control H-reflexes in order to identify the so-called early facilitation (or "short-latency facilitation") indicated by the first increase in the amplitude of the conditioned H-reflex (Fig. 2). This early facilitation is considered to be mediated via fast, presumably direct corticospinal pathways (Nielsen et al. 1993).

rTMS Intervention

During the rTMS intervention, stimuli were applied at 1 Hz for 20 min while subjects were sitting upright at rest. The stimulation intensity was set to 1.2 MT, as observations imply that the reduction in MEP size induced by 1-Hz stimulation is longer with longer train duration (Maeda et al. 2000) and at higher stimulation intensity (Fitzgerald et al. 2002). It was the aim to induce a relatively long-lasting suppression of the corticospinal excitability as the subsequent assessment by means of H-reflex conditioning took several minutes to accomplish. rTMS was applied in a similar setup as described above for both Experiment I and II.

Data Analysis and Statistics

In Experiment I, MEP and H/M recruitment curves were assessed in 9 subjects before and after rTMS. In order to allow comparison across subjects and over time, all evoked responses were normalized to the corresponding M_{max}. Also stimulation intensities were normalized: TMS stimulation intensities were normalized to the individual motor threshold (MT) at baseline established as the intensity that evoked MEP amplitudes >50 μV in 3 of 5 trials. Peripheral nerve stimulation intensities were normalized to the individual M-wave threshold at baseline established in a corresponding procedure. For each subject, the responses evoked at each stimulation intensity were averaged. Before statistical comparison, all datasets were tested for normal distribution by a Kolmogorov–Smirnov test. The resulting MEP and H-reflex recruitment curves were compared using a two-way repeated-measures ANOVA with TIME (4 levels: pre (baseline), post, 10 min post and 20 min post) and STIMULATION INTENSITY (7 levels: 0.9–1.5 × MT and M-wave threshold respectively) as factors. The obtained M_{max} responses were compared for an effect of TIME using a one-way repeated-measures ANOVA. Post hoc pairwise comparisons were performed as Bonferroni-corrected tests.

In Experiment II, the unconditioned (control) H-reflexes and the conditioned H-reflexes were also expressed as peak-to-peak amplitudes of the unrectified EMG. Ten conditioned H-reflexes were averaged for each ISI after both CMS- and M1-conditioning. Additionally, 10 control (unconditioned) H-reflexes were averaged. The control H-reflexes served as a reference for the conditioned H-reflexes. The intraindividual mean of the conditioned H-reflex (at each ISI) was divided by the intraindividual mean of the unconditioned control H-reflex and ISI-curves after M1- and CMS-conditioning were plotted for each subject (a representative subject is displayed in Fig. 2). As there is interindividual variability in the occurrence of the onset of the early facilitation (dependent on the subjects' anthropometry, i.e., trunk and leg lengths and possibly due to differences in the nerve conduction velocities), the early facilitation was determined in the premeasurement for each subject separately and compared with the amplitude of the early facilitation obtained in the postmeasurement using the same ISI. Thereby, the ISI indicating the individual early facilitation was defined as the first significant increase of the mean value of the conditioned H-reflex with respect to previous values (beginning at ISI −9 ms with CMS-conditioning and at ISI −5 ms with M1-conditioning) using nonparametric Wilcoxon tests (in line with Taube et al. 2011). In 3 subjects, there was no significant early facilitation after CMS-conditioning. In these subjects, the early facilitation was visually determined by the authors of the study. Furthermore, subsequent statistics were executed twice: one time including and a second time excluding these 3 subjects (indicated in the Result section; all figures display the data of all subjects).

For the statistical analysis, the early facilitation and the ISI before (−1) and the ISI after this facilitation (+1) were taken into consideration. The effect of rTMS was evaluated using a two-way repeated-measures ANOVA with TIME and ISI as factors. The amount of early facilitation in the premeasurement of both M1- and CMS-conditioning was compared with the early facilitations obtained in the postmeasurement by means of Bonferroni-corrected two-sided paired Student's *t*-tests after having tested that the present data followed a normal distribution (Kolmogorov–Smirnov test).

All variables were expressed as mean ± standard deviation (SD) unless indicated otherwise.

Differences were regarded significant at $P < 0.05$ for all tests. SPSS software 19.0 (SPSS®, Chicago, IL, USA) was used for the statistical analysis.

Results

MEP Recruitment Curves

There was a main effect of TIME ($F_{3,144} = 4.4$, $P = 0.014$) and a main effect of STIMULATION INTENSITY ($F_{6,144} = 10.4$, $P < 0.001$). Additionally, there was a significant TIME × STIMULATION INTENSITY interaction ($F_{18,144} = 2.528$, $P = 0.038$; see Fig. 1*A*). Bonferroni post hoc pairwise comparisons revealed that the evoked MEP amplitudes across all stimulation intensities, were significantly lower immediately after rTMS compared with pre (baseline) ($t = 2.883$, $P = 0.025$). Further tests of interactions between TIME and STIMULATION INTENSITY demonstrated that MEP amplitudes were significantly depressed immediately following rTMS compared with pre (baseline) at the stimulation intensities 1.2 ($t = 2.776$, $P = 0.02$), 1.3 ($t = 2.971$, $P = 0.011$), 1.4 ($t = 2.527$, $P = 0.039$), and 1.5 ($t = 3.562$, $P = 0.002$). Ten minutes following rTMS, MEP amplitudes increased again with the only significant difference from post-rTMS at 1.5 ($t = 2.731$, $P = 0.032$) and no difference compared with baseline measurements. Twenty minutes following rTMS, MEP amplitudes were back at baseline levels.

H/M Recruitment Curves

For the H/M recruitment curves, there was a main effect of STIMULATION INTENSITY ($F_{8,192} = 10.4$, $P < 0.001$) but not a main effect of TIME ($F_{3,192} = 0.78$, $P = 0.52$). There was also no significant STIMULATION INTENSITY × TIME interaction ($F_{24,192} = 0.756$, $P = 0.787$; Fig. 1*B*). Thus, there were no changes in the H-reflex amplitudes for different stimulation intensities over time, and thus no significant differences in the H-reflex recruitment curves could be detected after rTMS compared with the values before rTMS. There were also no differences in the evoked maximal compound potential M_{max} between recruitment curves obtained at baseline, immediately following, 10, or 20 min after rTMS.

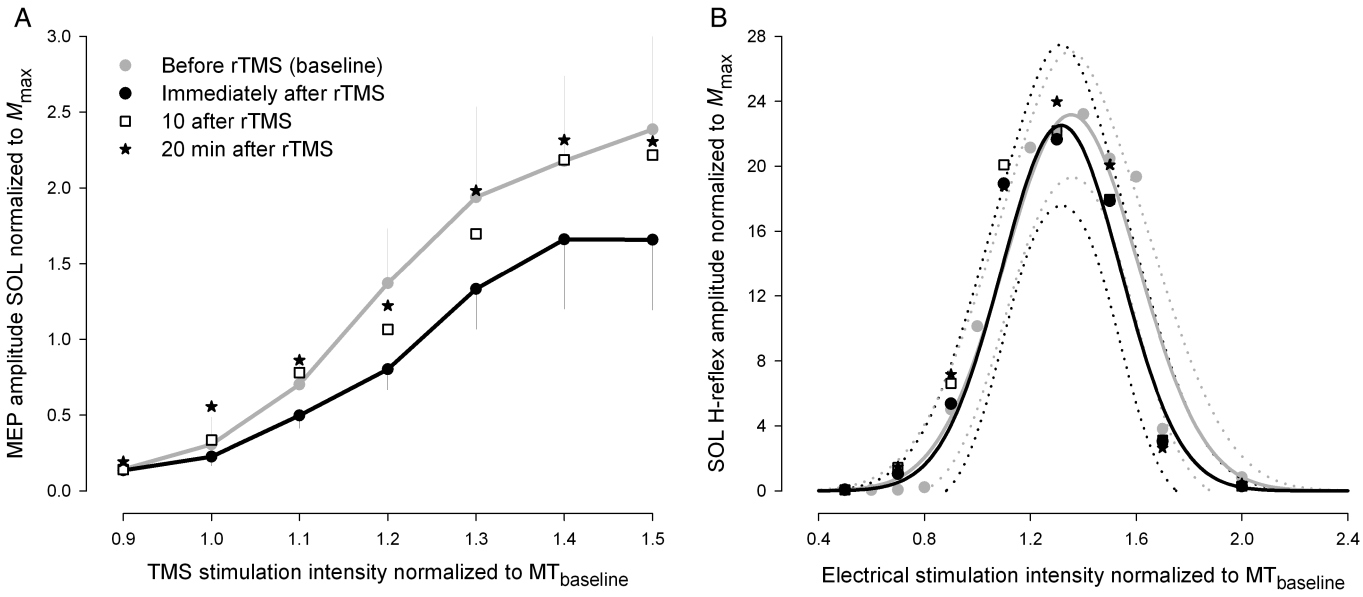

Figure 1. Results from Experiment I. (*A*) TMS stimulus-response curves obtained from SOL motor-evoked potentials evoked before, immediately after, 10, and 20 min after 20 min of 1-Hz rTMS at 1.2 MT. Stimulation intensities are normalized to the individual motor threshold and MEP amplitudes are normalized to M_{max}. Data are presented as group mean ± SEM, $^*P < 0.05$. (*B*) H-reflex recruitment curves obtained from SOL EMG at identical times. Data are presented as group mean. Recruitment curves are presented ± confidence bands.

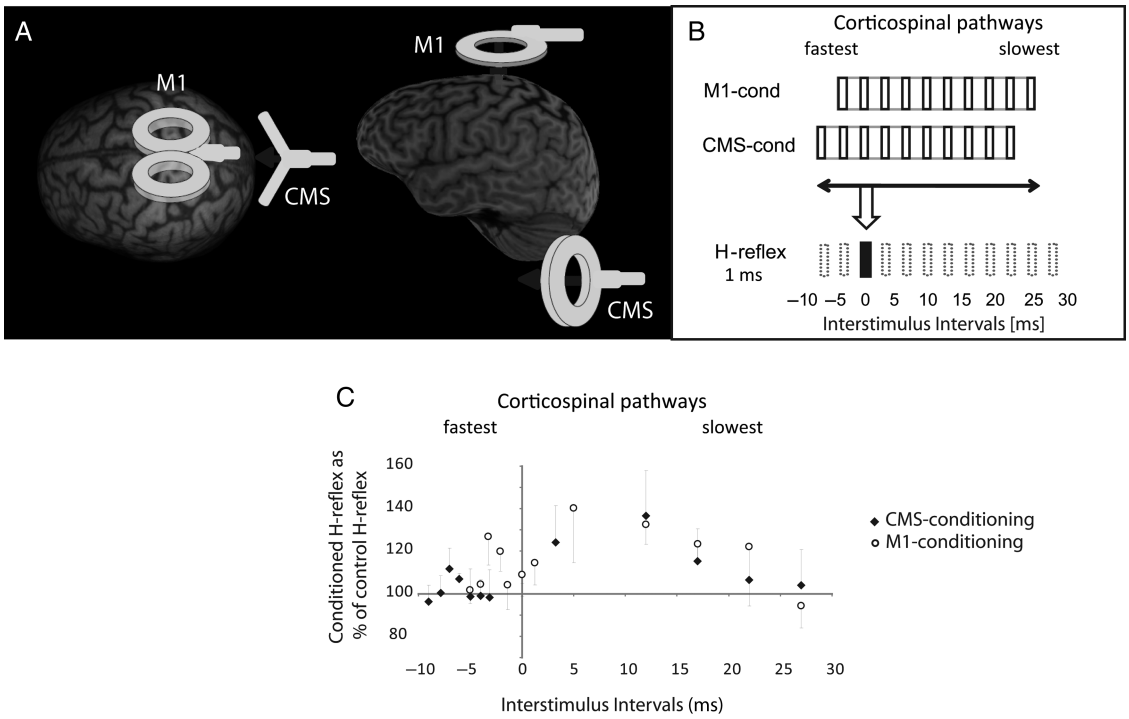

Figure 2. Procedure of M1- and CMS-conditioning. (*A*) Schematic drawing of M1- and CMS-conditioning procedure. One coil was placed over M1 (M1) and the other over the cervicomedullary junction (CMS). Conditioning of the SOL H-reflex by magnetic stimulation of the motor cortex (M1-conditioning) and by magnetic cervicomedullary stimulation (CMS-conditioning) was applied in a random order during the same measurement. (*B*) Descending volleys after magnetic stimulation of the motor cortex (M1-cond.) and the cervicomedullary junction (CMS-cond.) are dispersed for some milliseconds. In contrast, the peripheral nerve stimulation (H-reflex) produces 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 backward so that slower corticospinal pathways can be tested (late facilitation). In the present study, interstimulus intervals (ISIs) from −9 to +1 were tested; the later ISIs are only displayed to complete the picture. (*C*) H-reflex conditioning curves after M1- and CMS-conditioning. The same ISIs are displayed as in (*B*), and it can be seen that the early facilitation occurs between ISI −7 and ISI −2 followed by a late facilitation starting around ISI +5. The conditioned H-reflexes were expressed as percentage of the control (unconditioned) H-reflexes. The first positive deflection from the baseline (100%) was taken to indicate the start of the early facilitation. In most subjects, the early facilitation after CMS-conditioning started 3–4 ms earlier than the facilitation obtained with M1-conditioning due to the shorter travel distance. The magnitudes of the early facilitations were compared before and after 20-min rTMS.

Occurrence of the Early Facilitation After M1- and CMS-Conditioning

When the H-reflex was conditioned by M1 stimulation in Experiment II, subjects displayed the early facilitation around ISI −3.5 ms (mean ISI for the onset of the early facilitation: −3.54 ± 0.66 ms), whereas CMS-conditioning produced the early facilitation roughly 4 ms earlier (mean ISI for the onset of the early facilitation: −7.46 ± 0.66 ms) (an example of 1 subject is displayed in Fig. 2*C*). These values are comparable with those previously reported using the same technique (Taube et al. 2011): M1-conditioning: −3.69 ± 0.65 ms; CMS-conditioning: −7.19 ± 0.59 ms. As the early facilitation was assessed within the first 1 ms with both M1- and CMS-conditioning, it is believed to reflect activity of direct, monosynaptic corticospinal pathways (Nielsen et al. 1993, 1995; Nielsen and Petersen 1995a, 1995b; Petersen et al. 1998). In the following, the results therefore mainly concentrate on this early facilitation and other ISIs are only displayed as control ISIs in order to show that rTMS did not influence the properties of the motoneuron pool in response to the descending volleys in general.

Changes in the Amplitude of the Early Facilitation After rTMS

There was a significant TIME × INTERSTIMULUS INTERVAL effect of rTMS for both M1- ($F_{2,24} = 6.23$, $P = 0.006$, Fig. 3*A*) and CMS-conditioning (all subjects: $F_{2,24} = 5.34$, $P = 0.012$, Fig. 3*B*; only subjects with significant early facilitation ($n = 10$): $F_{2,18} = 4.65$, $P = 0.024$). After the rTMS intervention, the early facilitation obtained by M1-conditioning was significantly reduced (pre 149% vs. post 132%; $t = 4.64$, $P = 0.001$; Fig. 4*A*, *D*). Similarly, the early facilitation was significantly suppressed when tested with CMS-conditioning (pre 117% vs. post 111%; $t = 2.64$, $P = 0.04$; Fig. 4*B*,*E*; only subjects with significant early facilitation: pre 122% vs. post 114%; $t = 2.90$, $P = 0.035$). When comparing the effect of rTMS on the early facilitations, M1-conditioning revealed a greater inhibitory effect than CMS-conditioning ($t = 3.26$, $P = 0.007$). The amplitudes of the control H-reflexes remained at 20% of M_{max} in the postmeasurement, and comparable values were obtained before and after the rTMS intervention (Fig. 4*C*,*F*).

Discussion

We have shown in this study that low-frequency rTMS suppressed soleus MEP recruitment curves, but had no effect on the H/M recruitment curves. This suggests that the depression of the MEPs is not caused by changes in motoneuronal excitability, but must be explained by changes in transmission upstream from the motoneurons. The rTMS-induced reduction in the early facilitation of the H-reflex evoked by both M1- and

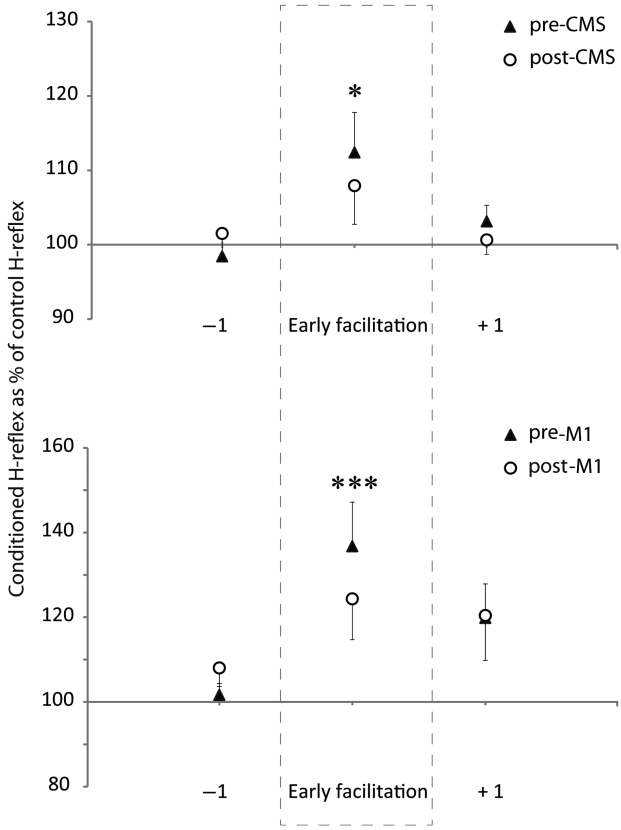

Figure 3. Results from Experiment II. The effects of low-frequency rTMS on the early facilitation obtained with CMS-conditioning (*A*) and M1-conditioning (*B*). The interstimulus interval (ISI) representing the early facilitation as well as the ISI before (−1) and the ISI after the early facilitation (+1) are displayed. rTMS significantly reduced the early facilitation of both M1- and CMS-conditioning whereas surrounding ISIs (−1 and +1) were not affected.

CMS-conditioning suggests that reduced efficiency of the CM synapse is a contributing factor to the MEP depression.

Corticospinal and Motoneuronal Excitability

One of the most extensively studied effects of rTMS is its influence on corticospinal excitability indicated by augmented MEPs after high-frequency rTMS and reduced MEPs after low-frequency rTMS (Pascual-Leone et al. 1994; Chen et al. 1997; Fitzgerald et al. 2006), similar to what we observed here. TMS (Di Lazzaro et al. 2008), PET (Conchou et al. 2009), and fMRI studies (Bestmann et al. 2003) have indicated that changes in cortical circuits contribute to these changes. However, rTMS is likely to influence not only cortical structures but also spinal circuits. Indeed, Valero-Cabré et al. (2001) reported increased flexor carpi radialis H-reflexes lasting 10 min following 1-Hz rTMS while Perez et al. (2005) and Berardelli et al. (1998) found reduced H-reflexes lasting around 1 s following 5-Hz rTMS in the soleus muscle and forearm muscles, respectively. In patients with multiple sclerosis, both findings were confirmed: high-frequency rTMS inhibited H-reflex responses whereas low-frequency rTMS facilitated the soleus H-reflex (Centonze et al. 2007). These observations indicate that changes in corticospinal excitability observed following rTMS may—at least for a short period—be influenced by changes in spinal circuits.

In Experiment I of the present study, we obtained H/M recruitment curves before, immediately after as well as 10 and 20 min after rTMS with 1 Hz at 1.2 MT. In contrast to the above-cited studies, the H/M recruitment curves remained unaltered at any time. This may be related to the relatively long time needed for testing different stimulation intensities in the present study, and/or the stimulation intensity in general. However, as we tested the whole range of the motoneuron pool, this finding suggests that suprathreshold low-frequency rTMS to the M1-representation of the soleus muscle did not induce longer lasting changes in the excitability of the motoneuron pool. Consequently, the depression of the compound MEPs and the early facilitation of the soleus H-reflex elicited by M1- and CMS-conditioning are unlikely to be caused by changes in motoneuronal excitability but are more likely explained by changes in transmission upstream from the motoneurons. This reasoning seems even more likely taking into account the abovementioned studies showing facilitated H-reflex responses after low-frequency rTMS (Valero-Cabré et al. 2001; Centonze et al. 2007). If anything, we would have expected to find facilitated H-reflex responses, which would have blurred rather than strengthened the suppressive effects obtained with the low-frequency rTMS used in the present study.

Potential Changes in the Efficiency of CM Synapses

The comparison of M1- and CMS-conditioning effects revealed greater (−17% vs. −6%) and more consistent (12 of 13 subjects vs. 10 of 13 subjects) suppression after M1-conditioning. It is however, not possible to quantitatively compare the effects after M1- and CMS-conditioning as the stimulation intensity could not be perfectly matched between conditions. Based on previous studies showing modulation of cortical circuitries by means of low-frequency rTMS (Di Lazzaro et al. 2008; Conchou et al. 2009), resulting in decreased excitability of M1 (for review Fitzgerald et al. 2006), it is most likely that intracortical processes may have contributed to the depression of the early facilitation evoked by M1-conditioning. However, they are unlikely to also explain the depression of the early H-reflex facilitation evoked by CMS-conditioning due to the subcortical site of stimulation. As the motoneuron excitability remained unchanged and the control H-reflexes were matched in pre- and postmeasurements (Fig. 4*F*), altered corticospinal transmission is likely to be involved. The early facilitation of the H-reflex is thought to be caused by activation of direct, monosynaptic corticospinal (CM) projections to the spinal motoneurons (Nielsen et al. 1993; Nielsen and Petersen 1995b). This and the fact that the ISIs before and after the early facilitation remained unchanged (Fig. 3*A*,*B*) indicate that the most likely site of the depression is the synapse of the CM neuron on the spinal motoneuron. This conclusion seems even more likely when considering the observation of direct recordings from the cervical epidural space showing reduction of later I-waves but not of the first I-wave after 1-Hz rTMS (Di Lazzaro et al. 2008; for summary see Fig. 8 in Di Lazzaro et al. 2010). As the first I-wave is separated by several milliseconds (~3–5 ms) from later I-waves and as the early facilitation is in all likelihood caused by activation of pathways transmitting this first excitation, not only the reduction of the early facilitation after CMS- but also M1-conditioning may strongly rely on mechanisms taking place at the CM synapses. Although corticospinal synapses have been shown to be unaffected by presynaptic

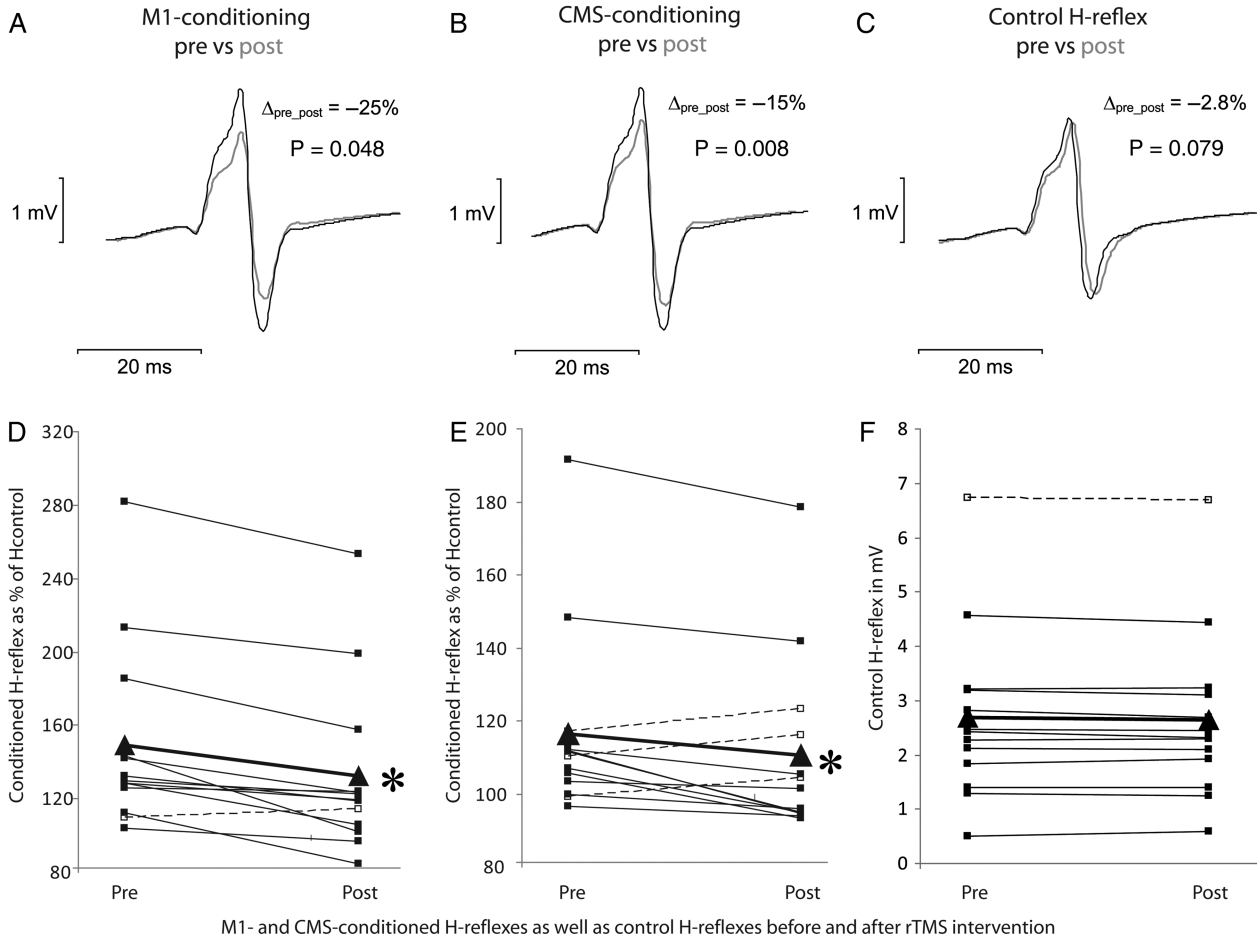

Figure 4. Results from Experiment II. The effects of low-frequency rTMS on the early facilitation obtained with M1-conditioning (*A* and *D*) and CMS-conditioning (*B* and *E*). Furthermore, the size of the control H-reflex is displayed in (*C*) and (*F*) to illustrate that the control H-reflex was kept constant. The first row (*A*, *B*, *C*) displays data (averages of 10 traces) from one single representative subject, whereas the second row (*D*, *E*, *F*) shows the mean data of all participating subjects before (pre) and after (post) rTMS. Each dot represents the mean of 10 responses and the triangles (triangle) represent the overall mean. It can be seen that rTMS significantly reduced not only the cortically conditioned H-reflexes (*A*, *D*) but also the responses after CMS-conditioning (*B*, *E*). *P*-values in the first row refer to the data of the single subject whereas the stars (asterisk) in the second row indicate significant suppression of the mean ($^*P < 0.05$).

inhibition elicited by stimulation of sensory afferents (Rudomin et al. 1975; Nielsen and Petersen 1994), this does not rule out the possibility that other populations of interneurons may inhibit the synapses of CM axons. It has also been known for some time that CM synapses show activity-dependent short-term plastic changes similar to other synapses in the nervous system including potentiation at high frequencies of activation and relative depression at longer intervals between action potentials (Porter and Lemon 1993). More recently, transmission from M1 neurons to α-motoneurons has been shown to change rapidly; possibly at least partly due to changes in the efficacy of CM synapses (Davidson et al. 2007).

Taylor and Martin (2009) and Leukel et al. (2012) demonstrated that "spinal PAS" consisting of repeated pairs of cortical and peripheral nerve stimulation may alter corticospinal transmission and suggested that changes in the efficiency of CM synapses could be one contributing factor. However, no conclusive evidence for this possibility was presented as previous studies did not assess alterations at the time of early facilitation, which reflects, at least when it is assessed within the first millisecond after its onset, activity in direct, monosynaptic corticospinal projections (Nielsen et al. 1993, 1995; Nielsen and Petersen 1995a, 1995b; Petersen et al. 1998).

The effects observed in the present study may also have contributed to some recently observed behavioral consequences following application of rTMS. In this previous study, we used an identical rTMS protocol to investigate interference effects during motor learning. rTMS (1 Hz) was applied both at suprathreshold and subthreshold intensities but only suprathreshold rTMS-induced behavioral aftereffects (Lundbye-Jensen et al. 2011). Therefore, it was suggested that the detrimental effect of suprathreshold rTMS on motor learning may be caused at least in part by subcortical or spinal mechanisms. Consequently, the observed changes in corticospinal transmission in the present study may at least partly explain these behavioral consequences of suprathreshold rTMS. From a physiological point of view, the finding of activity-dependent changes of CM synapses in response to rTMS may extend and specify the previously made observation that strong voluntary contractions depress the corticospinal transmission (Petersen et al. 2003). In addition, protocols using spinal PAS suggest that transmission cannot only be downregulated, but also be upregulated (Taylor and Martin 2009; Leukel et al. 2012). Thus, synaptic plasticity within the major pathway for voluntary contractions and more specifically within the direct corticospinal projections may be used to adapt transmission in an activity-dependent way.

The finding of the present study highlights that interventions influencing the corticospinal pathway may lead to changes in excitability, synaptic efficacy and thus transmission at different levels, cortical as well as spinal but importantly also at the specific level of the CM synapse. It is important that this is acknowledged when interpreting both behavioral and electrophysiological effects of different interventions. In the present study, the effects were elicited by a low-frequency suprathreshold rTMS protocol, but it is indeed likely that other electrophysiological neuroenhancement protocols and behavioral interventions such as motor practice could also be accompanied by changes at this level of the motor system.

Conclusion

In line with previous studies, the present study demonstrates that low-frequency rTMS suppresses corticospinal excitability. Whereas previous studies suggested that this effect relates to changes at a cortical level, the present study demonstrates that the effects of rTMS are not restricted to the motor cortex. Based on the current observation of suppressed early facilitation after cervicomedullary conditioning of the soleus H-reflex, it can be concluded that rTMS with suprathreshold intensity most likely also influences the synaptic efficiency of direct corticospinal pathways projecting to spinal motoneurons.

Notes

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