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Modulation of I-wave generating pathways by TBS: a model of plasticity induction

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Key points

- Mechanisms underlying plasticity induction by repetitive transcranial magnetic stimulation protocols such as intermittent theta-burst stimulation (iTBS) remain poorly understood.
- Individual response to iTBS is associated with recruitment of late indirect wave (I-wave) generating pathways that can be probed by the onset latency of TMS applied to primary motor cortex (M1) at different coil orientations.
- We found an association between late I-wave recruitment (reflected by AP-LM latency, i.e. the excess latency of motor evoked potentials (MEPs) generated by TMS with an anterior-posterior (AP) orientation over the latency of MEPs evoked by direct activation of corticospinal axons using latero-medial (LM) stimulation) and changes in cortical excitability following iTBS, confirming previous studies.

- AP-LM latency significantly decreased following iTBS, and this decrease correlated with the iTBS-induced increase in cortical excitability across subjects.
- Plasticity in the motor network may in part derive from a modulation of excitability and recruitment of late I-wave generating cortical pathways.

Abstract

Plasticity-induction following theta burst transcranial stimulation (TBS) varies considerably across subjects, and underlying neurophysiological mechanisms remain poorly understood, representing a challenge for scientific and clinical applications. In human motor cortex (M1), recruitment of indirect waves (I-waves) can be probed by the excess latency of motor evoked potentials (MEPs) elicited by TMS with an anterior-posterior (AP) orientation over the latency of MEPs evoked by direct activation of corticospinal axons using latero-medial (LM) stimulation, referred to as "AP-LM latency" difference. Importantly, AP-LM latency has been shown to predict individual responses to TBS across subjects. We, therefore, hypothesized that the plastic changes in corticospinal excitability induced by TBS are the result, at least in part, of changes in excitability of these same I-wave generating pathways. We investigated in 20 healthy subjects whether intermittent TBS (iTBS) modulates I-wave recruitment as reflected by changes in the AP-LM latency. As expected, we found that AP-LM latencies before iTBS were associated with iTBS-induced excitability changes. A novel finding was that iTBS reduced the AP-LM latency, and that this correlated significantly with changes in cortical excitability observed following iTBS: subjects with the largest reductions in AP-LM latencies had the largest increases in cortical excitability following iTBS. Our findings suggest that plasticity-induction by iTBS may derive from the modulation of I-wave generating pathways projecting onto M1, accounting for the predictive potential of I-wave recruitment. The excitability of I-wave generating may serve a critical role in modulating motor cortical excitability and hence represent a promising target for novel rTMS protocols.

Introduction

Theta burst stimulation (TBS) can transiently change the excitability of human motor cortex by inducing early-stage long-term potentiation/depression (LTP/LTD) like effects at cortical synapses. However, the after-effects of TBS vary considerably between subjects (Hamada *et al.*, 2013; Hinder *et al.*, 2014; López-Alonso *et al.*, 2014; Nettekoven *et al.*, 2015). Many factors contribute to these inter-individual differences such as, e.g., age, time of day, or circulating hormones (Suppa *et al.*, 2016; Guerra *et al.*, 2018). Hamada and colleagues reported that individual responses to TBS can be predicted by the differential recruitment of cortical pathways by the TMS pulse (Hamada *et al.*, 2013). These pathways generate different indirect waves (I-waves) of neural activity descending the corticospinal tract after a TMS pulse has been applied to the primary motor cortex (Day *et al.*, 1989; Di Lazzaro *et al.*, 1998). I-wave recruitment can be non-invasively assessed by onset latencies of motor-evoked potentials (MEPs) elicited by TMS pulses with different current orientations: posterior-anterior (PA) currents preferentially result in short-latency responses, while anterior-posterior (AP) currents evoke MEP with longer latencies, and latero-medial (LM) directed currents at high intensities evoke direct waves with shortest latencies (Day *et al.*, 1989; Sakai *et al.*, 1997; Di Lazzaro *et al.*, 1998; Hamada *et al.*, 2013). We refer to the excess latency of MEPs produced by AP-TMS over the latency of MEPs evoked by LM-TMS to as "AP-LM latency" difference and the excess latency of PA-TMS over LM-TMS as "PA-LM latency" difference. AP-LM latencies, reflecting *late* (i.e., long onset latency) I-wave recruitment, have been shown to strongly correlate with the plasticity effect induced by TBS across subjects (Hamada *et al.*, 2013). Since the biphasic (i.e., PA–AP) TMS pulse applied during TBS preferentially activates neurons during the reverse stimulation phase (AP) (Maccabee *et al.*, 1998; Di Lazzaro *et al.*, 2001), the MEPs evoked by AP stimulation might represent activation of the same elements as stimulated with TBS (Hamada *et al.*, 2013). This leads to the question whether plasticity induction by TBS may partially derive from selective changes in the elements activated by AP stimulation, i.e., cortical pathways underlying *late I-wave* recruitment. Support for this hypothesis stems from Di Lazzaro and colleagues, who showed that excitability enhancing intermittent TBS (iTBS) enhances late but not early I-waves in spinal recordings of descending activity (Di Lazzaro *et al.*, 2008). Moreover, we recently observed that AP-LM latencies are closely associated with the functional connectivity between M1 and a network of premotor areas as assessed via functional magnetic resonance imaging (fMRI): Subjects featuring late I-waves (i.e., long AP-LM latency) showed weaker premotor-M1 connectivity (Volz *et al.*, 2015). Importantly, functional M1 connectivity has previously been

shown to be readily increased by iTBS, especially in responders to iTBS (Nettekoven *et al.*, 2014, 2015). Thus, iTBS induced increases in M1 connectivity may reflect decreased recruitment of late I-wave generating pathways following iTBS.

We therefore hypothesized that iTBS reduces I-wave latency especially for subjects in whom iTBS induces cortical plasticity, as reflected by increased cortical excitability. In effect, we can imagine that AP-TMS (reflecting late I-wave recruitment) activates a neuron or chain of neurons that excite corticospinal cells (Lemon, 2008; Di Lazzaro *et al.*, 2012; Di Lazzaro & Ziemann, 2013). The weaker the synaptic connections in this pathway the longer the latency of the response. Strengthening such synaptic connections via iTBS will reduce synaptic delays and speed transmission, thus shortening the latency of AP-MEPs. To address this hypothesis, we investigated the effect of iTBS applied over M1 or a control site (parieto-occipital vertex) on MEP latencies evoked by different current directions and MEP amplitudes elicited by conventional biphasic PA pulses. We expected a relationship between the reduction of AP-LM latency and increases in cortical excitability across subjects following iTBS applied over M1 compared to control stimulation.

Materials and Methods

Ethical approval

20 right-handed healthy subjects (15 female and 5 male) were enrolled in the study after providing written informed consent in accordance to the Declaration of Helsinki. All participants had no history of neurological or psychiatric disease and no contraindication to TMS (Rossi *et al.*, 2009). The ethics committee at the medical faculty of the University of Cologne approved the study (Ethical approval number: 14-141). The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database.

Experimental design

We used a single-blinded, vertex-stimulation controlled, within-subject, crossover design to assess the effects of iTBS on TMS latencies. iTBS was applied either over left M1 or vertex

(control stimulation) in two different sessions that were separated by at least seven days in order to prevent carry-over effects. The order of sessions was counterbalanced across subjects. We also assessed fMRI activity directly after MEP assessment in each session. However, fMRI data were not considered in the analyses of the present paper.

Transcranial Magnetic Stimulation

The assessment of MEP amplitudes and latencies was performed using a Magstim 200² stimulator (The Magstim Co. Ltd) equipped with a 70 mm figure-of-eight coil eliciting monophasic pulses. A Magstim SuperRapid² stimulator (The Magstim Co. Ltd) with a figure-of-eight coil (70 mm standard coil, Magstim) generating biphasic pulses was used to apply iTBS. The position of the TMS coil was monitored and recorded throughout the experiment using a Brainsight² computerized frameless stereotaxic system (Rogue Research Inc., Montreal, Canada).

Ag/AgCl surface electrodes (Tyco Healthcare, Neustadt, Germany) placed in a belly-to-tendon montage were used to record the EMG signals from the right first dorsal interosseous (FDI) muscle. The EMG signal was filtered (0.5 Hz high-pass and 30–300 Hz band-pass), amplified and digitized using a Power-Lab 26T and the LabChart software package (ADInstruments Ltd, Dunedin, New Zealand).

The resting motor threshold (RMT) was defined as the lowest stimulator intensity resulting in a MEP with a minimum amplitude of 50 μV in at least 5 out of 10 trials (Rossi *et al.,* 2009). The active motor threshold (AMT) was defined as the lowest stimulator intensity resulting in a MEP with a minimum amplitude 200 μV in at least 5 out of 10 trials, while subjects performed constant contraction of the FDI muscle at ~10% of maximum strength (monitored by a force transducer, ADInstruments Ltd., Sydney/Australia). Of note, motor thresholds were assessed using monophasic pulses at different coil orientations (RMTpa, AMTpa, AMTap, AMTlm) to individualize respective stimulation intensities for TMS applied with currents oriented in AP, PA or LM direction. While stimulation intensities used to record MEPs for orientation-dependent latency assessment were individualized using the respective AMT, cortical excitability before and after iTBS was probed via 20 MEPs evoked by monophasic PA-TMS applied at 120% of the individual RMTpa. The AMT was also assessed with biphasic pulses

generated by the Magstim SuperRapid^2 (in "standard" PA orientation) to individualize iTBS intensity (80% of biphasic AMT).

To determine maximum grip force and monitor contraction during MEP recordings, a grip force sensor was placed between the bases of the thumb and index finger in pronation position of the hand, which results in effective recruitment of the FDI muscle during thumb–index abduction. Maximum contraction force was estimated by averaging over 3 repetitions performed by the subjects with breaks of several seconds between repetitions to prevent fatigue.

We used iTBS as introduced by Huang and colleagues (Huang *et al.*, 2005). iTBS was applied either over the left M1 (i.e., the "hotspot") or over the parieto-occipital vertex as control stimulation (Herwig *et al.*, 2007, 2010). Stimulation intensity was individualized to 80% active motor threshold (AMT, as determined using the Magstim SuperRapid^2).

I-wave recruitment by single pulse TMS

Single monophasic TMS pulses were applied using different coil orientations to probe I-wave recruitment. Posterior–anterior (PA) oriented currents elicit I-waves of short onset latency. Anterior–posterior (AP) oriented currents typically result in I-waves with longer onset latencies and latero-medial (LM) oriented currents applied at high stimulation intensities result in shortest latency responses, i.e. direct waves (D-waves; Day *et al.,* 1989; Di Lazzaro *et al.,* 1998; Hamada *et al.,* 2013). While latencies evoked by PA currents are relatively consistent, the latency of MEPs elicited by AP-TMS are typically more variable across subjects (Hamada *et al.,* 2013). Like in previous studies (Hamada *et al.,* 2013; Volz *et al.,* 2015), we used the latency difference between LM and AP or PA evoked MEP onsets as a measure of I-wave recruitment. This latency difference reflects the excess time caused by activation of cortical pathways relative to D-waves proposedly resulting from direct axonal stimulation of corticospinal pyramidal cells (Hamada *et al.,* 2013).

The TMS coil was oriented in the following ways to evoke distinctly oriented currents. PA-TMS: the coil was positioned postero-laterally forming an angle of ~45° with the midline. AP-TMS: the coil was held at 180° relative to PA-TMS. LM-TMS: the coil was held with the handle pointing to the left while forming a 90° angle to the midline (Fig. 1). The coil position eliciting MEPs with maximal amplitudes following PA-TMS at minimum stimulation intensities was

defined as "TMS hotspot". The same hotspot was used for all coil orientations and iTBS application.

MEP onset latencies were determined during constant contraction of the FDI muscle (~10% of the maximum contraction, with online feedback visualizing the generated force). Stimuli were applied at following intensities: PA-TMS: 110% AMTpa, AP-TMS: 110% AMTap, and LM-TMS: 150% AMTlm. In subjects whose 150% AMTlm did not reach 50% of maximum stimulator output (MSO), 50% MSO was used as stimulation intensity to assure D-wave recruitment by LM-TMS (Werhahn *et al.*, 1994). To ensure methodological validity of our results, stimulation parameters applied in the present study were identical to those previously used to empirically assess distinct I-wave circuits (cf. Hamada *et al.* 2013).

20 MEPs were recorded for PA- and AP-TMS and 10 MEPs were elicited by LM-TMS. After each block of 10 stimuli, subjects were instructed to relax their hand muscles to avoid fatigue. Recording latency measurements took 10–15 min. Importantly, all subjects successfully performed constant FDI-contraction at the given intensity throughout MEP recordings without significant fluctuations in grip strength due to the low force level.

Statistical analysis

MEP onset latencies were determined by an automated method to minimize a potential observer bias, using a custom-made MATLAB script (MATLAB 2016b, The MathWorks, Inc., Natick, United States). For each trial, the MEP onset was defined as the earliest time point following stimulation where EMG signals exceeded the average level plus 2 standard deviations (SDs) of the pre-stimulus EMG signal (−100 to 0 ms of TMS). Of note, this analysis was performed after data collection ("offline") rather than in real time during data collection to prevent the induction of a potential bias of the investigator performing the TMS assessment.

Changes in both TMS-latencies and MEP-amplitudes were analyzed using two-factorial repeated-measures analyses of variance (rm-ANOVA) including the factors: STIMULATION ("M1", "control") and TIME (pre iTBS, post iTBS) using the ezANOVA-package for R (Lawrence, 2015). In case of significant main or interaction effects, post-hoc t-tests were performed.

Results

Motor thresholds

Motor thresholds evaluated with different current orientations and monophasic pulses did not differ between sessions (all p>0.1: RMTpa: p=0.11; AMTpa: p=0.38; AMTap: p=0.65; AMTlm: p=0.18, two-sided Student's t-tests). Furthermore, significant inter-correlation coefficients indicated a high re-test reliability of motor thresholds (RMTpa: r=0.932, p<0.001; AMTpa: r=0.923, p<0.001; AMTap: r=0.805, p<0.001; AMTlm: r=0.860, p<0.001). Likewise, the AMTs determined by biphasic pulses for determination of iTBS intensity were similar (p=0.57) and highly correlated between the two sessions (r=0.856, p<0.001). Hence, MEP recordings and iTBS application were performed at comparable intensities for each subject in both sessions rendering a potential bias of diverging threshold-adapted stimulation intensities unlikely.

Cortical excitability

Cortical excitability before and after iTBS was assessed via monophasic PA-TMS applied at 120% of the individual RMTpa. iTBS aftereffects on cortical excitability were assessed via normalized MEP amplitudes relative to the baseline (post/pre stimulation; for example see Huang *et al.,* 2005; Hamada *et al.,* 2013; Nettekoven *et al.,* 2014).

While normalized MEP amplitudes significantly increased after M1-stimulation (p=0.035, one-sample two-sided t-test), but not after control stimulation (p=0.463), no significant difference was found when comparing normalized MEP amplitudes for control and M1 stimulation directly (Fig. 2A, p=0.181, two-sided t-test). Thus, in line with previous negative findings (Hamada *et al.*, 2013), iTBS did no significantly modulate cortical excitability across all subjects.

In summary, albeit we found evidence that iTBS effectively modulated cortical excitability compared to baseline, the effect was not strong enough compared to control stimulation, probably due to differential responsiveness of the subjects to M1-iTBS as expected (Hamada *et al.,* 2013; Cárdenas-Morales *et al.*, 2014; Nettekoven *et al.,* 2015).

TMS latencies

For PA-LM latencies, a two-factorial rm-ANOVA showed no significant effects (STIMULATION: $F_{(1,19)}$=0.071, p=0.792; TIME: $F_{(1,19)}$=2.062, p=0.167; STIMULATION x TIME: $F_{(1,19)}$=0.097, p=0.758) indicating that PA-LM latencies did not differ between sessions or before and after iTBS. In contrast, for AP-LM latencies, we observed a significant interaction STIMULATION x TIME ($F_{(1,19)}$=10.090, p=0.005), without significant main effects (STIMULATION: $F_{(1,19)}$=0.243, p=0.628; TIME: $F_{(1,19)}$=2.234, p=0.151) (Fig. 2B). Post-hoc tests showed that the significant interaction was driven by a significant decrease in AP-LM latency after M1- compared to control stimulation (p=0.005). Hence, iTBS applied over M1 selectively reduced AP-LM, but not PA-LM latencies compared to control stimulation (for a depiction of individual AP-LM and PA-LM latencies before and after iTBS see Suppl. Fig. 1). Stimulation effects on direction-dependent latencies may in principle be influenced by concurrent changes in the amplitudes of MEPs evoked by TMS with differential current directions. Importantly, MEP amplitudes evoked with AP, PA or LM stimulation did not changes significantly after stimulation (all p>0.1, uncorrected). Moreover, changes in amplitudes elicited with different current directions did not correlate with latency changes observed following iTBS. Therefore, the observed effects on directionality-dependent latencies were not biased by changes in MEP amplitudes induced by iTBS.

Cortical excitability and I-wave recruitment

Pre-stimulation AP-LM latency significantly correlated with the relative increase in MEP-amplitudes (r=0.467, p=0.038). Hence, subjects featuring longer AP-LM latencies showed greater increases in MEP-amplitudes following iTBS to M1, but not following control stimulation (Fig. 3A, r=0.188, p=0.428), replicating earlier findings by Hamada and colleagues (Hamada *et al.*, 2013).

AP-LM latencies at baseline featured a significant negative correlation with iTBS-induced decrease in AP-LM latencies (Fig. 3B, r=-0.507, p=0.022). Hence, subjects featuring long AP-LM latencies had the strongest decreases in AP-LM latencies. Furthermore, these subjects also showed a more pronounced increase in MEP amplitudes as indicated by a significant negative correlation between the iTBS-induced change in AP-LM latencies and MEP-amplitudes (Fig. 3C, r=-0.596, p=0.006).

In summary, we here observed for the first time that iTBS lead to a selective reduction in AP-LM latencies. This reduction was strongest in subjects who featured pronounced increases in MEP-amplitudes after iTBS. Of note, such a relationship was missing for baseline AP-LM values in the control session with both changes in MEP amplitudes ($r=0.188$, $p=0.428$) and in AP-LM latencies ($r=-0.374$, $p=0.105$) following control stimulation, thus corroborating the specificity of this finding.

Discussion

Application of iTBS to M1 significantly reduced AP-LM latency. The amount of latency change correlated with the effect of iTBS: participants with the largest MEP increases after iTBS showed the greatest reduction in AP-LM latency. We hypothesize that iTBS modulates I-wave recruitment potentially by strengthening synapses in the I-wave pathway activated by AP pulses. This increases the amplitude of MEPs while the resulting increased efficiency of synaptic transmission reduces the AP-LM latency.

Plasticity induction and I-wave generating pathways

rTMS after-effects on cortical excitability of the human brain are thought to derive from the induction of LTP-like or LTD-like effects at synapses in the stimulated tissue (Huang *et al.*, 2005). However, it remains unknown whether plasticity induction primarily occurs via changes in synaptic transmission in specific pathways or subtypes of neurons, or whether all stimulated neurons are affected. The present study addressed this question using the iTBS protocol. Previous work by Hamada and colleagues showed that an individual's response to iTBS could be predicted from measurement of the latency of MEPs evoked by AP-TMS (Hamada *et al.*, 2013). Since this direction of TMS primarily evokes late I-wave inputs to corticospinal neurons, the suggestion was that the synapses between neurons in this pathway might be the locus of the plastic changes induced by iTBS. That is, the more efficiently iTBS targeted the AP-pathway the greater the chance of producing plastic changes in its synaptic connections. Indeed, work by Di Lazzaro and colleagues showed that iTBS enhances late but not early I-waves in spinal recordings of descending activity (Di Lazzaro *et al.*, 2008).

The present findings add to this work by showing that following iTBS there is a small, but significant reduction in the AP-LM latency (Fig. 2B) which was related to the amount of plasticity observed: Individuals who showed the largest increases in MEP amplitude had the greatest reduction in AP-LM latency (Fig. 3C). This was not due to the increase in size of MEPs since latency measures were made using a constant response amplitude of approximately 1 mV. The reduction of AP-LM latency by iTBS was also inversely correlated with baseline AP-LM latency determined before stimulation (Fig. 3B). Hence, subjects with long AP-LM latencies (i.e., 'canonical' responders to iTBS cf. Hamada *et al.*, 2013), not only showed the strongest increases in cortical excitability (Fig. 3A) but also the most pronounced decreases in AP-LM latency (Fig. 3B).

In summary, we found that the response to iTBS may not only be predicted by probing I-wave recruitment, but that application of iTBS specifically seems to modulate the cortical circuitry generating late I-waves. Alternatively, iTBS-induced plasticity may result from modulation of I-wave generating pathways primarily activated by PA stimulation. However, no significant change was observed in the excess latency of MEPs produced by PA-TMS over the latency of MEPs evoked by LM-TMS, i.e. PA-LM latency, following iTBS, nor did PA-LM latencies correlate with iTBS-induced changes in cortical excitability. Taken together, this renders a key role of I-wave generating pathways primarily recruited by PA stimulation rather unlikely. Conversely, iTBS primarily induced plasticity in subjects prone to recruiting late I-waves following AP-TMS, which decreased in onset-latency after iTBS application. Of note, the notion that cortical pathways distinctly recruited by AP- and PA-TMS may contribute to motor plasticity is supported by a recent study showing that PA- and AP-inputs participate in the induction of cortical plasticity after different paired associative stimulation protocols (PAS, Hamada *et al.*, 2014). Our finding does not only offer an explanation of the capacity of AP-LM latency to predict the susceptibility to plasticity-inducing rTMS protocols, but also grants novel insights into the putative neurobiological mechanisms underlying the effects of non-invasive brain stimulation in general (Grefkes & Fink, 2012). Of note, late I-wave recruitment has not only been associated with plasticity-induction by TBS, but has also been reported to predict plastic changes observed after anodal transcranial direct current stimulation (tDCS) (Wiethoff *et al.*, 2014; McCambridge *et al.*, 2015). This convergence of findings points to a general mechanism involving the modulation of activation properties of late I-wave generating pathways, that is not limited to TBS, but potentially hold implications for the modulation of human cortical excitability in a more general fashion.

Modulation of I-wave generating circuitry by iTBS

The neural mechanisms underlying the generation of different I-waves remain unknown, and several competing models have been introduced, ranging from oscillating properties of the corticospinal cells to distinct circuits of excitatory and inhibitory interneurons impacting on corticospinal target cells or the activation of synaptic inputs at different distances to the cell soma in M1 layer 5 (Esser *et al.*, 2005; Di Lazzaro & Ziemann, 2013; Rusu *et al.*, 2014; Triesch *et al.*, 2015). One popular model of I-wave generation hypothesizes that different pathways feature distinct numbers of interneurons and intercalated synapses from the first neuron onto the corticospinal neuron (CSN), with less synapses (monosynaptic) resulting in shorter latencies (i.e., early I-waves) and more synapses (oligosynaptic) leading to long-latency, i.e., late I-waves (Lemon, 2008; Di Lazzaro *et al.*, 2012; Di Lazzaro & Ziemann, 2013). Since subjects predominantly exhibiting late I-waves upon M1 stimulation showed an inter-related increase in cortical excitability and shortening of AP-LM latencies (Fig. 3), the late I-wave pathway seems to be modulated by iTBS. According to the oligo-synaptic I-wave model, iTBS-induced changes may occur in late I-wave generating pathway projecting from premotor areas onto the CSN located in or close to M1 (Shimazu *et al.*, 2004; Lemon, 2008; Volz *et al.*, 2015). A lack of excitability increases in subjects in whom AP-TMS primarily recruits early I-waves, may derive from the fact that the same oligo-synaptic I-wave pathway is already pre-activated and hence cannot be further optimized for rapid signal transmission (resulting in low latencies). Support for this hypothesis stems from a recent study reporting that responders to iTBS featured both lower functional connectivity before stimulation and stronger stimulation induced increases in connectivity, in line with a ceiling on iTBS after-effects (Nettekoven *et al.*, 2015). Of note, the observed reduction in AP-LM latency after iTBS was <1 ms and hence is small enough to principally stem from reduced excitatory postsynaptic potential (EPSP) rise times (Sayer *et al.*, 1990). An alternative mechanism underlying late I-wave generation may lie in the preferential activation of GABAergic interneurons, specifically of the neurogliaform cell (NGFC) type (Di Lazzaro *et al.*, 2018). TBS may modulate the activity and excitability of such GABAergic interneurons, thereby altering the response to AP-LM stimulation and potentially contributing to changes in cortical excitability and plasticity induction. Support for this hypothesis stems from animal studies showing differential TBS effects on the activity of distinct subtypes of inhibitory GABAergic interneurons (Benali *et al.*, 2011; Funke & Benali, 2011; Volz *et al.*, 2013).

Future studies are needed to further clarify the cortical mechanisms of late I-wave recruitment and their role in induction of motor plasticity.

Conclusions

AP-LM latencies decreased in subjects who featured long AP-LM latencies and showed pronounced increases in cortical excitability after iTBS. These findings are in line with the notion that iTBS-induced increases in cortical excitability may in part result from the modulation of late I-wave generating circuitry. Our current findings thus explain the predictive power of AP-LM latencies for the response to iTBS and constitute a mechanism of plasticity induction by TBS in the human cortex. These insights might help to develop novel stimulation protocols aiming at modulating the excitability of I-wave generating pathways to increase the efficiency of plasticity-induction via rTMS across subject.

Additional Information

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Figure legends

Figure 1: Coil orientation-dependent TMS latencies. (A) Schematic representation of coil orientations and typical example of MEPs during constant contraction of the TMS target muscle (FDI). Arrow indicates the timing of TMS and arrow head indicates the onset of MEPs (Calibration bars, 1 mV, 20 ms). (B) Individual PA-LM and AP-LM latency differences before iTBS application.

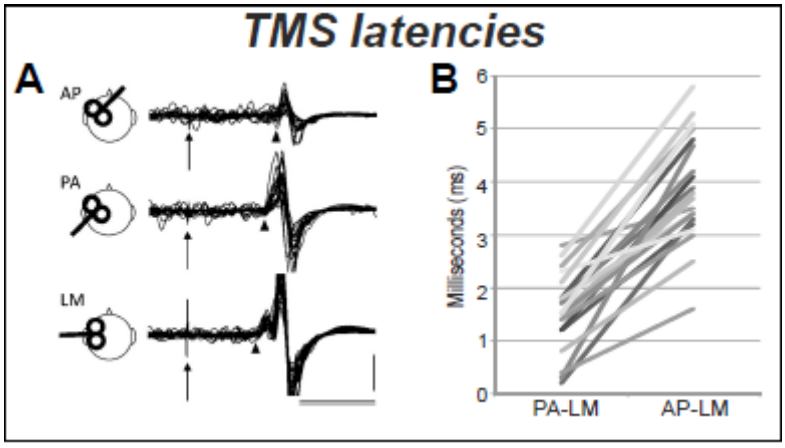


Figure 2: (A) Individual normalized MEP amplitudes for M1 and control stimulation. iTBS applied to M1 did not result in a significant increase in MEP-amplitudes compared to control stimulation across our cohort of subjects, in line with previous observations (Hamada et al. 2013). (B) Individual AP-LM latencies significantly decreased after iTBS applied to M1 compared to control stimulation (STIMULATION x TIME interaction: $F_{(1,19)}$=10.090, p=0.005, *post-hoc t-test: p=0.005).

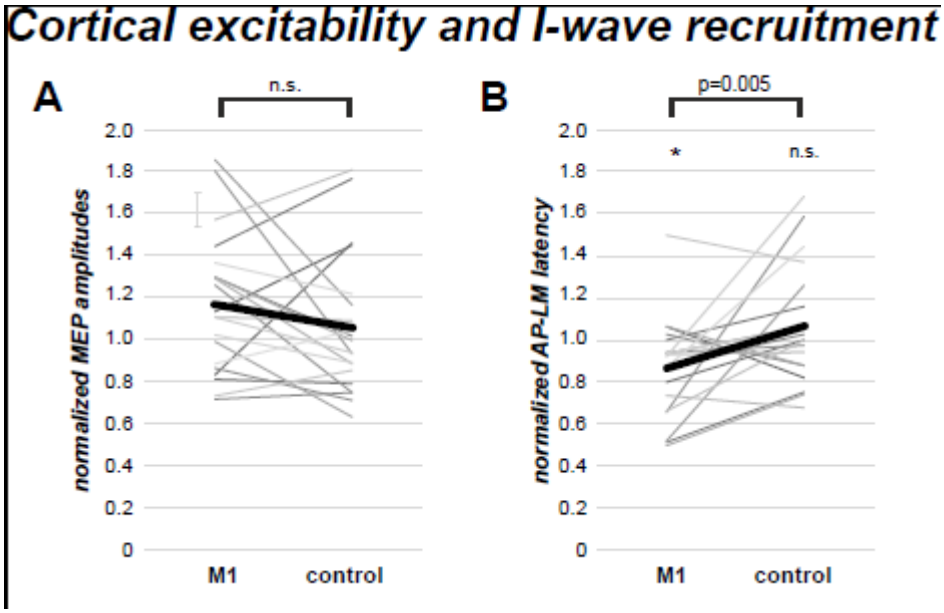
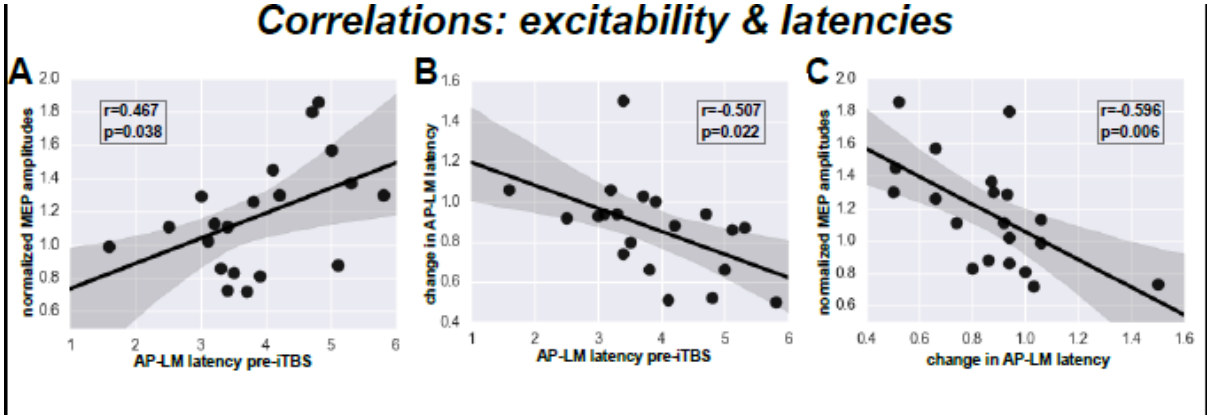


Figure 3: (A) AP-LM latencies obtained before iTBS application significantly correlated with changes in MEP amplitudes replicating earlier findings [1]. Subjects featuring longer AP-LM latencies showed strongest increases in MEP amplitudes after iTBS. (B) AP-LM latencies obtained before iTBS application also significantly correlated with iTBS-induced changes in AP-LM latencies. Subjects with long AP-LM latencies before iTBS featured most pronounced decreases in AP-LM latencies after iTBS.

(C) iTBS induced change in cortical excitability significantly correlated with decreases in AP-LM latencies. Hence, subjects with pronounced increases in cortical excitability (i.e., responders) also featured reduced AP-LM latency after stimulation. In summary, these findings establish a strong link between changes in cortical excitability and AP-LM latencies after iTBS. In subjects who recruit late I-waves after AP-TMS, iTBS resulted in increased cortical excitability alongside a shortening of AP-LM latencies.



Supplementary Figure 1: Individual AP-LM (A) and PA-LM (B) latencies before *(pre)* and after *(post)* iTBS applied to M1 or the parieto-occipital vertex (control). While AP-LM latency significantly decreased following M1- but not control-stimulation (*p=0.005), no significant changes were observed for PA-LM latencies.

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Lukas J. Volz works in clinical neurology and clinical neuroscience with a focus on neuromodulation and motor learning after stroke, heading the Network Plasticity research group at the Department of Neurology, University of Cologne. After investigating the effects of rTMS on the rat brain for his doctoral thesis with Klaus Funke in Bochum, he studied mechanisms of neuroplasticity in the human brain as a postdoc at the Max-Planck Institute for Neurology and the Department of Neurology in Cologne, with Christian Grefkes and Gereon Fink. Before returning to Cologne, he extended his expertise in neuroimaging studying reward processing and motor learning at the SAGE center for the Study of the Mind and Brain at the University of California, Santa Barbara with Mike Gazzaniga and Scott Grafton.