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Pulsed facilitation of corticospinal excitability

by the sensorimotor mu-alpha rhythm

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Abstract

Alpha oscillations (8-14 Hz) are assumed to gate information flow in the brain by means of pulsed inhibition, i.e., the phasic suppression of cortical excitability and information processing once per alpha cycle, resulting in stronger net suppression for larger alpha amplitudes due to the assumed amplitude asymmetry of the oscillation. While there is evidence for this hypothesis regarding occipital alpha oscillations, it is less clear for the central sensorimotor mu-alpha rhythm. Probing corticospinal excitability via transcranial magnetic stimulation (TMS) of the primary motor cortex and the measurement of motor evoked potentials (MEP), we have previously demonstrated that corticospinal excitability is modulated by both amplitude and phase of the sensorimotor mu-alpha rhythm. However, the direction of this modulation, its proposed asymmetry, and its underlying mechanisms remained unclear. We therefore used real-time EEG-triggered single- and paired-pulse TMS in healthy humans of both sexes to assess corticospinal excitability and GABA-A-receptor mediated short-latency intra-cortical inhibition (SICI) at rest during spontaneous high amplitude mu-alpha waves at different phase angles (peaks, troughs, rising and falling flanks) and compared them to periods of low amplitude (desynchronized) mu-alpha. MEP amplitude was facilitated during troughs and rising flanks, but no phasic suppression was observed at any time, nor any modulation of SICI. These results are best compatible with sensorimotor mu-alpha reflecting asymmetric pulsed facilitation but not pulsed inhibition of motor cortical excitability. The asymmetric excitability with respect to rising and falling flanks of the mu-alpha cycle further reveals that voltage differences alone cannot explain the impact of phase.
Significance statement

The pulsed inhibition hypothesis, assuming alpha oscillations to actively inhibit neuronal processing in a phasic manner, is highly influential and has substantially shaped our understanding of alpha oscillations. However, some of its basic assumptions, in particular its asymmetry and inhibitory nature, have hardly been tested directly. Here we explicitly investigated the asymmetry of modulation and its direction for the human sensorimotor mu-alpha rhythm. Notably, we found clear evidence of pulsed facilitation but not inhibition in the human motor cortex, challenging the generalizability of the pulsed inhibition hypothesis and advising caution when interpreting sensorimotor mu-alpha changes in the sensorimotor system. This study also demonstrates how specific assumptions about the neurophysiological underpinnings of cortical oscillations can be experimentally tested non-invasively in humans.
Introduction

Alpha (8-14 Hz) oscillations are the most prominent rhythm observable during wakefulness in the human scalp electroencephalogram (EEG) (Berger, 1929). They are strongly expressed in all sensory regions (Haegens et al., 2015) and presumably involve both thalamic and cortical generators (Lopes da Silva et al., 1980; Vijayan and Kopell, 2012). According to the pulsed inhibition hypothesis (Klimesch et al., 2007; Jensen and Mazaheri, 2010), alpha cycles reflect bouts of inhibition, rhythmically suppressing bottom-up processing of sensory input, restricting associated gamma (40-100 Hz) oscillations (Tallon-Baudry and Bertrand, 1999) to interleaved periods of disinhibition. Importantly, alpha has been proposed to be asymmetric (Mazaheri and Jensen, 2008; Schalk, 2015), with larger amplitudes reflecting stronger inhibition and shortened periods of disinhibition, resulting in fewer gamma cycles and reduced information processing capacity (Jensen et al., 2014).

Indeed, alpha power and phase modulate gamma oscillations in human visual (Osipova et al., 2008) and motor cortex (Yanagisawa et al., 2012), and neural spiking in monkey motor and somatosensory cortex (Haegens et al., 2011). Also visual cortical excitability, indexed by perceptual performance or the probability of transcranial magnetic stimulation (TMS) to induce phosphenes has been inversely linked to occipital alpha power (Thut et al., 2006; Romei et al., 2008b; Romei et al., 2008a; van Dijk et al., 2008) and is modulated by its phase (Busch et al., 2009; Mathewson et al., 2009; Dugue et al., 2011). Accordingly, transcranial alternating current stimulation (TACS) in the alpha range phasically suppressed visual stimulus-induced gamma power in concurrent magnetoencephalography (MEG) recordings, with the extent of phasic suppression predicting the accompanying decrease in visual detection performance (Herring et al., 2019).

For the sensorimotor mu-alpha rhythm, the link to cortical excitability is less consistent. In primary somatosensory cortex (S1), both negative linear (Jones et al., 2010; Anderson and Ding, 2011) and inverted u-shape relationships (Linkenkaer-Hansen et al., 2004; Zhang and Ding, 2010; Anderson and Ding, 2011; Ai and Ro, 2014) have been observed between pre-stimulus mu-alpha
power and tactile perception or somatosensory evoked potentials. In the primary motor cortex (M1), earlier studies either observed negative relationships in small samples (Zarkowski et al., 2006; Lepage et al., 2008; Sauseng et al., 2009), or no relationship at all (reviewed in Madsen et al., 2019), whereas more recent studies suggest a positive linear relationship with motor evoked potential (MEP) amplitude (Hussain et al., 2018; Thies et al., 2018; Ogata et al., 2019). Our group previously observed mu-alpha phase to modulate corticospinal excitability, with larger MEPs evoked during troughs compared to peaks of mu-alpha waves (Schaworonkow et al., 2018; Stefanou et al., 2018; Zrenner et al., 2018; Schaworonkow et al., 2019). However, it remained unknown (i) whether this phasic modulation reflects asymmetric pulsed inhibition, asymmetric pulsed facilitation, or a symmetric combination of both (Figure 1A), and (ii) whether cortical excitability depends on phase or merely the instantaneous voltage amplitude (Schalk, 2015). To answer these questions, we employed real-time EEG-triggered single- and paired-pulse TMS to measure corticospinal excitability (MEP amplitude) and GABA-A-receptor mediated short-latency intracortical inhibition (SICI) (Kujirai et al., 1993) at rest (i.e., with relaxed muscles and in absence of any motor task) at four different phase angles (peak, falling flank, trough, rising flank) of a robustly expressed (i.e., high power) spontaneous mu-alpha rhythm and compared them to a baseline state of spontaneously desynchronized (i.e., low power) mu-alpha at random phase when the rhythm is virtually absent (Figure 1B). If mu-alpha reflects asymmetric pulsed inhibition, its less excitable peaks should reflect inhibition and attenuate MEPs relative to low power periods and troughs alike, possibly accompanied by a rhythmic increase of SICI. If mu-alpha reflects asymmetric pulsed facilitation instead, MEPs should be increased during troughs relative to low power periods and peaks, and no modulation of SICI should be observed. A symmetric scenario would result in some combination of the above. Further, if phase per se matters irrespective of voltage amplitude, excitability may differ for rising and falling flanks despite comparable absolute voltages.

< Figure 1 about here >
Materials and Methods

Subjects. Twenty-three (N = 23) healthy, right-handed volunteers (26.1 ± 5.8 years; 11 females), who were free of medication and had no neurologic or psychiatric history or any contraindications against TMS (Rossi et al., 2011), participated after providing written informed consent. The study protocol conformed to the Declaration of Helsinki and was approved by the local ethics committee of the University Hospital Tübingen. Subjects were recruited based on the following inclusion criteria: (i) a clear mu-alpha frequency peak (i.e., a distinct peak between 8 and 14 Hz in the power spectrum with an amplitude \( \geq 2 \times \) the background 1/f noise, as visually identified in the eyes-open EEG resting-state power spectrum; see below) to ensure sufficient signal-to-noise-ratio for real-time power and phase targeting; (ii) the existence of a TMS motor hot spot allowing to consistently evoke MEPs with a resting motor threshold (RMT) \( \leq 75\% \) maximum stimulator output (MSO) to ensure sufficiently long stimulation periods without coil overheating. In total, 23 of 36 screened subjects fulfilled these criteria, were included, and completed the study.

Procedures. Subjects participated in a single session, consisting of several preparatory measures and the main experiment. Preparatory measures (see below for details) included: mounting of EEG and EMG electrodes, arrangements for TMS neuronavigation, EEG resting-state recording (3 min) for calibration of real-time detection criteria, motor hot spot search, as well as automated determination of resting motor threshold (RMT), stimulation intensity (SI) producing MEPs of 1 mV peak-to-peak amplitude, and CS intensity producing 50% of maximal SICI based on a SICI curve with varying CS intensities. During the main experiment, both single-pulse TMS (TS alone) and paired-pulse TMS (CS + TS at 2 ms inter-stimulus interval, ISI) was delivered, assessing corticospinal excitability and GABA-A-receptor mediated intracortical inhibition respectively. TMS was automatically triggered in real-time (see below for details) to target 5 different mu-alpha states: (1) low mu-alpha power periods (i.e., 1-20 % of the individual mu-alpha power distribution) at random phase, or high mu-alpha power periods (i.e., 81-100 % of the individual mu-alpha power distribution) at four different phase angles of the mu-alpha rhythm, i.e., either (2) the peak (0°), (3) the falling
flank (90°), (4) the trough (180°), or the rising flank (270°). These 10 different experimental conditions (5 mu-alpha rhythm states x 2 trial types) were pseudorandomly intermingled (by concatenating permutations of the 10 conditions). The experiment was split into multiple blocks, separated by ~10 min breaks to allow for coil cooling and relaxation time for the participant. To account for slow power drifts with time on task (Benwell et al., 2018), in the first 16 subjects, the break was also used to perform a re-calibration of mu-alpha power thresholds (see below) based on 3 min resting-state EEG recordings, whereas in the last 7 subjects a continuous re-calibration was implemented in form of a sliding distribution of mu-alpha power values based on the last 60 s of clean data (excluding 1.5 s intervals post-TMS), as this procedure had been shown in the meanwhile to prevent unnecessarily long inter-trial intervals (ITI) that occur when the algorithm waits for the power criterion to be met in the face of slow mu-alpha power fluctuations (Thies et al., 2018). This resulted on average in slightly shorter and more homogenous ITIs for the last seven compared to the first 16 subjects (3.7 ± 0.7 s vs. 4.6 ± 1.1 s), but did not produce any differences between experimental conditions. Block duration varied based on individual stimulation intensity (i.e., max. time until coil required cooling) and individual endogenous mu-alpha rhythm fluctuations (i.e., actual average ITI due to EEG-triggered TMS), resulting on average in 4.6 ± 1.1 blocks (M ± SD) with 15.2 ± 4.0 min duration and a total number of 95.3 ± 13.1 trials (min: 70, max 123) acquired per condition.

**EEG recordings.** 64-channel EEG via extra-flat TMS-compatible sintered Ag/AgCl electrodes (Multitrodes, EasyCap, Germany) and 2-channel EMG were recorded in DC mode with 1000 Hz anti-aliasing low-pass filter and digitized at 5 kHz using a TMS-compatible 24-bit amplifier (NeurOne Tesla with Digital-Out Option, Bittium, Finland). EMG was recorded from the relaxed right first dorsal interosseus (FDI) muscle in belly-tendon montage via a bipolar channel of the same amplifier.

**Transcranial magnetic stimulation (TMS).** TMS was applied to the left M1 via four Magstim 200² stimulators, connected to a single 70 mm figure-of-eight coil via the Magstim 4-into-1 module.
(Magstim Ltd, UK) to allow paired-pulses with 2 ms inter-stimulus interval (ISI) and inter-trial intervals (ITI) below 4 s (recharge time of a single Magstim 200² unit). Coil position was determined to produce consistent MEPs in the target muscle and was maintained using neuronavigation (Localite GmbH, Germany). Monophasic stimuli induced a posterolateral-to-anteromedial current in the brain tissue. Stimulation intensity (SI) for the suprathreshold test stimulus (TS) was set to elicit MEP amplitudes around 1 mV (SI1mV: 60.3 ± 10.8 % MSO), and SI for the subthreshold conditioning stimulus (CS) 2.0 ms earlier was set to produce 50 % of maximal possible SICI (31.5 ± 5.6 % MSO or 65.6 ± 9.1 % RMT) as determined from the SICI curve (see below) to allow a bidirectional modulation of SICI by the mu-alpha rhythm, while preventing floor or ceiling effects.

EEG resting-state recording. Resting-state EEG was recorded for 3 min with subjects having their eyes open and fixating a crosshair in ~2 m distance as well as keeping their muscles relaxed. Power spectra were calculated by a Hanning-windowed fast Fourier transform (FFT) for consecutive, non-overlapping 1 s data segments, and individual mu-alpha frequency was determined as frequency bin of maximal power in the 8-14 Hz range of the 1/f corrected power spectrum. Further, individual power thresholds for low and high mu-alpha power conditions were determined as the 20 % and 81 % percentile, respectively, from the individual distribution of mu-alpha power values during the 3 min recording.

TMS threshold hunting. Resting motor threshold (RMT) and stimulation intensity inducing approx. 1 mV MEPs on average (SI1mV) were determined using a fully automated adaptation of the Simple Adaptive Parameter Estimation by Sequential Testing (SA-PEST) procedure (Taylor et al., 1983; Awiszus, 2003; Borckardt et al., 2006), which we implemented in MATLAB using our real-time EEG/EMG system to read out the MEP response to the last TMS pulse and adjust the SI for the next pulse accordingly to reach a fluctuating equilibrium with half of the MEPs being smaller or larger than the target value, respectively (i.e., 0.05 mV for RMT and 1 mV for SI1mV). After a fixed number of 40 trials, SI was averaged over the last 20 as an estimate of the respective threshold.
SICI curve. SICI, calculated as ratio of the MEP evoked by CS+TS relative to the TS alone, was calculated for 10 different CS intensities (ranging from 45% to 90% MSO in steps of 5% with a fixed TS intensity at 11mV) intermingled in pseudorandomized order with 20 trials per CS intensity and 20 trials of the TS alone. Based on the SICI curve interpolated from all these intensities, the CS intensity was then determined that caused approx. 50% of the maximal possible inhibition in a given individual.

Real-time EEG-TMS. The real-time EEG-TMS system is described elsewhere in detail (Zrenner et al., 2018). Briefly, a Simulink Real-Time (R2016a, Mathworks) model processed the EEG data at 1 kHz and triggered TMS whenever the respective power and phase criteria were met (see Figure 2 for a schematic overview of the real-time processing pipeline). Real-time EEG processing involved: (1) reading in digitized data of 64 EEG- and 2 EMG-channels from the NeurOne system, (2) downsampling to 1 kHz, (3) buffering the last 512 ms data with a sliding window, (4) spatial filtering with a C3-centered Hjorth-montage (C3 – mean(CP1, CP5, FC1, FC5)) (Hjorth, 1975) to create a single virtual channel; and for power targeting: (5) calculating a Hanning-windowed FFT of the last 512 ms sliding data segment, (6) extracting the frequency bin including individual mu-alpha peak frequency (10.9 ± 1.1 Hz M±SD), (7) comparing the current mu-alpha power value to the power criteria targeted in the current trial (with power percentiles determined either from the resting-state calibration preceding the current run (first 16 subjects) or from a sliding distribution of mu-alpha power values (last 7 subjects), see details below); and for phase targeting: (8) band-pass filtering the last 512 ms sliding data segment of the raw C3-Hjorth signal by a two-pass (zero-phase) finite impulse response filter (FIR) filter with order 128 and a pass-band of the individual mu-alpha frequency ± 2 Hz, (9) removing the 64 ms corrupted by filter edge artefacts on each side of the buffer, (10) forward predicting the signal based on the remaining 384 ms by an autoregressive model (Yule-Walker, order 30) for 128 ms (McFarland and Wolpaw, 2008; Chen et al., 2013), thus providing ± 64 ms around “time zero” (i.e., “now”), (11) determining whether the data point at time zero is a maximum turning point (i.e., a peak), a minimum turning point (i.e., a trough), a negative-to-positive zero crossing (i.e.,
a rising flank), or a positive-to-negative zero crossing (i.e., a falling flank), (12) comparing the current mu-alpha phase to the phase criterion targeted in the current trial; and eventually: (13) immediately triggering either a single or a paired TMS-pulse (depending on the current trial type) if both the current power and phase criteria are met for the data point at time zero. Scalp-to-Simulink data transmission delay was ~3 ms (no jitter), and processing time per real-time cycle and TMS trigger delays accumulated to ~1 ms; including a slight Simulink-to-MagStim trigger delay TMS thus was applied with an average delay of ~4.5 ms. A minimal ITI of 3 s was maintained to avoid corruption of power or phase estimates by TMS-related brain responses or artifacts from the previous trial.

< Figure 2 about here >

**Offline EEG analysis.** Post-hoc offline-analyses were only performed to validate detection performance of the real-time EEG analyses. Pre-TMS EEG data was processed offline, using the FieldTrip toolbox (Oostenveld et al., 2011) and custom MATLAB code (MathWorks, USA), to verify that TMS was correctly delivered to the intended mu-alpha states. EEG data was segmented (-1.5 to 1 s relative to TMS), baseline corrected (-0.502 to -0.002 s, avoiding the TMS pulse artifact), and re-referenced to the common average of all EEG electrodes. A virtual channel was added, representing the C3-centered Hjorth-montage (C3 – mean(CP1, CP5, FC1, FC5)) (Hjorth, 1975). Independent component analysis (ICA) was conducted on pre-TMS data segments (-1.002 to -0.002), downsampled to 1 kHz, to identify components reflecting eye movement artifacts and muscle noise based on their spatial topography, spectral profile, as well as their temporal profiles within and across trials (Chaumon et al., 2015). Subsequently, the same unmixing matrix was applied to the original data, previously identified bad components were removed (on average 2.1 ± 0.9 eye movement components and 3.7 ± 2.3 muscle components per subject), and data were projected back to channel space. Subsequently, semiautomatic artifact detection was used to reject trials with either EMG pre-innervation (amplitude > 50 μV in the 80-140 Hz band-pass filtered EMG signal) or EEG artifacts in C3-Hjorth (z-normalized signal > 5 SDs in the 1 Hz high-pass filtered EEG signal) in the pre-TMS period (on average 3.67 ± 1.51 trials per condition were rejected per subject). Although ITI
(4.33 ± 1.08 s, mean ± SD) did not differ significantly at the group level, neither between phase conditions (p > 0.2) nor between single and paired-pulse trials (p > 0.7), conditions were stratified per subject with respect to ITI to exclude any possible confound of MEP amplitude by variations in ITI (Julkunen et al., 2012; Vaseghi et al., 2015). Single-subject stratification iteratively removed trials with the longest ITI from conditions with the longest average ITI until a rmANOVA of ITI across conditions reached a p-value ≥ 0.2 (Thies et al., 2018). On average, 73.6 ± 1.9 trials remained per condition after bad trial rejection and stratification. To demonstrate power-specificity, power spectra were calculated per trial using a Hanning-windowed FFT of the pre-TMS interval (-0.502 to -0.002 s), zero-padded to 1 s, with a frequency resolution of 1 Hz, ranging from 1 to 35 Hz, and spectra were averaged per condition across trials and afterwards across subjects. To show the frequency-specificity to the targeted mu-alpha power, time-frequency representations (TFR) were calculated for the pre-TMS, for a time period from -1.5 to 1 s, with the post-TMS period being replaced by zeros to prevent any TMS-related responses and artifacts of the post-TMS period from corrupting power estimates in the pre-TMS period. We applied Welch’s method using a moving Hanning-windowed FFT with a dynamic window length of 3 cycles of a given frequency, a step size of 20 ms, and a frequency resolution of 1 Hz, ranging from 1 to 35 Hz. Since TMS was delivered in a mu-alpha power- and phase-triggered fashion, there was no unbiased baseline period preceding the TMS-pulses to allow commonly used normalization as relative change from baseline. TFRs for each subject were therefore z-normalized per condition with respect to the average across all conditions before calculating grand averages across subjects. To show topographical specificity of the targeted mu-alpha power, the topographical distribution of z-normalized pre-TMS mu-alpha power values (as extracted from the individual mu-alpha peak frequency bin and averaged across the -0.3 to -0.1 s time bins of the TFR) was plotted per condition. To illustrate phase-specificity, pre-TMS time-series were averaged across trials per subject and condition. Time-series were converted to phase-angle (in radians) according to the individual mu-alpha peak frequency before averaging across subjects to account for inter-individual differences in mu-alpha frequency and prevent phase-cancelation when averaging across
subjects. Average phase of TMS application was quantified per condition and subject. Since no detected target states were left unstimulated in order to maximize trial numbers, the phase at which TMS was actually applied could not be directly calculated due to signal corruption by TMS-related artifacts and evoked potentials. As second best alternative, phase was thus estimated for the uncorrupted time point exactly one individual mu-alpha cycle earlier. To increase precision, individual mu-alpha period was not determined from the initial resting-state power spectrum, but from the main experiment as the average inter-peak (and inter-trough) interval from the last three mu-alpha cycles preceding the TMS pulse (10.8 ± 1.1 Hz M±SD; absolute deviation from initial peak frequency estimation was 0.4 ± 0.4 Hz). Phase estimates revealed a delay corresponding to ~4.5 ms, attributable to technical factors (see Methods) which have been taken into account in newer versions of the real-time algorithm.

**Offline EMG analysis.** MEP peak-to-peak amplitudes from all remaining trials (73.6 ± 1.9 per condition, see above) were normalized block-wise as percent change from block average (across all conditions) and then averaged across blocks to take slow drifts in corticospinal excitability across blocks into account (Thies et al., 2018; Zrenner et al., 2018). SICI was calculated per mu-alpha rhythm state as ratio of the MEP evoked by paired-pulse TMS relative to single-pulse TMS, and was additionally normalized per subject as percentage of the maximal inducible SICI (from the SICI curve).

**Experimental Design and Statistical Analysis.** The experiment consisted of a single session per subject (N = 23, 11/12 female/male). The independent variable was the targeted *mu-alpha state*, realized as a within-subject factor with the following five power/phase combinations as levels: low/random, high/peak, high/rising, high/trough, and high/falling. The two dependent variables were (i) corticospinal excitability as indexed by MEP amplitude and (ii) GABA-A-receptor mediated inhibition as indexed by the 2 ms short intracortical inhibition (SICI) of MEP amplitudes. For both dependent variables, one-way repeated-measures ANOVAs were conducted with post-hoc paired t-test where applicable. Statistical analyses were conducted using MATLAB (functions RMAOV1 and...
ttest). A p-value of p < 0.05 was considered significant. Effect sizes for ANOVA ($\eta_p^2$, partial eta squared) and t-tests (Cohen’s $d_{av}$, based on the averaged SD) are provided (Lakens, 2013). In addition, we report the Bayes Factor, calculated using the JASP statistical software package (JASP Team, jasp-stats.org), for non-significant tests as BF$_{01}$ to quantify strength of evidence supporting the null-hypothesis (H0) and for significant tests as BF$_{10}$ (i.e. $1/BF_{01}$) to quantify strength of evidence supporting the alternative hypothesis (H1). According to Jeffreys (1961), a Bayes Factor of 1-3 reflects ‘anecdotal evidence’, 3-10 ‘substantial evidence’, 10-30 ‘strong evidence’, 30-100 ‘very strong evidence’, and >100 ‘decisive evidence’ for the H0 (BF$_{01}$) and H1 (BF$_{10}$), respectively. Data are reported as mean ± standard error of the mean (M ± SEM) if not stated otherwise. EEG data was merely analyzed as manipulation check, i.e., to demonstrate successful mu-alpha power and phase targeting.

Results

**Mu-alpha rhythm phasically facilitates MEP corticospinal excitability but not intracortical inhibition**

MEP amplitude was modulated as a function of mu-alpha power and phase ($F_{4,88} = 4.71$, $p = 0.002$, $\eta_p^2 = 0.18$, BF$_{10} = 107.92$; **Figure 3A; Table 1**). When averaged across phase conditions, MEPs triggered during periods of high mu-alpha power were larger than those obtained at random phase during low mu-alpha power ($t_{22} = 2.25$, $p = 0.03$, Cohen’s $d_{av} = 0.80$, BF$_{10} = 1.76$). Taking phase into account, MEPs were larger during the mu-alpha trough and rising flank than during the peak and falling flank (trough vs. peak: $t_{22} = 2.97$, $p = 0.008$, $d_{av} = 0.99$, BF$_{10} = 6.09$; trough vs. falling: $t_{22} = 2.83$, $p = 0.009$, $d_{av} = 0.85$, BF$_{10} = 5.04$; rising vs. peak: $t_{22} = 2.19$, $p = 0.04$, $d_{av} = 0.78$, BF$_{10} = 1.59$; with a trend for rising vs. falling: $t_{22} = 1.8$, $p = 0.08$, $d_{av} = 0.64$, BF$_{10} = 0.88$), but did not differ between trough and rising flank ($p > 0.4$, $d_{av} = 0.25$, BF$_{01} = 3.37$) or between peak and falling flank ($p > 0.6$, $d_{av} = 0.11$, BF$_{01} = 4.26$). Importantly, MEPs during high power trials were only increased with respect to low power trials when obtained during the trough and rising flank ($t_{22} = 2.94$, $p = 0.008$,
d_{av} = 1.08, BF_{10} = 6.18; rising: t_{22} = 2.25, p = 0.03, d_{av} = 0.90, BF_{10} = 6.71), but not during the peak and falling flank (peak: p > 0.4, d_{av} = 0.26, BF_{01} = 3.37; falling: p > 0.3, d_{av} = 0.34, BF_{01} = 3.04). In contrast, while being clearly expressed at all phase angles (Table 1), SICI did not differ significantly as a function of mu-alpha power or phase (F_{4.88} = 1.104, p = 0.36, \eta^2_p = 0.05, BF_{01} = 7.076; Figure 3B). MEP amplitudes were thus rhythmically facilitated during the trough and rising flank of high amplitude mu-alpha oscillations, while remaining comparable to periods of low mu-alpha amplitude during high amplitude peaks and falling flanks. This modulation was not mediated by variations in intracortical inhibition.

Real-time EEG-triggered TMS successfully targeted mu-alpha power and phase conditions

To ensure that TMS was correctly delivered to the intended mu-alpha states (Figure 4) and that no systematic confounds occurred, we performed additional offline analyses of the pre-TMS time period with respect to power spectra (Figure 4A), time-frequency representations (Figure 4B), topographical distribution of mu-alpha power (Figure 4C), time-locked EEG signal (Figure 4D) and estimated phase of actual TMS delivery (Figure 4E). These analyses revealed that on average power and phase were targeted as intended (with a technical delay of ~4.5 ms, corresponding to ~18° phase angle or 5% of the oscillatory cycle, see Methods), consistently across subjects (mean vector length across subjects was 0.96 for all phase targeted conditions and 0.15 for the random phase condition), and that neither adjacent frequencies nor oscillatory activity from other sources (such as occipital alpha) confounded the experimental variation of power and phase conditions.
We report evidence that the sensorimotor mu-alpha rhythm reflects asymmetric pulsed facilitation, rather than inhibition, of corticospinal excitability. Relative to a desynchronized, low power, mu-alpha state, MEP amplitudes were facilitated during high power troughs and rising flanks of the oscillation, but were not altered during peaks and falling flanks. Accordingly, we found no evidence for a link between GABA-A-receptor mediated intracortical inhibition and mu-alpha power or phase.

These results bear immediate conceptual consequences. Firstly, the observed pulsed facilitation of the motor cortex questions the universality of the *pulsed inhibition hypothesis* (Klimesch et al., 2007; Jensen and Mazaheri, 2010) beyond the realm of primary sensory regions. Secondly, the excitability difference between mu-alpha rising and falling flanks of comparable voltage amplitude challenges the *function-through-biased-oscillations hypothesis* (Schalk, 2015), which assumes that instantaneous voltage amplitude, rather the power or phase of an oscillation reflects cortical excitability.

**Mu-alpha rhythmically facilitates corticospinal excitability**

Mu-alpha troughs but not peaks were associated with facilitation of corticospinal excitability relative to periods of low mu-alpha power, but at no phase a relative inhibition could be observed. The resulting net facilitation of corticospinal excitability during the asymmetric mu-alpha oscillation corroborates recent findings of a weak positive relationship between mu-alpha power and MEP amplitude (Hussain et al., 2018; Thies et al., 2018; Ogata et al., 2019). While the larger excitability for troughs than peaks replicates previous findings (Schaworonkow et al., 2018; Stefanou et al., 2018; Zrenner et al., 2018; Schaworonkow et al., 2019), periods of spontaneous mu-alpha desynchronization (low power trials) had not yet been considered as baseline to determine the direction of phasic modulation. The only other study taking pre-TMS mu-alpha power into account used post-hoc trial sorting of peaks and troughs and a trial-by-trial linear mixed-effects model to include continuous power values (Hussain et al., 2018). Notably, no main effect of phase but only an
interaction with power was observed, driven by a positive relationship between mu-alpha power and
MEPs during troughs but not peaks. If mu-alpha peaks simply reflect the absence of pulsed
facilitation, but not active inhibition, as our results suggest, the amplitude of those peaks should
indeed not matter, whereas the amplitude of troughs would reflect the degree of pulsed facilitation
(Figure 1A).

Mu-alpha does not modulate GABA-A-receptor mediated intracortical inhibition

Beside the lack of a phasic decrease in corticospinal excitability relative to desynchronized periods,
there was also no evidence for a phasic modulation of GABA-A-receptor mediated inhibition as
indexed by SICI (Kujirai et al., 1993; Di Lazzaro and Ziemann, 2013). SICI presumably reflects the feed-
forward inhibition of corticospinal cells via activation of inhibitory interneurons by the first
subthreshold stimulus, as those interneurons likely have a lower excitation threshold (Di Lazzaro and
Ziemann, 2013). Given constant excitability of corticospinal neurons, SICI should thus change
whenever either the excitability of those inhibitory interneurons changes or the efficacy of their
GABA-A-ergic transmission (Ilic et al., 2002). However, since corticospinal excitability was phasically
modulated, comparable levels of relative SICI (% suppression of MEP) indicate variations in absolute
inhibition (mV MEP amplitude). The excitability of both pyramidal cells and inhibitory interneurons
thus seems proportionally facilitated during the mu-alpha trough, maintaining excitation-inhibition
balance (EIB).

Relevance of phase over instantaneous voltage amplitude

Despite similar absolute voltages at the zero crossings, corticospinal excitability was increased only
during rising but not during falling flanks. Although the direct comparison between both flanks
revealed a statistical trend only, these findings are not in support of the function-through-biased-
oscillations hypothesis (Schalk, 2015), which argues that the absolute voltage and not phase per se
explains phasic excitability changes. Our findings suggest that there is likely more to the oscillatory phase than absolute voltage. Since we exclusively used zero phase shift band-pass filters during real-time detection, and calculated post-hoc time-locked averages from the unfiltered raw signal, it is unlikely that the observed flank asymmetry was spurious produced by the asymmetric arch-like shape of the mu-rhythm (Cole and Voytek, 2017), which is characterized by different peak and trough duration but to our knowledge no particular asymmetry regarding its sharp rising and falling flanks. We can only speculate that the increase in corticospinal excitability during the rising flank may reflect a transient continuation of the neurophysiological process responsible for the facilitation during the trough itself.

**Potential mechanisms mediating mu-alpha related pulsed facilitation of corticospinal excitability**

Given the predictions of the pulsed inhibition hypothesis (Klimesch et al., 2007; Jensen and Mazaheri, 2010), our results may appear controversial at first. However, in the primary somatosensory cortex (S1), the relationship between mu-alpha rhythm power and cortical excitability seems to be more complex than in the visual system (see Introduction), and there may be no uniform phase-excitability relationship within the sensorimotor system. The origin of the sensorimotor mu-alpha rhythm is presumably rather postcentral (S1), as opposed to the more precentral (M1) sensorimotor mu-beta rhythm (Salmelin and Hari, 1994; Ritter et al., 2009; Stolk et al., 2019), and Stolk et al. (2019) have recently demonstrated in electrocorticographical (ECoG) recordings that the two rhythms are driven by different neuronal populations and are functionally segregated during movement selection. They even found that individual waves travel in opposite direction across the sensorimotor cortex, with alpha waves travelling from S1 to M1 and beta waves from M1 to S1 (Stolk et al., 2019). These traveling mu-alpha waves may in fact explain the considerable posterior-to-anterior mu-alpha phase shifts that are sometimes observable in the surface EEG, complicating the optimization of spatial filters for target signal extraction (Schaworonkow et al., 2018). Since the C3-Hjorth montage we used is likely more sensitive to radial sources from the crown of the postcentral gyrus (S1) than tangential
sources from the anterior wall of the precentral sulcus (M1), our mu-alpha target signal may originate from a different neuronal population (in S1) than the one whose excitability we probed with MEPs (in M1). Given that the tight sensory-to-motor interconnections involve large amounts of feedforward inhibition (Murray and Keller, 2011), it is possible that mu-alpha causes pulsed inhibition in S1 (as predicted by the pulsed inhibition hypothesis) but a rhythmic release of M1 from a general sensory-to-motor inhibition. Future studies should explicitly investigate the role of S1-M1 interactions for mu-alpha power and phase effects.

It is also possible that the mu-alpha related pulsed facilitation of corticospinal excitability observed in this experiment only holds for the case of spontaneous mu-alpha oscillations at rest, whereas relative inhibition may be observable in MEP and SICI during mu-alpha de- and re-synchronization in the context of motor tasks. Interestingly, such a state-dependent flip of effect direction has also been observed for TACS of the motor cortex at beta frequency (for a recent meta-analysis see Wischnewski et al., 2019), which paradoxically increased corticospinal excitability during rest (Feurra et al., 2011; Feurra et al., 2013) but not during motor imagery (Feurra et al., 2013), while having the expected inhibitory or akinetic effect on motor performance (Pogosyan et al., 2009; Joundi et al., 2012). Then again, TACS at alpha frequency facilitated corticospinal excitability when applied during motor imagery rather than rest (Feurra et al., 2013). It has also been argued that beta-TACS induced synchronization of the relevant neuron populations in M1 may facilitate the recruitment of corticospinal neurons by the TMS pulse, synchronize the respective corticospinal volleys, and thereby increase MEP amplitude (Feurra et al., 2011). It is principally possible that also cortical synchronization by spontaneous alpha oscillations facilitates MEP amplitude via a similar mechanism.

Sensorimotor mu-alpha and mu-beta rhythms are physiologically and functionally separate rhythms that fluctuate independently (McFarland et al., 2000; Fransen et al., 2016; Stolk et al., 2019), and while beta was not investigated during this mu-alpha focused investigation, its precentral origin and clear motor task-related modulation make it a strong candidate for exerting power- and phase-
specific effects on corticospinal excitability. However, in our previous studies we could not identify any such effects of beta by means of post-hoc analyses, neither with respect to phase (Zrenner et al., 2018) nor power (Thies et al., 2018), and real-time beta-triggered TMS may be needed to answer that question in the future. Interestingly, Stolk et al. (2019) found the 1/f slope in the power spectrum, a putative power-spectral index of synaptic excitation-inhibition balance (EIB) (Gao et al., 2017), to indicate effector-specific and spatially focal shifts in EIB towards excitation during mu-beta power decreases in a motor imagery task, whereas the link between mu-alpha power and inhibition was spatially unspecific. A (potentially task-specific) dissociation between the EIB profile of mu-alpha and -beta oscillations, and their putative impact on corticospinal excitability, as indexed by the MEP, warrants future investigation.

Conflicting evidence regarding the impact of mu-alpha power and phase on corticospinal excitability

Previous publications have either revealed no relationship of mu-alpha power with corticospinal excitability (Lepage et al., 2008; Berger et al., 2014; Keil et al., 2014; Schulz et al., 2014; Iscan et al., 2016; Madsen et al., 2019), a negative relationship for near-threshold stimulation intensities in very small samples (Zarkowski et al., 2006; Sauseng et al., 2009), or, more recently, a weak positive relationship (Hussain et al., 2018; Thies et al., 2018; Ogata et al., 2019). The impact of mu-alpha phase on corticospinal excitability was larger during troughs than peaks in all studies from our group (Schaworonkow et al., 2018; Stefanou et al., 2018; Zrenner et al., 2018; Schaworonkow et al., 2019), while one recent real-time EEG-triggered TMS study from another group did not observe this phasic modulation (Madsen et al., 2019). It should be noted that the samples from the above cited studies by our group (each with a different research question) partially overlapped. Of the total of N = 53 subjects, 31 subjects participated in a single study, 10 subjects in two studies, 8 subjects in three studies, and 4 subjects in four studies. For the current study, 7 subjects did not participate in any of the other studies, whereas 16 subjects also participated (before or afterwards) in one or more of the
other studies. Importantly, subjects were only included for their good mu-alpha peak in the power spectrum, while we were completely blind with respect to their individual expression of a phase effect. Madsen et al. argued that several previous studies also failed to find a mu-alpha phasic modulation of MEP amplitude (cf. their Table 1). However, the cited studies investigated corticomuscular coherence (van Elswijk et al., 2010; Keil et al., 2014; Schulz et al., 2014) or pre-stimulus power (Iscan et al., 2016), rather than mu-alpha phase; and van Elswijk et al. (2010) found phase effects in EMG (though not EEG) even during isotonic contraction, and Berger et al. (2014) reported EEG-MEP phase-amplitude correlations also in the mu-alpha range during rest. There are several issues, already mentioned by Madsen et al., that may be relevant for obtaining the observed phase effects. Firstly, we preselected subjects based on the presence of a distinct mu-alpha peak in the C3-Hjorth power spectrum (here ~64% of the screened subjects were included, but, importantly, no subject was removed thereafter). While such an inclusion criterion may reduce generalizability, it is necessary to ensure the correct implementation of the independent variable (i.e., mu-alpha phase). Without the presence of a clear oscillation, the most perfect detection algorithm will accurately target the meaningless phase of band-pass filtered 1/f noise (even for the upper percentiles of individual power values). Secondly, we consistently used C3-Hjorth montages, whereas Madsen et al. projected a dipole with radial orientation from the assumed cortical motor hot spot, potentially resulting in stronger contribution of more anterior sources (cf. their Figure 2). Thirdly, their inter-trial intervals (ITI) were much longer (mean 11.9 s = 0.08 Hz) than ours (here: mean ± SD, 4.33 ± 1.08 s = 0.23 Hz), and the large MEPs observed after particularly long ITIs (Julkunen et al., 2012) may have occluded the phase effect. Importantly, the irregular stimulation at ~0.23 Hz has unlikely produced an “inhibitory brain state” (Madsen et al., 2019), and our stratification approach ensured equal ITIs across all phase conditions.

Conclusion
Our findings are best explained by a scenario of pulsed facilitation of corticospinal excitability by power and phase of the sensorimotor mu-alpha rhythm, thus questioning whether the pulsed inhibition hypothesis (Klimesch et al., 2007; Jensen and Mazaheri, 2010) generalizes to the sensorimotor cortex and challenging the function-through-biased-oscillations hypothesis (Schalk, 2015). Future studies should test whether the observed pulsed facilitation actually relies on a rhythmic release from default sensory-to-motor inhibition.

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### Table 1. Mean ± 1 SEM for MEP amplitudes and short-latency intracortical inhibition (SICI) per condition: raw MEP amplitudes, normalized MEP amplitudes, SICI, and normalized SICI.

| power/phase | low/random | high/peak | high/falling | high/trough | high/rising |
|-------------|------------|-----------|--------------|-------------|-------------|
| MEP (mV raw) | 1.26 ± 0.10 | 1.31 ± 0.11 | 1.33 ± 0.12 | 1.44 ± 0.13 | 1.39 ± 0.12 |
| MEP (% normalized) | -5.06 ± 2.25 | -2.65 ± 1.60 | -1.74 ± 1.78 | 5.82 ± 1.94 | 3.63 ± 1.74 |
| SICI (% of TS) | -35.28 ± 4.02 | -33.23 ± 3.88 | -35.10 ± 4.01 | -36.39 ± 3.55 | -32.26 ± 4.45 |
| SICI (% of max. SICI) | -47.58 ± 4.72 | -44.67 ± 5.27 | -46.15 ± 5.68 | -50.06 ± 3.90 | -42.96 ± 5.89 |
Figure 1. Scenarios for a rhythmic modulation of corticospinal excitability by the sensorimotor mu-alpha rhythm and illustration of detection criteria for real-time EEG-triggered TMS. (A) Three different possible scenarios of rhythmic modulation of corticospinal excitability by the sensorimotor mu-alpha oscillation: asymmetric pulsed inhibition, producing stronger inhibition with increasing amplitude as predicted by the ‘pulsed inhibition hypothesis’ (top); symmetric pulsed inhibition and facilitation, both stronger with increasing amplitude (middle); or asymmetric pulsed facilitation, producing stronger facilitation with increasing amplitude (bottom). (B) EEG-triggered single-pulse TMS (test stimulus, TS, alone to assess MEPs) and paired-pulse TMS (with preceding conditioning stimulus, CS+TS at 2 ms ISI to assess short-latency intracortical inhibition, SICI) targeting periods of low (1-20 % percentile) and high (80-100 % percentile) mu-alpha power. The low power condition was targeted at random phase, whereas for the high power condition, either peak (0°), falling flank (90°), trough (180°), or rising flank (270°) of the ongoing mu-alpha rhythm were targeted. TS = test stimulus; CS = conditioning stimulus.
Figure 2. Overview of real-time EEG-triggered TMS processing pipeline. See Methods for details.

Abbreviations: IMF = individual mu-alpha frequency; FIR = Finite Impulse Response; FFT = Fast Fourier Transform; TMS = Transcranial Magnetic Stimulation.
Figure 3. MEP amplitude but not SICI is modulated by power and phase of the sensorimotor mu-alpha rhythm. (A) Normalized MEP amplitude (% change from block average across conditions; mean ± 1 SEM) was modulated by both mu-alpha power and phase ($F_{4,88} = 4.71, p = 0.002$). While MEPs for high power peaks and falling flanks did not differ from the low power random phase condition, MEPs during high power troughs and rising flanks were significantly increased relative to both low power trials as well as high power peak and rising flank conditions. Significance of post-hoc comparisons is indicated as follows: # $p < 0.1$, * $p < 0.05$, ** $p < 0.01$. (B) Normalized SICI (ratio of conditioned to unconditioned MEP as % of individual max. SICI); mean ± 1 SEM) is modulated neither by mu-alpha power nor by mu-alpha phase (all $p > 0.3$).
Figure 4. Mu-alpha power- and phase conditions were successfully targeted. (A) Pre-TMS power spectra (FFT) of the C3-Hjorth signal for single- (blue) and paired-pulse trials (red), separately for all power/phase conditions. A clear mu-alpha peak can be observed in all high power conditions but not in the low power condition. (B) Pre-TMS time-frequency representations (TFR) of oscillatory power in the C3-Hjorth signal, calculated separately for single- and paired all power/phase conditions and z-normalized across conditions. TFRs show a modulation of mu-alpha power preceding TMS onset (at 0 ms), with a relative increase for high power trials and a relative decrease for low power trials. Note that the apparent broad-band bursts of oscillatory power (vertical bands) in the high power condition are explained by the fact that each of those trial types was time-locked to a specific phase of the non-sinusoidal mu-alpha oscillation. Also note that the apparent decrease in modulation shortly...
before TMS results from to zero-padding of the post-TMS interval to prevent corruption of pre-TMS
interval by overlapping of the sliding window (length: 3 cycles per frequency) with TMS-related
activity or artifacts. (C) Topographical maps of the z-normalized pre-TMS mu-alpha power
modulation (time window [-0.3 -0.1] from B). The topographies verify that a local power increase
over left sensorimotor cortex was targeted, and estimates were not confounded by the stronger
parieto-occipital alpha oscillation. (D) Time-locked C3-Hjorth signal relative to delivery of TMS (black
vertical line) for single- (blue) and paired-pulse trials (red) with the time-axis transformed to phase
angle (in radians) of the individual mu-alpha peak frequency before averaging across subjects to
prevent phase smearing due to variation in individual mu-alpha frequency. While expectedly no
oscillation is visible in random phase low power trials, TMS was successfully delivered to peaks,
falling flanks, troughs and rising flanks in high power trials. (E) Subject-wise average phase angles on
the unitary circle and resulting mean vectors for estimated stimulation phase for each experimental
condition and separately for single- (blue) and paired-pulse trials (red); due to TMS-related
artifacts/potentials, stimulation phase was estimated for the mu-alpha cycle (see methods for
details). The obvious phase offset of ~18° between targeted and stimulated phase corresponds to
~4.5 ms only, and is entirely owed to technical delays (see methods for details).