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Short-interval and long-interval intracortical inhibition of TMS-evoked EEG potentials

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Abstract

Background: Inhibition in the human motor cortex can be probed by means of paired-pulse transcranial magnetic stimulation (ppTMS) at interstimulus intervals of 2-3 ms (short-interval intracortical inhibition, SICI) or ~100 ms (long-interval intracortical inhibition, LICI). Conventionally, SICI and LICI are recorded as motor evoked potential (MEP) inhibition in the hand muscle. Pharmacological experiments indicate that they are mediated by GABAA and GABAB receptors, respectively.

Objective/Hypothesis: SICI and LICI of TMS-evoked EEG potentials (TEPs) and their pharmacological properties have not been systematically studied. Here, we sought to examine SICI by ppTMS-evoked compared to single-pulse TMS-evoked TEPs, to investigate its pharmacological manipulation and to compare SICI with our previous results on LICI.

Methods: PpTMS-EEG was applied to the left motor cortex in 16 healthy subjects in a randomized, double-blind placebo-controlled crossover design, testing the effects of a single oral dose 20 mg of diazepam, a positive modulator at the GABAA receptor, vs. 50 mg of the GABAB receptor agonist baclofen on SICI of TEPs.

Results: We found significant SICI of the N100 and P180 TEPs prior to drug intake. Diazepam reduced SICI of the N100 TEP, while baclofen enhanced it. Compared to our previous ppTMS-EEG results on LICI, the SICI effects on TEPs, including their drug modulation, were largely analogous.

Conclusions: Findings suggest a similar interaction of paired-pulse effects on TEPs irrespective of the interstimulus interval. Therefore, SICI and LICI as measured with

TEPs cannot be directly derived from SICI and LICI measured with MEPs, but may offer novel insight into paired-pulse responses recorded directly from the brain rather than muscle.

Keywords: Paired-pulse transcranial magnetic stimulation; TMS-evoked EEG potential; short-interval intracortical inhibition; long-interval intracortical inhibition; GABA; diazepam; baclofen

1. Introduction

Paired-pulse transcranial magnetic stimulation (ppTMS) represents a non-invasive approach employed in clinical practice to probe the level of GABAergic inhibition in the human cortex [1-4]. Paired-pulse protocols deliver two consecutive TMS pulses, a conditioning and a test stimulus (CS and TS, respectively), which at given intensities and interstimulus intervals (ISIs), can probe intracortical interneuronal circuits of inhibition and excitation [5]. The stimulation paradigm of short-interval intracortical inhibition (SICI) is assumed to reflect GABA_A receptor (GABA_AR) mediated inhibition, specifically those GABA_AR subtypes bearing the $\alpha 2$ or $\alpha 3$ subunits [6, 7], whereas long-interval intracortical inhibition (LICI) is thought to reflect GABA_B receptor (GABA_BR) mediated inhibition, based on studies that demonstrated a significant LICI increase by the specific GABA_BR agonist baclofen [8, 9]. However, ppTMS measures have so far been assessed with motor evoked potential (MEP) amplitude modulation, i.e. an electromyographic (EMG) readout, that only provides indirect information on brain responses, and effects at subcortical and/or spinal level may contribute to MEP modulation [10].

PpTMS protocols coupled to electroencephalographic recordings (ppTMS-EEG) allow direct assessment of processes of neural inhibition and excitation at the level of motor cortex (M1) [11-16], including the possibility to test non-motor regions as well [15, 17]. The impact of the CS-TS sequence can be quantified by evaluating the amplitude modulation of the TMS-evoked EEG potentials (TEPs) which by pharmacological characterization have been shown to reflect both excitatory and inhibitory neural processes [18-20]. First ppTMS-EEG studies of M1 that have investigated the cortical

correlates of SICl reported inconsistent results: Ferreri and colleagues showed an increase of the N100 and a suppression of P70 and P180 in the stimulated hemisphere [12]. In contrast, no modulation of these potentials was found in the work from Paus and colleagues [11]. A more recent study showed a suppression of the P30, N45 and P60 in the stimulated hemisphere, and the suppression of P30 and P60 correlated significantly with ppTMS-induced MEP inhibition [15]. The authors suggested that these two TEP components may represent a direct relationship with MEP inhibition induced by SICl.

Pharmacology is a powerful tool to draw direct links between specific TEP components and neurotransmission pathways targeted by drugs with specific modes of action [4]. For pharmaco-physiological characterization of SICl of TEPs as measured by ppTMS-EEG, we tested the effects of a single oral dose of 20 mg of diazepam, a positive allosteric modulator at GABAARs, and 50 mg of baclofen, a specific GABABR agonist in a placebo-controlled, double-blinded, pseudo-randomized crossover design in healthy subjects. Finally, we sought to compare the paired-pulse TMS-EEG signatures of SICl of this study and LICl from our previous study [14] to improve our knowledge on ppTMS-EEG as a possible tool to better identify functional brain network abnormalities caused by altered inhibition in disease.

2. Methods

2.1 Subjects

Sixteen healthy male subjects (mean age 25.6 ± 4.5 yrs, age range 19-35 yrs) participated in the study after giving written informed consent. Female participants were

excluded due to menstrual cycle dependent effects on TMS measures of cortical excitability [21]. All subjects were right-handed according to the Edinburgh Handedness Inventory (mean laterality score 75%) [22]. Physical examination was performed before and after each session. In addition, participants were screened for contraindications to TMS [23]. A history of neurological or psychiatric disease, use of CNS active drugs, abuse of any drugs including nicotine and alcohol, or a contraindication to the study medication (baclofen and diazepam) were considered as exclusion criteria. The study was approved by the local Ethics Review Committee of the Medical Faculty of the Eberhard Karls University Tübingen, Germany (protocol number 199/2014BO2), and was conducted in accordance with the latest version of the Declaration of Helsinki.

2.2 Experimental design

The ppTMS protocol for testing SICl was applied during EEG recording before and after the intake of the study drug in a pseudo-randomized, placebo-controlled, double-blind crossover design. Subjects attended three experimental sessions separated by at least one week to rule out carry-over effects. During each study visit, the motor threshold at rest (RMT) was determined and TMS-EEG recordings were performed at baseline (i.e. before drug intake) and 90 min after the administration of a single oral dose of diazepam (20mg, Diazepam-ratiopharm®, ratiopharm GmbH), baclofen (50mg, Lioresal®, Novartis Pharma) or placebo (P-Tabletten, Lichtenstein). Diazepam is a classical benzodiazepine targeting $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ subunit-containing GABAARs, whereas baclofen is a selective GABABR agonist. Drug dosages and the time point of

post-drug measurements were chosen in line with previous experiments indicating a significant modulation of ppTMS-EMG/EEG parameters [8, 9, 14, 18] and according to the known drug pharmacokinetics [24], and prescribed information from Novartis Pharma).

2.3 Stimulation protocols

A figure-of-eight coil (external diameter of each wing, 90 mm) connected to a Magstim 200² magnetic stimulator (Magstim Company, UK) was used to stimulate the left (dominant) M1. The optimal coil location was identified as the position that consistently elicited largest MEPs in the right abductor pollicis brevis (APB) muscle by slightly suprathreshold single-pulse TMS. MEPs were recorded by means of surface EMG using Ag-AgCl cup electrodes in a belly-tendon montage. The EMG signal was band-pass filtered (20 Hz to 2 kHz) and digitized at an A/D rate of 10 kHz (CED Micro 1401, Cambridge Electronic Design, UK). To maintain a constant coil orientation throughout the experiment, the APB hotspot was marked on the EEG cap with a felt pen. RMT was measured following the relative frequency method and defined as the minimum intensity, given as percentage of maximum stimulator output (%MSO), that elicited MEPs > 50 μ V peak-to-peak amplitude in at least five out of ten subsequent trials [23]. SICI was measured according to an established protocol, employing a subthreshold CS at an intensity of 70% RMT applied 2.0 ms before a suprathreshold test stimulus TS at an intensity of 100% RMT. As in our previous study [14], we avoided use of suprathreshold TS intensity in order to limit re-afferent somatosensory feedback, which

can contaminate the EEG signal, in particular the late TEP components [11]. One previous TMS-EMG studies demonstrated significant MEP inhibition with these settings [25].

Single pulses at intensities of 70% RMT (CS-70%) and 100% RMT (TS-100%), and paired pulses (CS-70% RMT followed by TS-100% RMT) were applied (125 trials each) in randomized order every 5 s with 25% variation to limit anticipation of the next trial. Single pulse CS-70% was recorded to correct for CS-70% effects on TS-100% evoked TEPs in the ppTMS trials [14].

Finally, RMT was tested pre- and post-drug administration to assess possible drug-induced changes in RMT and re-adjust stimulation intensity to keep it at 100% RMT.

2.4 TMS-EEG recordings

The EEG signal was obtained from 64 Ag/AgCl electrodes mounted on an elastic cap according to the International 10-20 system (BrainCap-Fast'n Easy 64ch, Brain Products GmbH, Munich, Germany). TEPs were recorded via TMS-compatible EEG amplifiers (BrainAmp DC, Brain Products GmbH). Data were digitized at a sampling frequency of 5 kHz, and electrode impedance was maintained below 5 k Ω during data acquisition. Electrooculography recordings were obtained by placing two additional electrodes outside of the outer canthus and above the right eye. Participants were seated on a comfortable reclining chair. A masking noise was played through earplugs to avoid contamination of TEPs by auditory evoked cortical potentials elicited by the TMS-induced click [26, 27].

In line with our previous study [14], SICI was assessed by subtracting the average single-pulse CS-70% evoked EEG response from the average ppTMS evoked EEG response (paired-pulse corrected). SICI was then calculated by subtracting the average single-pulse TS-100% evoked response from the paired-pulse corrected EEG response, aligned to the TS (for details, please refer to [14]).

2.5 TMS-EMG recordings

To corroborate previous TMS-EMG findings of significant SICI with a CS intensity of 70% RMT, a TS intensity of 100% RMT and an interstimulus interval of 2 ms [28], we tested 10 healthy subjects, right-handed according to Edinburgh Handedness Inventory (5 female; mean age, 32.4 ± 10.0 yrs) applying the same inclusion/exclusion criteria as in the TMS-EEG experiment. The voluntarily relaxed right APB was selected as target muscle. In each subject, we tested 55 TS and 55 CS-TS trials, randomly intermixed, with an intertrial interval of $5 \text{ s} \pm 25\%$.

2.6 Data processing and analysis

Preprocessing and analysis of EEG recordings were performed using BrainVision Analyzer (version 2, BrainProducts GmbH, Munich, Germany), MATLAB 2012b and the Fieldtrip open source MATLAB toolbox (www.ru.nl/fcdonders/fieldtrip/) [29]. EEG data was first re-referenced to the linked mastoids (channels TP9-TP10) and down-sampled to 1 kHz. The continuously recorded EEG time series were segmented each for single-

pulse (TS-100% and CS-70%) and paired-pulse trials between -500 ms to 600 ms post-stimulus. To eliminate TMS ringing artifacts, the EEG signal was linearly interpolated enclosing an epoch of ± 12 ms around the TMS pulse (with respect to TS in the ppTMS trials) [30]. Single trials were then visually inspected to reject those containing large artifacts (e.g., blinks, muscle activity). After data export to MATLAB, the artifact-free EEG signals for single-pulse CS-70% (mean number of trials across subjects $\pm$ SEM before and after diazepam: 114 ± 11 and 108 ± 16 ; baclofen: 108 ± 18 and 107 ± 13 ; placebo: 109 ± 18 and 111 ± 14), single-pulse TS-100% (mean number of trials across subjects before and after diazepam: 109 ± 6 and 97 ± 14 ; baclofen: 103 ± 11 and 107 ± 8 ; placebo: 105 ± 8 and 105 ± 9) and paired-pulse TMS (mean number of trials across subjects before and after diazepam: 103 ± 12 and 97 ± 15 ; baclofen: 102 ± 13 and 103 ± 9 ; placebo: 105 ± 10 and 98 ± 13) were baseline corrected by subtracting the mean EEG signal -500 ms to -100 ms before the application of TMS. Trials were linearly detrended, bandpass filtered between 2 and 80 kHz and a 50 Hz notch filter was applied. Independent component analysis (ICA) decomposition was performed separating the components related to brain signals and artefacts to remove the latter from EEG recordings. Components were visually inspected considering time course, topographical distribution and amplitude allowing removal of residual muscle activity [31] appearing mostly in the fronto-temporal region ipsilateral to the stimulation site, TMS-related artifacts showing exponential decays, and recharge artifacts around 200 ms after the TMS pulse [32-34].

A band-pass filter between 1 and 45 Hz was applied to smooth TEPs. According to the literature [18, 35], five reproducible TEP components (P, positive deflection; N, negative

deflection) were defined depicting non-overlapping time windows of interest (TOI): P25 (15-35 ms), N45 (36-57 ms), P70 (58-80 ms), N100 (81-144 ms) and P180 (145-205 ms), based on the grand average butterfly plot of the single-pulse TMS-evoked EEG responses. TOIs were kept identical irrespective of TMS (single- or paired-pulse) or drug condition.

2.7 Statistics

To evaluate drug effects on RMT, a repeated-measures analysis of variance (rmANOVA) was performed with DRUG (3 levels: diazepam, baclofen, placebo) and TIME (2 levels: pre-drug, post-drug) as within-subject factors. Normal distribution was indicated by the Kolmogorov-Smirnov test, and Mauchly's test showed that sphericity could be assumed.

TEP amplitude modulations were analyzed for single-pulse and paired-pulse conditions. Multiple dependent sample t-tests were conducted separately for different conditions and TOIs. The effects of SICI at baseline were obtained by comparing the paired-pulse corrected data with the single-pulse TS-100% data. To analyze drug-induced changes, TEPs in the single-pulse conditions (TS-100% and CS-70%) and in the SICI paradigm were contrasted post-drug versus pre-drug. Finally, we sought to compare the two GABA-mediated paired-pulse protocols of SICI and LICI. LICI, acquired in our previous work, was tested according to an established protocol [36], where a CS is applied 100 ms before the delivery of a TS. To reduce/limit somatosensory re-afferent feedback, the intensity for each stimulus also in this case was set at 100% RMT [37, 38]. In this case,

multiple independent t-tests were run for comparing SICI and LICI. Cluster based permutation analysis was performed as established in FieldTrip to correct for multiple comparisons [39]. Details are outlined in our previous studies [14, 18, 19]. If not indicated otherwise, all data are presented as means $\pm$ SEM. For all tests, a p-value < 0.05 was considered to indicate a significant difference.

3. Results

Study drugs were generally well tolerated. Reported main adverse effects were drowsiness (diazepam $n=11$; baclofen $n=7$) and dizziness (diazepam $n=2$; baclofen $n=4$), which did not affect the capability of the subjects to fully comply with the requirements of this study.

3.1 Drug effects on Resting Motor Threshold

RmANOVA of drug effects on RMT showed significant main effects of TIME ($F_{(1, 15)} = 15.27$, $p = 0.001$) and interaction of DRUG*TIME ($F_{(2, 30)} = 7.06$, $p = 0.003$). No significant effects were found for DRUG ($F_{(2, 30)} = 0.24$, $p = 0.79$). The interaction DRUG*TIME was explained by a significant RMT increase by diazepam (pre-drug: 37.31 ± 1.30 %MSO, post-drug: 40.75 ± 1.86 %MSO, paired t-test: $t(15) = 3.91$, $p = 0.001$), and by baclofen (pre-drug: 37.94 ± 1.30 %MSO, post-drug: 39.00 ± 1.37 %MSO; $t(15) = 2.35$, $p = 0.03$). In contrast, RMT remained unchanged in the placebo condition (pre-drug: 39.31 ± 1.69 %MSO, post-drug: 39.50 ± 1.86 %MSO; $t(15) = 0.34$, $p = 0.74$).

3.2 Drug effects on single-pulse TEPs

At baseline, TEPs with the typical components (i.e. P25, N45, P70, N100 and P180) were elicited by both TS-100% and CS-70% single pulses (**Figure 1A-B**). The cluster-

based permutation analysis showed no significant differences for TEPs elicited by TS-100% and CS-70% between DRUG conditions (all $p > 0.05$).

Diazepam increased the N45 ($p = 0.03$) and suppressed the N100 and P180 ($p < 0.001$ and $p = 0.01$, respectively) over channels contralateral to the stimulated left M1 (at TS-100%), in line with our previous studies [18]. Similarly, the CS-70% evoked N45 was increased ($p = 0.005$), and the N100 was decreased ($p = 0.01$) by diazepam (**Figure 1C-D**).

Baclofen, in contrast, increased the TS-100% evoked N100 ($p = 0.04$) over channels close to the stimulated site as described previously by our group [18], whereas no significant modulation was observed for TEPs evoked by CS-70% (**Figure 1E-F**).

3.3 Short-interval intracortical inhibition (SICI) of TEPs

SICI was calculated as the difference between paired-pulse corrected responses (corrected for CS effects) and single-pulse TS-100% evoked responses (**Figure 2A**). The topographical distribution of scalp voltages of the paired-pulse corrected TEPs covered the same regions where single-pulse evoked scalp voltages are distributed (**Figure 2B-C**). SICI significantly reduced late TEPs (N100 and P180, both $p < 0.001$; **Figure 2D-E**). In contrast, earlier TEPs (P25, N45, P70) were not significantly altered by SICI (**Figure 2**). Single subject data of SICI effects for the N100 and P180 potentials were largely consistent, with only few subjects showing opposite effects (**Figure 3**).

3.4 Drug effects on SICI of TEPs

Cluster-based permutation analysis showed that diazepam suppressed SICI of the N100 over frontal sites of the right (non-stimulated) hemisphere ($p = 0.03$; **Figure 4A**). In contrast, baclofen increased SICI of the N100 over frontal sites of both hemispheres ($p = 0.007$; **Figure 4B**). Importantly, comparison of pre-drug SICI did not show significant results between drug conditions (all $p > 0.05$) and, thus, cannot explain the differential modulatory effects of diazepam vs. baclofen on SICI of N100.

3.5 Short-interval intracortical inhibition (SICI) of MEPs

There was on average a SICI effect (mean (CS-TS MEP) / mean (TS MEP)) of 0.71 ± 0.14 , with all 10 but one subject showing MEP inhibition. A paired sample, one-tailed t-test on individual means of MEPs elicited by CS-TS vs. TS conditions ascertained the significance of MEP amplitude reduction ($t(9) = -1.84$, $p = 0.048$).

3.6 Comparison of SICI and LICI of TEPs

We used data from our previous study where we calculated LICI as the difference between paired-pulse corrected TEPs (corrected for CS effects) and TS-100% TEPs [14]. **Figure 5** shows the superimposition of SICI and LICI waveforms (**Figure 5A**) and the distribution of their scalp voltages for each TEP component (**Figure 5 B-C**). Cluster-based permutation analysis did not show significant differences for any of the TEPs (all

$p > 0.05$), except for the P70 potential which had higher amplitudes in LICI ($p = 0.02$; **Figure 5D**).

4. Discussion

We have studied short-interval intracortical inhibition of TMS-evoked EEG potentials in a systematic way by measuring paired-pulse TMS evoked brain responses with high-density EEG, and by testing two different drugs (diazepam, baclofen) targeting GABA_AR and GABA_BR mediated inhibitory neurotransmission for pharmacological characterization of SICI. The EEG readout of SICI is reflected by an amplitude reduction of late TEP components compared to single-pulse responses (i.e. N100 and P180). Moreover, diazepam and baclofen specifically modulated SICI of N100 in opposite directions. Finally, the EEG fingerprint comparison between SICI and LICI showed a highly similar pattern for the two GABAergic paired-pulse protocols.

4.1 Methodological considerations

To the best of our knowledge only three previous studies have characterized SICI using EEG and results are rather inconsistent, highlighting the methodological challenges of paired-pulse TMS protocol evaluation with EEG [11, 12, 15]. These studies either did not show any modulation of TEPs by SICI [11], an increase of the N100 and suppression of P70 and P180 in the stimulated hemisphere [12] or a suppression of early potentials (i.e., P25 and P60) [15]. A substantial difference with respect to our approach is that none of those studies subtracted the CS-evoked TEPs from the

ppTMS-evoked TEPs. We have argued previously that subtraction of both the single-pulse CS-evoked and TS-evoked EEG potentials from the ppTMS-evoked TEPs is necessary to uncover interactions between CS and TS in the ppTMS-evoked EEG response that are not explained by arithmetic summation [14].

Further, the above-mentioned studies used different approaches to extract TEP information. With 60 EEG channels and a group of five subjects, Paus et al. did not find relevant differences between TEPs evoked by single pulses and ppTMS. The TEP amplitude maxima were statistically compared by means of a *t*-test [11]. In a more recent study with 32-channel EEG, a General Estimating Equation model was employed to statistically evaluate the TMS-evoked response at fixed latencies [12]. Finally, Cash and colleagues, with 64-channel EEG, calculated the Area Under the Curve (AUC) by means of the sum of the instantaneous amplitudes as estimated by means of a Hilbert transform [15]. In our study a well-established cluster analysis was adopted to evaluate significant differences TEPs amplitudes [39].

Another important factor which may have contributed to the diversity of results is the intensity of stimulation, which plays a critical role in TMS-EEG studies [13, 40, 41] and influences TMS-evoked activity and connectivity in healthy individuals [38]. Furthermore, TMS of suprathreshold intensity results in contamination of the TMS-evoked EEG response, in particular the late TEP components, through somatosensory re-afference originating from the TMS-evoked muscle twitch [11]. To overcome these problems, we chose, as in our previous study [14], 100% RMT for TS intensity, rather than a clearly suprathreshold TS intensity as has been applied in all of the previous TMS-EEG SICI studies [11, 12, 15].

To verify whether our 70-100% paired-pulse TMS-EEG set-up could effectively elicit an MEP inhibition in the APB muscle, we conducted TMS-EMG experiment. We found significant SICI under these conditions. This result is consistent with previous findings (see Fig. 1B in [28]). We can therefore conclude that the SICI protocol implemented in our ppTMS-EEG experiment elicited corticospinal inhibition.

Finally, the above mentioned methodological differences, together with the lack of masking noise [15] to avoid TMS-evoked auditory cortical potentials, may explain why the previous TMS-EEG studies on SICI testing have been inconsistent.

4.2 Macroscopic evaluation of paired-pulse TMS protocols

Single-pulse pharmaco-TMS-EEG studies demonstrated that GABAAR and GABABR mediated inhibitory neurotransmission contributed, respectively, to the N45 and N100 TEP components [18, 19]. Since pharmaco-TMS-EMG studies have provided consistent evidence that SICI represents a marker of GABAAR-mediated inhibition, and LICI reflects GABABR-mediated inhibition (for recent review, [4]), one could have put forward the expectation that a ppTMS SICI protocol would enhance the N45 potential, and a ppTMS LICI protocol would enhance the N100 potential. However, in contrast to this line of thoughts, the present study demonstrated that the N45 amplitude showed a trend towards a decrease by the ppTMS SICI protocol (cf. **Figure 2**), and our previous study showed that N100 amplitude was also decreased when using the ppTMS LICI protocol [14]. Further, we showed close similarity between SICI- and LICI of TEPs, i.e., SICI and LICI led to highly analogous modulation of the spatio-temporal evolution of TEPs

(**Figure 5**), except the P70 component, which was larger in LICI. The physiology of the P70 is not completely clear, however, the existing evidence suggests a link with somatosensory feedback from muscle twitches caused by suprathreshold TMS [38]. Therefore, a stronger P70 in LICI may be explained by the fact that we used 100%RMT CS in the ppTMS LICI protocol [14], in contrast to 70%RMT CS in the ppTMS SICI protocol of this study. In addition, we adjusted the stimulation intensity to compensate for any drug-induced RMT changes, and to ensure a comparable excitation of the corticospinal system. However, RMT adjustments may constitute a potential confounder in TMS-EEG analysis, as neuron populations unrelated to RMT changes but involved in the network activation revealed by the TEP, may respond differently to changes in stimulation intensity [41-43]. Further, somatosensory re-afferent feedback due to the TMS-evoked muscle twitch has been recently shown as a potential confounding factor [37, 38].

In agreement with our results, TMS-EEG signatures of ppTMS SICI (ISI = 3 ms) were very similar to those of another ppTMS protocol (ISI = 11 ms), i.e. a virtually identical increase in N45 and N100 at the site of stimulation [12]. In TMS-EMG recordings, the latter ppTMS protocol typically results in an increase in MEP amplitudes, referred to as intracortical facilitation [12, 44, 45]. Together with our present findings these data strongly suggest that ppTMS-induced modulation of EEG potentials cannot be deduced in any straightforward manner from ppTMS-induced modification of MEP amplitude. This is not really surprising if the notion is called to attention that only the N15-P30 TEP complex showed a significant relation to MEP amplitude [15, 46]. However, we did not find a significant remaining P30 TEP component in the SICI grand average TEP or T-

statistic map (cf. **Figure 2D-E**). In summary, TMS-evoked EEG potentials, in particular later TEP components, seem to reflect more complex brain responses caused by propagation of the initial response at the site of stimulation to remote brain areas [26, 47, 48]. As the interaction of CS- and TS-evoked EEG responses seem to be largely uniform irrespective of the interstimulus interval (3 ms vs. 11 ms in [12]; 2 ms vs. 100 ms in the present study and [14]), it can be concluded that ppTMS-EEG protocols are not identical to the findings of ppTMS-EMG protocols that would result in SICI, ICF and LICI of MEP amplitudes at interstimulus intervals of 2-3 ms, 11 ms and 100 ms, respectively [36, 44, 45]. Furthermore, the underpinning physiological mechanisms of this rather uniform interaction of CS and TS in ppTMS-evoked EEG responses is presently unclear and will require further clarification by experimental work. This uniformity is corroborated further by the present and previous [14] pharmacological experiments, which show a decrease of SICI of N100 by diazepam in the non-stimulated hemisphere (cf. **Figure 4A**) and an increase of SICI of N100 by baclofen close to the stimulation site (**Figure 4B**), and virtually identical effects of opposite modulation of LICI N100 by both drugs (Figures 6-7 in [14]). These drug effects on SICI and LICI of N100 seem largely driven by their effects on the single-pulse TMS-evoked N100, i.e. a decrease of N100 by diazepam in the non-stimulated hemisphere and an increase by baclofen at or close to the site of stimulation (present study, [18]), as the TS100%-evoked EEG response is subtracted from the ppTMS-evoked response for assessing SICI (see Methods).

To conclude, these findings suggest a largely uniform interaction of paired-pulse effects on TEPs irrespective of the interstimulus interval. Therefore, SICI and LICI as measured

with TEPs cannot be directly derived from SICI and LICI measured with MEPs, but may offer novel insight into paired-pulse responses recorded directly from the brain rather than muscle.

5. Conclusions

We found that SICI reduced late TEPs (N100 and P180), but did not affect earlier TEPs (P25, N45, P70). Diazepam reduced SICI of the N100 TEP, whereas baclofen increased it. A direct comparison of the ppTMS-EEG signatures of SICI with LICI (from our previous study) showed largely identical TEP modulation, including highly similar pharmacological profiles.

These findings suggest a largely uniform interaction of paired-pulse effects on TEPs irrespective of the interstimulus interval. Therefore, SICI and LICI as measured with TEPs are not directly translatable to SICI and LICI measured with MEPs.

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Figure Legends

Figure 1: Single-pulse TMS-evoked EEG potentials (TEPs) at subthreshold and supra- threshold TMS intensities. Grand-averaged baseline TEPs before the intake of diazepam (DZP, blue), placebo (PBO, red) and baclofen (BAC, black) elicited by single-pulse TMS at 100% RMT (**A**, TS-100%) and 70% RMT (**B**, CS-70%) over left primary motor cortex. (**C**) Diazepam increased the N45 and decreased the N100 and P180 components primarily over the right hemisphere (i.e. contralateral to the stimulation site); (**E**) baclofen increased the N100 component over the stimulated left hemisphere only. Data reproduce findings from [18]. (**D**) Diazepam increased the N45 and decreased the N100 components, whereas baclofen had no effects on TEPs elicited by sub-motor threshold stimuli (**F**, CS-70%). Black bars underneath traces represent time windows of significant drug-induced changes in TEPs. Shades indicate ± 1 SEM. T-statistic maps are calculated for post-drug versus pre-drug TEP amplitude differences. Blue colors in topographical plots represent either an increase in surface voltage negativity or a reduction in positivity, whereas red colors indicate a reduction in negativity. **C**, **D** and **E** plots show the grand-average across significant channels, which are indicated by black dots in the t-statistic maps. **A**, **B** and **F** plots show TEPs averaged across all channels. For further details, see Materials and Methods.

Figure 2. Spatiotemporal EEG characteristics of SICI. (**A**) Grand-averaged TEPs at baseline (i.e., before drug intake) evoked by single-pulse TMS (TS-100%, blue) and paired-pulse TMS (CS70-TS100, corrected for CS effect, red). Distribution of scalp voltages (μ V) of each TEP component (P25, N45, P70, N100 and P180) for single-pulse TS-100% (**B**) and paired-pulse (**C**) trials. (**D**) Grand-average of SICI at baseline (paired-pulse minus single-pulse TS-100% TEPs); black bars underneath represent time windows of significant CS-induced changes in TS-evoked TEPs (shades indicate ± 1 SEM). (**E**) T-statistic maps of TEP amplitude differences (paired-pulse minus single-pulse TS-100% TEPs) for the significant TEP components N100 and P180. Black dots indicate channels, which showed significant differences between paired-pulse and

single-pulse conditions. Blue colors indicate a decrease in positivity, while red colors indicate a decrease in negativity.

Figure 3. Individual SICl of N100 and P180 TEPs. Data are grand-averages across the 3 pre-drug conditions and indicate consistent suppression of N100 and P180 potentials (paired-pulse minus single-pulse TS-100% amplitudes) across subjects. Error bars indicate group mean $\pm$ SEM.

Figure 4. Effects of diazepam and baclofen on SICl of TEPs. Grand-averaged SICl recorded before (blue) and after (red) the intake of diazepam (**A**) and baclofen (**B**) (shades indicate ± 1 SEM). Diazepam reduced SICl of the N100 TEP, whereas baclofen increased it. Black bars underneath represent time windows of significant drug effects. In the right column, topographical maps of voltage distributions and t-statistic maps are shown for drug effects on SICl of the N100. SICl curves in **A** and **B** are averaged across significant channels, which are indicated by black dots in the t-statistic maps. Blue colors indicate a decrease in positivity, while red colors indicate an increase in positivity.

Figure 5. Comparison of spatiotemporal characteristics of SICl and LICl of TEPs. Grand-average of SICl (blue) and LICl (red) of TEPs are shown in **A**. The LICl data are from our previous publication (Premoli et al., 2014b). Topographical distribution of surface voltages for SICl and LICl (paired-pulse minus single-pulse TEPs) of the most pronounced TEP components (P25, N45, P70, N100 and P180) are illustrated in **B** and **C**. (**D**) shows T-statistic maps of TEP amplitude differences (SICl minus LICl). Whereas LICl significantly increased the P70 TEP component (**C**), SICl showed no effect (**D**), rendering the difference SICl minus LICl negative. Black dots indicate channels, which showed a significant difference between SICl and LICl. None of the other TEP components showed any difference between SICl and LICl.

A

B

D

Highlights

- TMS-EEG measures TMS-evoked responses (TEPs) directly from the brain
- Paired-pulse TMS (ppTMS, ISI of 2 ms) results in inhibition of N100 and P180 TEPs
- Diazepam reduces this N100 inhibition, while baclofen enhances it
- PpTMS (ISIs of 2 vs. 100 ms) leads to virtually identical N100 and P180 inhibition
- TMS-EEG offers novel insight into ppTMS responses recorded directly from the brain