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Test–retest reliability of single and paired pulse transcranial magnetic stimulation parameters in healthy subjects

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ABSTRACT

Objective: To determine the influence of different factors on test–retest reliability of frequently used transcranial magnetic stimulation (TMS) parameters while controlling for potential confounders in healthy subjects.
Methods: TMS was applied in 93 healthy volunteers (61% male) twice (mean retest interval of 34.0 ± 25.6 (SD) days) between 7 am and 2 pm by four investigators (sessions n investigator A = 47, investigator B = 95, investigator C = 28, investigator D = 16). Women were assessed in their follicular phase. Test stimulus (TS), resting motor threshold (RMT), short latency intracortical inhibition (SICI), intracortical facilitation (ICF) and cortical silent period (SCP) were analyzed.
Results: Good test–retest reliabilities were observed for TS (r = .880) and RMT (r = .826), moderate for visual and automated analyzed CSP durations (resp. r = .466, r = .486), and poor for ICF (r = −.159). Reliable change indexes are reported. Gender (e.g. automated CSP women: r = .538 vs. men: r = .422), re-test interval and method of CSP-analysis did not influence reliabilities.
Conclusions: In a large sample of healthy volunteers we found good to moderate test–retest reliabilities in all but one TMS-parameter. Automated analysis of the CSP did not prove to be more reliable than visual determination.
Significance: This study contains analyses of re-test reliability in TMS considering several confounding factors. For the first time it presents reliable change indices for all frequently used TMS parameters.

1. Introduction

Cortical excitability is frequently assessed using transcranial magnetic stimulation (TMS) for clinical and research purposes [39]. However, to allow the detection of relevant and clinically meaningful changes in TMS parameters and thus cortical excitability following an intervention, sufficient test–retest reliability is required. Confounding factors that may systematically influence TMS results and hence reduce reliability should be recognized and considered when developing TMS-based research protocols.

Several studies have looked at variability in TMS before. Those studies have found overall good reliabilities of TMS parameters in single and paired-pulse settings in healthy volunteers [4,7,14,24] as well as in stroke patients [5,48] and amputees [19].

Resting motor threshold (RMT) was commonly found to be very reliable [4,25,26,35] with few contradictory results [11]. The RMT is defined as excitability of cortico-cortical axons and their connection to pyramidal cells [51] and mainly mediated by voltage-gated sodium channels.

Studies regarding reliability of the cortical silent period (CSP) are sparse [4,14,41]. CSP, here measured as contralateral interruption of tonic contractions of the hand muscle, represents both spinal inhibitory processes during the first 50 ms and cortical mechanisms including motor cortex inhibition later than 100 ms [50]. The latter is thought to reflect inhibition of pyramidal cell $GABA_B$ receptors through interneurons [47,51,52].

Studies on reliability of short latency intracortical inhibition (SICI) and facilitation (ICF) have been inconclusive [2,24,30,46]. Both SICI and ICF are measured using a paired-pulse design. A subthreshold conditioning stimulus (CS) is followed by the suprathreshold individual test stimulus (TS) with varying interstimulus intervals (ISI). ICF can be

Abbreviations: CSP, Cortical silent period; EMG, Electromyography; GABA, Gamma-aminobutyric acid; ICF, Intracortical facilitation; ISI, Interstimulus interval; MEP, Motor evoked potencial; NMDAR, N-methyl-d-aspartate receptor; RMT, Resting motor threshold; RCI, Reliable change index; SICI, Short intracortical inhibition; TMS, Transcranial magnetic stimulation; TS, Test stimulus.

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elicited with ISI of 6–25 ms while SICI is elicited at 1–6 ms [37]. Both, SICI and ICF prove intracortical excitability. Yet, they probably depend on different mechanisms. Both seem to be altered by GABAA dependent inhibition while ICF also responds to NMDAR changes [45].

The variability in TMS parameters might stem from inherent differences between participants [6,26]. In women, TMS has revealed that menstrual cycle phase affects cortical excitability and inhibition [6,18,43]. However, only few TMS studies on retest-reliability included women [9,11,24,42] and only one specified the phase of the menstrual cycle during which TMS was performed [42]. De Gennaro et al. [11] argued that low reliabilities reported were not result of including women as the male participant also showed low reliabilities. Additionally, investigators' skill and expertise in neurophysiological research were suggested to be relevant [4], but investigator effects have not yet been formally studied.

Lately, the proposed method of choice for determination of the CSP duration has been automated computer analysis rather than visual analysis to reduce variability [10,15,21]. Yet, to the best of our knowledge, there is no study comparing both methods regarding test–retest reliabilities, hence no superiority claim can be made.

Moreover, studies on retest reliability have discussed several factors potentially influencing measures of cortical excitability, including age, daytime, handedness [9,24,26,34,36,41,46] and period of menstrual cycle in women [17,18,43].

We therefore chose to control for these latter factors: age by including only young adults, daytime by measuring only during the morning, handedness by only including right-handed individuals and menstrual cycle by measuring during the follicular phase to yield precise data on our main aim on retest reliability. Reliable change indices have not been reported in such a big sample, yet, they are relevant for evaluation of data despite group significance according to their clinical importance.

Based on the pertinent literature we hypothesized, that retest reliability would be strong for RMT and CSP and moderate for SICI and ICF. Furthermore, that automated analysis of CSP would yield higher reliabilities than visually determined CSP and that gender effects on reliability would be absent due to the restrictions in assessing TMS parameters in women.

2. Experimental procedures

2.1. Subjects

Exclusion criteria involved a history of neurological and/or psychiatric disease and use of central nervous system active drugs. Only right-handed subjects with a score ≥80 on the Edinburgh handedness inventory [31] were included to have a homogenous sample for stimulation site, namely only the suspected dominant left hemisphere. Participants consented to refrain from caffeine intake or smoking for 12 h prior to and during assessments. Female participants underwent both TMS sessions during the follicular phase as determined by individual calendars. They were instructed during the screening how regularity of the cycle and follicular phase was defined. They individually determined their follicular phase and contacted the investigators accordingly.

The study conformed to the declaration of Helsinki and was approved by the local ethics committee of the Philipps-University Marburg, Germany. Written informed consent was obtained from all participants.

Ninety-six participants were included in the study. After excluding three participants (one due to technical problems, two did not finish the study) results from 93 volunteers (female n = 36, 38.7%; male n = 57, 61.3%, age: 23.74 ± 3.38 years, range: 19–36 years) were analyzed.

2.2. Investigators

Four investigators applied TMS in this study due to organizational reasons (sessions n investigator A = 47, investigator B = 95, investigator C = 28, investigator D = 16). All investigators received training by two experienced supervisors. For training purposes, all investigators applied TMS and analyzed data of several volunteers before they acquired data for the present study. Throughout the whole study, one experienced investigator was always available for support.

2.3. Sessions

Each participant completed two sessions (T1, T2). These were conducted at minimum of 14 days apart. TMS was repeated on average 34.0 ± 25.6 days after the first session (range 14–173 days). The two sessions represent baseline measurements for an experimental study on carbamazepine induced acute changes of cortical excitability that was published elsewhere [28]. All participants were assessed between 7 am and 2 pm. On average, the second session started 30 min earlier than the first (10:19 h ± 1.26 h vs. 9:49 h ± 1:21 h, p = .005).

2.4. Transcranial magnetic stimulation

Subjects were comfortably seated in an armchair with the head fixed in a custom plastic foam headrest. TMS was delivered through a focal figure-of-eight shaped magnetic coil (70 mm external loop diameter) connected to two Magstim 200 magnetic stimulators via a BiStim-module (all Magstim, Whitland, Dyfed, UK). The coil was placed flat on the head over the left motor cortex, at an approximate angle of 45° to the sagittal plane, inducing a current in the brain roughly perpendicular to the central sulcus, flowing from posterior to anterior, as this has been reported to be the most effective way to activate the corticospinal system transsynaptically [3]. Motor evoked potentials were recorded using surface EMG Ag/AgCl electrodes placed over the right abductor digiti minimi muscle (ADM) in a belly-tendon montage. The raw signal was amplified, filtered (20 Hz–10 kHz) and recorded with a PC using a commercially available data-collection and averaging program (Magnetix®, Center of Sensorimotor Research, Munich, Germany) for offline analysis. The optimal coil placement was determined by recording motor evoked potentials (MEP) while varying the coil position. The coil position leading to the highest peak-to-peak amplitude of the MEP ('hot spot') was marked with a semi-permanent pen directly on the scalp to ensure accurate coil positioning throughout the testing.

All sessions followed a fixed sequence of TMS measurements: First, TS and RMT, then the paired-pulse parameters, SICI and ICF, were obtained in random order. In all paired pulse TMS procedures, the interval between trials was randomly changed between 4 and 6 s, in single pulse procedures the inter-trial interval was 5 s. The protocol concluded with determination of the CSP.

2.5. TMS-parameters

TMS is a well-known non-invasive stimulation technique and it was applied ensuring high standards [13,33]. The parameters were specified as follows:

1. The resting motor threshold (RMT) was defined as the lowest stimulator output intensity that induced MEP peak-to-peak amplitude greater than 50 μV in at least five of ten consecutive trials. Complete muscle relaxation was monitored via audiovisual feedback. A step-by-step intensity resolution of the maximal stimulator output was used for determination of the individual RMT using the maximum likelihood threshold hunting (MLTH) procedure for TMS (Friedemann Awiszus, Magdeburg [1]).
2. Short intracortical inhibition (SICI) and intracortical facilitation (ICF) were obtained with paired-pulse TMS. A conditioning and a test stimuli were applied with different fixed interstimulus intervals (ISI). The conditioning stimulus was set to an intensity of 75% of the RMT as this does not produce changes of excitability in the spinal

cord [12,22]. The intensity of the following suprathreshold test stimulus was adjusted to produce MEPs of approximately 1.5 mV peak-to-peak amplitude if delivered without preceding conditioning stimulus (test stimulus, TS). SICI was obtained at short ISIs of 3 ms, leading to a decreased MEP as compared to a MEP induced by a non-conditioned test stimulus. ICF was obtained using ISIs of 10 ms, leading to an increased MEP [3,22,29,38,47]. Fifteen trials of single non-conditioned test stimuli and fifteen paired stimuli of each ISI, generated in random order by the computer program, were recorded. The average of the 15 trials was used to define the amplitude of the peak-to-peak MEP for each condition. The conditioned response was defined as the mean amplitude of the conditioned responses belonging to each ISI, expressed as percentage of the mean amplitude of the unconditioned test response. For better comparability, this percentage was subtracted from 100% for SICI [SICI: 100% − (conditioned response/unconditioned response × 100%); ICF: conditioned response/unconditioned response × 100%; [45]]

3. The cortical stimulation-induced silent period (CSP) was measured during 20 trials at a stimulus intensity of 110% of the RMT. Participants were instructed to hold a voluntary muscle contraction of approximately 30% of their maximal force, controlled by audio-visual feedback.
   CSP duration was determined offline in two ways, visually and automated, for all participants. For visually guided analysis, the CSP duration was defined as the time from TMS stimulus artifact to the first reoccurrence of voluntary EMG activity exceeding 25% of muscle activity prior to the stimulus. This was always determined by the same investigator in order to minimize variability. Duration was determined offline in the Magnetix® program.
   Additionally, automated analysis of the CSP was carried out with custom software (CSPDuration©, C. Bauer, Schopp, Germany) based on the method introduced by Garvey et al. [15]. This analysis was conducted after analyzing all data manually to avert influence on the latter and was conducted by a different and experienced investigator.
   Briefly, the twenty individual curves are single-trial full-wave rectified and averaged. Via an algorithm, the duration of the CSP is determined by mean pre-stimulus EMG, mean consecutive difference and a constant of 2.66. CSPDuration© default for averaging was 51 points. The program ignores calculated durations of the CSP of the original algorithm that are shorter than 30 ms or have a starting point more than 250 ms post MEP. The beginning of the CSP was set after the MEP.

One single-value per person per session was calculated for RMT and by averaging separately for SICI, ICF, visual CSP, and automated CSP.

2.6. Statistical analysis

Analysis was computed with PASW® Statistics 20® (SPSS, IBM Company, Chicago, Illinois). Data are displayed as Mean ± SD.

Comparison of daytime of measurement during first and second session was calculated by paired t-tests.

Reliability for all TMS parameters was calculated using Pearson's product moment correlation coefficient (r) as suggested by Rousson et al. [40]. For the reliability-analysis outliers were not considered in the calculation. They were first selected by eye in the scatterplot and confirmed statistically (>mean ± 2 SD). This resulted in excluding one participant for RMT analysis (n = 92) and two participants for SICI (n = 91) analysis.

The level of significance was set to $p = .05$ (two-tailed).

For several combinations of correlations (e.g. automated vs. visual CSP total sample) at intercorrelation = 0, it was analyzed whether the correlation coefficients differ statistically according to Meng et al. [27]. The program used is available online from Hahn and Stöber [16].

Reliable change indices (RCI) were computed (n = 93) according to Jacobson & Truax [20] and Chelune et al. [8]. Briefly, test–retest reliability coefficients were computed between session 1 and session 2, as well as standard errors (SE) for all TMS parameters. From these data, the Sdiff (standard error of the difference between sessions 1 and 2) was calculated, and RC (reliable change) index scores were derived. If individual measurements fall in between the respective confidence interval, this means that changes should be considered as random. The formula was $RC = (X_2 - X_1) / S_{diff}$ where $S_{diff} = \sqrt{(2 * (S_E)^2)}$ and $S_E = S1\sqrt{(1 - rx_1x_2)}$.Confidence intervals of 90%, 95% and 99% are presented.

3. Results

3.1. Overall test–retest reliability

The reliability analysis disclosed significant correlations between sessions 1 and 2 for all TMS-parameters (Table 1) except ICF ($r = -.159$, $p = .127$). Good correlations for TS ($r = .880$) and RMT ($r = .826$, Fig. 1) were reached.

3.2. Visual vs. automated CSP

Considering the whole sample, both visual and automated CSP were moderate in reliability (respectively $r = .466$, $r = .486$, Fig. 1c & d). The correlations did not differ significantly ($p > .1$). Fig. 2a-b shows the individual data points for all subjects.

3.3. Reliable change indices

For the purpose of determination of relevant changes in future TMS-studies reliable change indices (RCI) were computed (see Table 1). Smaller intervals prove better estimation of the mean of the population.

3.4. Potential confounding factors

3.4.1. Re-test interval

Looking at differences in reliabilities due to different time intervals we divided the whole sample by using a median split (<28 days vs. ≥28 days). The earlier re-measured group was measured on average 20.2 days (±4.9 days) after the first TMS, the latter group on average 49.9 days (±30.3 days). The respective correlations were for TR (early: $r = .992$ vs. late: $r = .825$), RMT ($r = .850$ vs. $r = .798$), SICI ($r = .230$ vs. $r = .532$), ICF ($r = -.068$ vs. $r = -.258$), Visual CSP ($r = .386$ vs. $R = .572$) and automated CSP ($r = .426$ vs. $R = .565$). Testing for significant differences of the correlations, TS was more reliable when measured early while SICI was more reliable when re-measured

Table 1
Test–retest reliabilities and reliable change (RC) index scores for different confidence intervals (CI).

Test	Test–retest reliability	S_{dif}	RCI (90% CI)	RCI (95% CI)	RCI (99% CI)
TS	.880	2.97	±4.87	±5.82	±7.63
RMT	.826	4.04	±6.63	±7.92	±10.38
SICI	.383	31.26	±51.27	±61.27	±80.34
ICF	−.159	40.09	±65.75	±78.58	±103.03
Vis_CSP	.466	26.12	±42.84	±51.20	±67.13
Aut_CSP	.486	15.46	±25.35	±30.30	±39.73

TS: test stimulus, RMT: resting motor threshold, SICI: short intracortical inhibition; SICF: short intracortical facilitation; Vis_CSP: visually analyzed cortical silent period; Aut_CSP: automatically analyzed cortical silent period; S_{dif} = standard error of difference between the two test scores, RCI = reliable change index, CI = confidence interval.

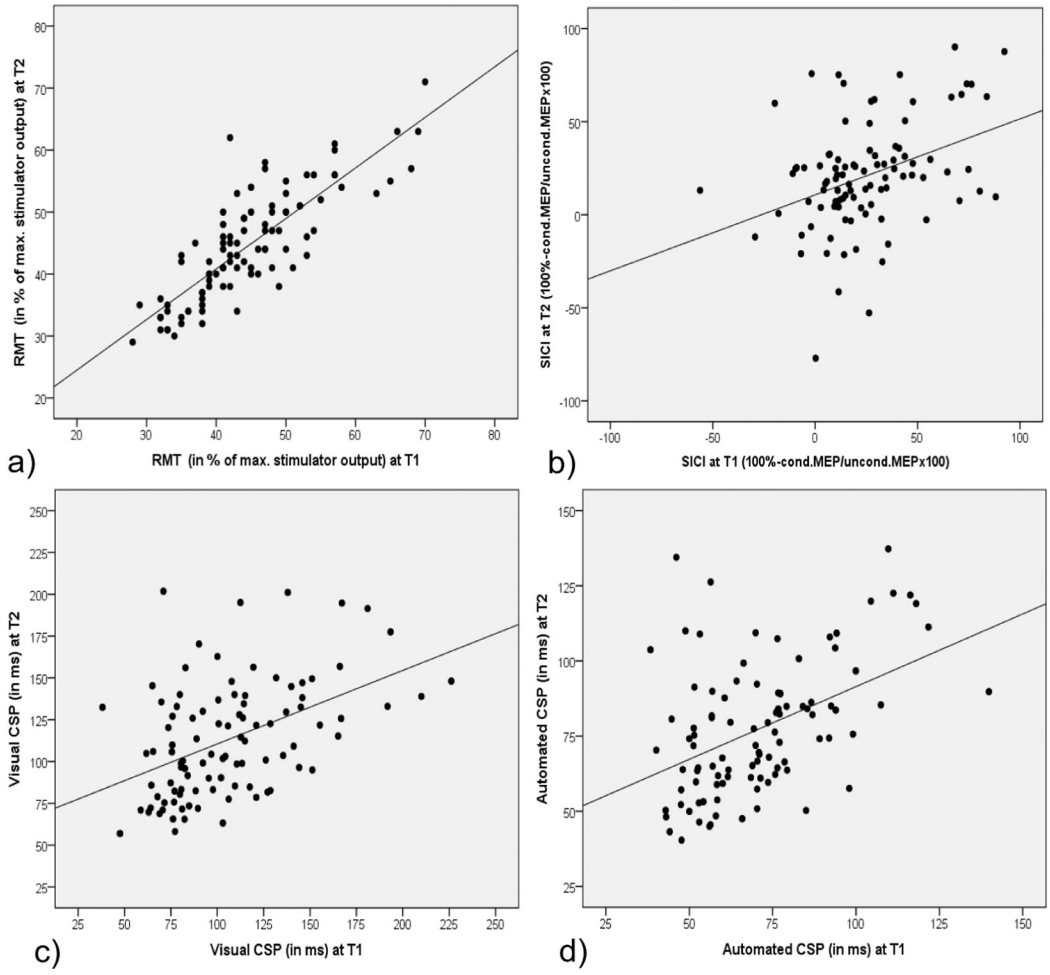

Fig. 1. a–d. Correlations between the two sessions. a) resting motor threshold (RMT, r = .880, p < .001), b) short intracortical inhibition (SICI, r = .383, p < .001); c) visually determined duration of cortical silent period (CSP, r = .466, p = .001); d) automatically determined duration of cortical silent period (Automated CSP, r = .486, p = .001). T1 = first session, T2 = second session.

late (p = .003; p = .021 respectively). All other correlations, including automated and visual CSP, did not differ significantly between earlier and later re-measurements.

3.4.2. Investigator

Multivariate analysis of variance (MANOVA) with investigator as 4-staged between-subject factor and session as within-subject factor indicated a significant main investigator effect (p < .001, Table 2), while no interaction was found (p > .1). In univariate analysis the investigator effect held only for the RMT (F (3, 89) = 5.404, p = .002). Post-hoc tests revealed that investigator D obtained higher RMTs than investigators A (p = .001) and B (p = .003) and by trend also C (p = .059). None of the ANOVA for the interaction was significant.

3.4.3. Gender

Reliabilities on TS (women: r = .867, p < .001; men: r = .894, p < .001) and RMT (women: r = .819, p < .001; men: r = .837, p < .001) were comparably high. Moderate reliabilities were reached on SICI (women: r = .333, p = .05; men: r = .410, p = .002), visual CSP (women: r = .477, p = .004; men: r = .408, p = .002) and automated CSP (women: r = .538, p = .001, men: r = .422; p = .001), see Fig. 3a–d. None of the correlation coefficients differed significantly between men and women.

4. Discussion

This study evaluated test–retest reliability during single and paired pulse TMS in a large sample of healthy right-handed male and female participants and determined influences of visual versus automated analysis of the CSP, short versus long retest interval and gender. We controlled our study for potential influences of daytime of TMS measurement, age, handedness and period of the menstrual cycle in women.

We found overall strong correlations between the two sessions for TS and RMT. Both visual and automated analysis of CSP, the latter as adapted from Garvey et al. [15], provided evidence for moderate retest reliability of this parameter with no substantial difference between the two methods. The short intracortical inhibition (SICI, at ISI 3 ms) yielded moderate correlations while the intracortical facilitation (ICF, at ISI 10 ms) was not reliable.

In line with other research the RMT was a very reliable parameter [4] which supported its frequent use as key variable in TMS research. This was also the case for short and long retest intervals.

Most of the studies on test–retest reliability of TMS either do not provide any data on CSP [2,6,7,11,24–26,35,42,49] or do not specify whether offline determination of duration of CSP was conducted visually or using an automated algorithm [4,14,41]. While lately the proposed method of choice is automated CSP analysis, to our knowledge, so far no

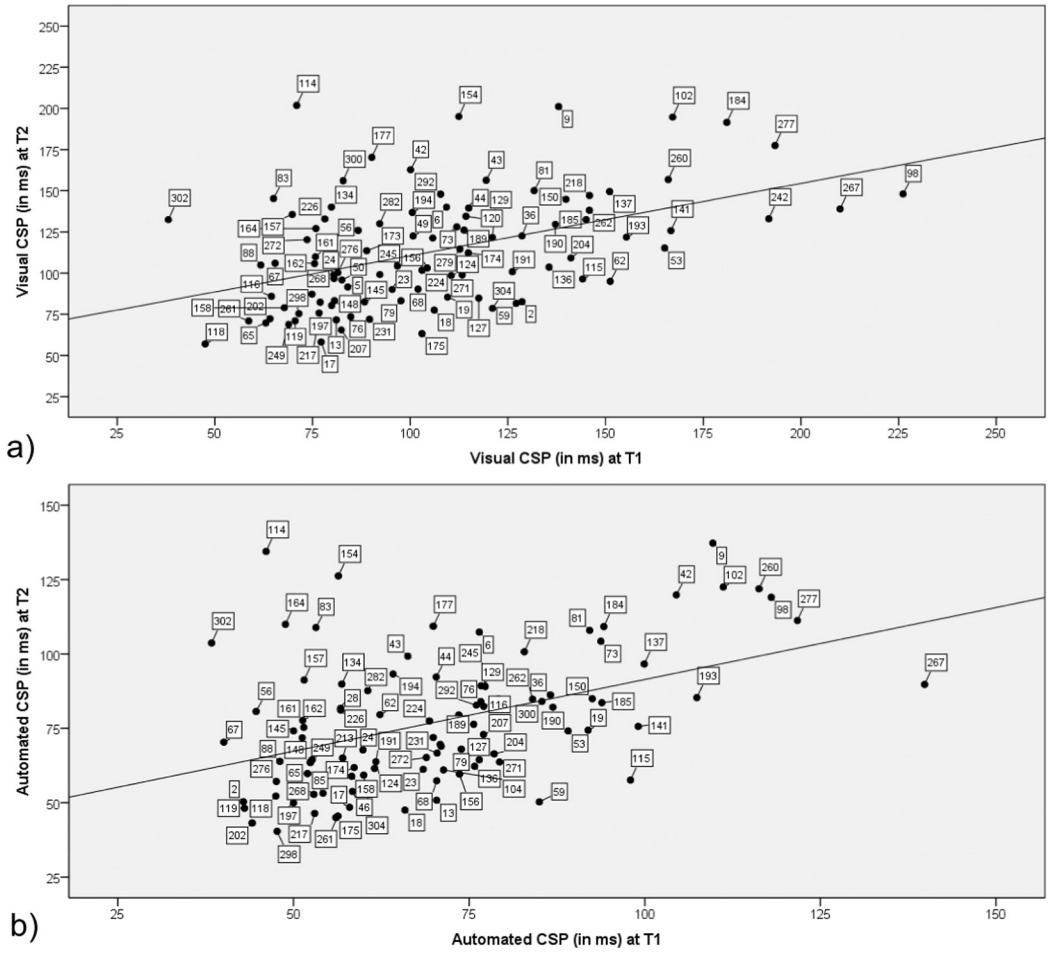

Fig. 2. a–b. Enumerated scatterplots for visual and automated analysis of cortical silent period (CSP) for the individual subjects (identified by the individual subject number).

comparisons on retest reliability were published. By providing data on two methods of CSP analysis we could show that both displayed moderate retest reliability and automated CSP analysis not being significantly more reliable, yet exhibiting a smaller confidence interval.

Table 2
Effects of MANOVA with the between-group factor "investigator A vs. B vs. C vs. D" and the within-group factor "session 1 vs. 2" on TS, RMT, SICI, SICF, visual CSP and automated CSP.

Source of variation	Dependent variable	F	df1	df2	p	eta^2
Main effect: "investigator A vs. B vs. C vs. D"	Multivariate analysis	4.07	18	258	.000	.223
	TS	.061	3	89	.980	.002
	RMT	5.40	3	89	.002	.154
	SICI	1.27	3	89	.290	.041
	SICF	.676	3	89	.569	.022
	Vis_CSP	1.02	3	89	.388	.033
	Aut_CSP	.069	3	89	.976	.002
Interaction effect: "session × investigator"	Multivariate analysis	1.06	18	258	.397	.070
	TS	.324	3	89	.808	.011
	RMT	.228	3	89	.877	.008
	SICI	.959	3	89	.416	.031
	SICF	.542	3	89	.655	.018
	Vis_CSP	.204	3	89	.114	.064
	Aut_CSP	.603	3	89	.615	.020

MANOVA: multivariate analysis of variance, TS: test stimulus, RMT: resting motor threshold, SICI: short latency intracortical inhibition, SICF: short latency intracortical facilitation, Vis_CSP: visual cortical silent period, Aut_CSP: automated cortical silent period.

The difference of re-test reliability in SICI and ICF is interesting. Our finding of low reliabilities of the ICF is in concordance with few earlier studies. High inter-subject variability in ICF [2] or less replicable ICF compared to SICI [24] were previously described, both in smaller cohorts. The authors put forward several potential biological and technical reasons for that. Those include the different thresholds for SICI and ICF with the latter being slightly higher, test MEP amplitudes as well as mathematical calculation of ICF. Additionally, our data suggest that the difference in reliability in ICF and SICI might be due to different mechanisms of NDMA and GABAa in those double-pulse parameters.

Future studies should include more than one ISI and/or administer more than 15 trials for each ISI to better capture average facilitation during ICF. The best time interval for retesting has to be determined in future studies to yield optimal balance between consistency and minimized learning transfer. Additionally, the use of individual SICI and ICF thresholds might diminish variability [32]. Attentional problems during the double-pulse paradigms could be ruled out in our study because the double pulse paradigms were tested before CSP. Between measurements participants were verbally activated to ensure that there was no loss in attention. Still, increased adherence to or better standardization of instructions for the participants regarding the level of muscle contraction might diminish activation-induced changes and the resulting lower reliabilities in CSP measurements compared to RMT.

The data suggest that one month (=28 days) of interval between the TMS measurements in TMS-naïve healthy participants without any special intervention allows for good reliabilities. This conforms to a recent study that also demonstrated good reliabilities on day 14 and day 28 [23].

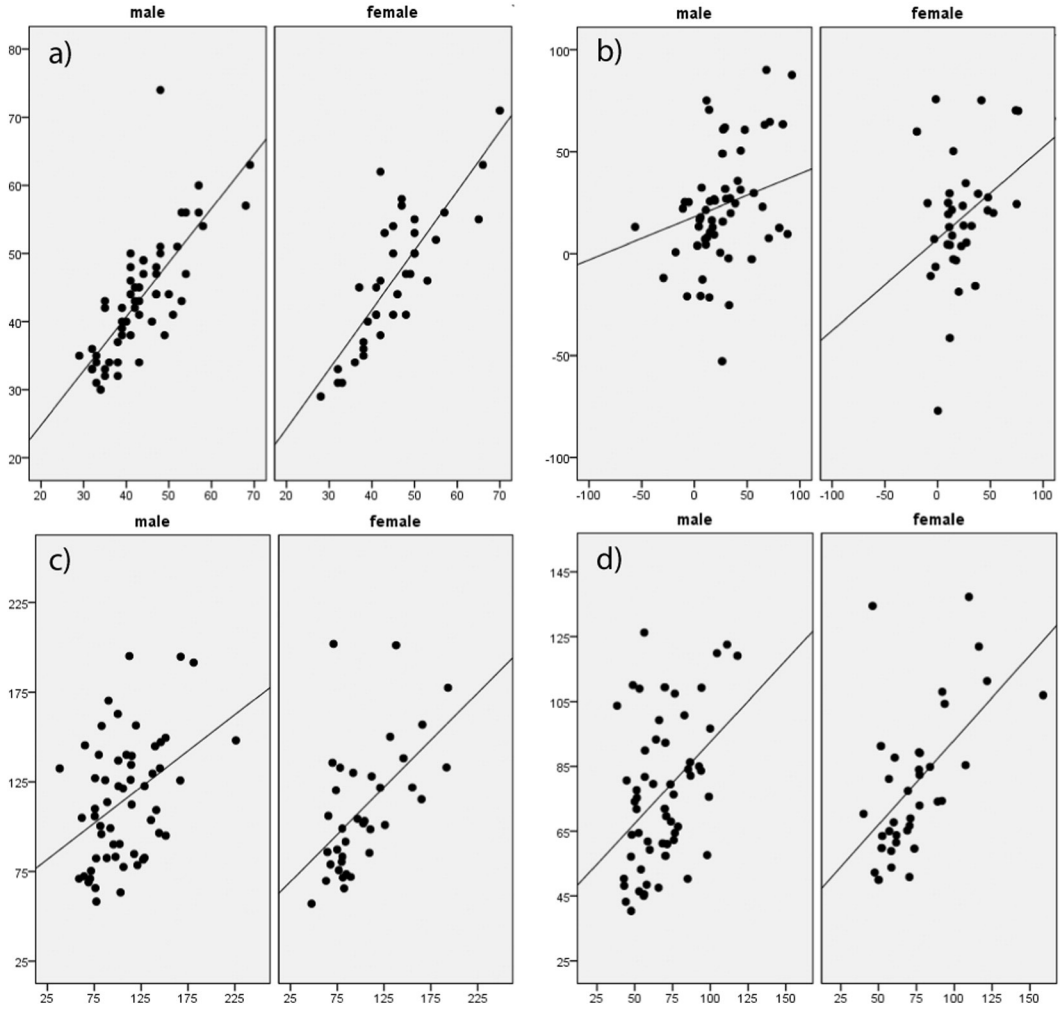

Fig. 3. a–d. Scatterplots of correlations for male and female subjects a) RMT, b) SICI, c) visual CSP, d) automated CSP. T1 is displayed on the x-axis, T2 is displayed on the y axis.

Cacchio et al. [4] partly attributed their strong correlations to the profound experience of their two investigators concerning TMS. Our data illustrates that overall the four investigators in our study reached good re-test reliabilities. As this study was not intended to assess inter-rater reliabilities we cannot conclude on this directly. Possibly, reducing heterogeneity in investigators should be achieved to further diminish influencing factors.

Several studies have underlined the confounding potential of hormone changes during the menstrual cycle in menstruating women [6,43,46], although this ovarian hormone dependent change was not present for the MEP threshold. The present study, to our knowledge, provides data of the largest cohort of women, all measured during their follicular phase to minimize effects of ovarian hormones. Given this standardization, reliabilities were equally good in women as in men, suggesting that, at least when measuring in the same phase of the hormonal cycle, reliabilities are high.

Reliable change indices have, to our knowledge, not yet been calculated for TMS. They can be used for determination of relevant changes between two TMS sessions. However, the provided reliable change indices are derived from a large healthy sample, and might not hold for neurological patients. They also depend on the quality of measurement and the corresponding retest reliabilities and might thus be different across labs.

Additional studies are needed to explore the retest reliabilities in elder vs. younger, right- vs. left-handed individuals and during different phases of the menstrual cycle as these variables were held stable during this study to minimize their potential impact on retest reliabilities.

A limitation of our current study is, that no MRI-guided neuronavigated TMS was conducted to ensure better coil repositioning which might influence re-test reliabilities of TMS positively [44].

In conclusion, this study corroborates strong retest reliabilities in single and paired-pulse TMS, especially for TS and RMT, and provides insights into the effects gender and retest intervals on test–retest reliability in a large sample of healthy right-handed individuals. The comparison of test–retest reliability of visual vs. automated analysis of CSP duration, carried out for the first time, demonstrated little dependence on the analysis method used regarding reliability. Reliable change indices were calculated for the first time for all captured TMS parameters.

These data can be helpful to determine the significance of observed changes in cortical excitability as well as for study design and sample size calculation in future research, in healthy populations.

Disclosure statement

Author contributions: AHe, AHa, KM and FR designed the research; AHe, CD, KB & HB performed the research; KM, SB & VM contributed to data acquisition, AHe & AHa analyzed the data, AHe, AHa & FR wrote the paper.

All authors have approved the final article.

The authors declare the following conflicts of interest:

AHe has no conflicts of interest to declare.

AHa has received honoraria for data analysis, manuscript preparation for an observational study from UCB and for a survey study from Sanofi-Aventis.

CD, KB, HB, SB, VM & KM have no conflicts of interest to declare.

Within the last two years FR has received honoraria as scientific advisor from GSK, EISAI, UCB, Pfizer, cerbomed. He has received speaker honoraria from UCB, GSK, EISAI, Desitin, Medtronic, Novartis, Shire and educational grants from Nihon-Kohden, UCB, Medtronics, Cyberonics and Cerbomed. FR has, however, no COI regarding this study.

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