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Research paper

Comparison between adaptive and fixed stimulus paired-pulse transcranial magnetic stimulation (ppTMS) in normal subjects

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ABSTRACT

Objectives: Paired-pulse TMS (ppTMS) examines cortical excitability but may require lengthy test procedures and fine tuning of stimulus parameters due to the inherent variability of the elicited motor evoked potentials (MEPs) and their tendency to exhibit a 'ceiling/floor effects' in inhibition trials. Aiming to overcome some of these limitations, we implemented an 'adaptive' ppTMS protocol and compared the obtained excitability indices with those from 'conventional' fixed-stimulus ppTMS.
Methods: Short- and long interval intracortical inhibition (SICI and LICI) as well as intracortical facilitation (ICF) were examined in 20 healthy subjects by adaptive ppTMS and fixed-stimulus ppTMS. The test stimulus intensity was either adapted to produce 500 μV MEPs (by a maximum likelihood strategy in combination with parameter estimation by sequential testing) or fixed to 120% of resting motor threshold (rMT). The conditioning stimulus was 80% rMT for SICI and ICF and 120% MT for LICI in both tests.
Results: There were significant ($p < 0.05$) intraindividual correlations between the two methods for all excitability measures. There was a clustering of SICI and LICI indices near maximal inhibition ('ceiling effect') in fixed-stimulus ppTMS which was not observed for adaptive SICI and LICI.
Conclusions: Adaptive ppTMS excitability data correlates to those acquired from fixed-stimulus ppTMS.
Significance: Adaptive ppTMS is easy to implement and may serve as a more sensitive method to detect changes in cortical inhibition than fixed stimulus ppTMS. Whether equally confident data are produced by less stimuli with our adaptive approach (as already confirmed for motor threshold estimation) remains to be explored.
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1. Introduction

Paired-pulse transcranial magnetic stimulation (ppTMS) is a non-invasive method to examine cortical excitability and has contributed to important discoveries in basic neuroscience and neuropharmacology (Ziemann et al., 2015; Hanajima and Yoshikazu, 2008). Recent studies have also demonstrated a potential contribution of ppTMS in the clinical setting where it may add important information to the diagnostic work-up of motor neuron disease (Menon et al., 2015a; Huynh et al., 2016) and epilepsy (Badawy et al., 2014; Bauer et al., 2014).

A typical ppTMS paradigm consists of a sub- or suprathreshold conditioning stimulus (CS) followed by a suprathreshold test stimulus (TS) which typically elicits a motor evoked potential (MEP) from the muscle. The chosen time interval between the paired stimuli, as well as the CS intensity, determine whether the CS will produce a facilitatory or inhibitory influence on the TS, e.g., short-interval intracortical inhibition (SICI), intracortical facilitation (ICF) and long-interval intracortical inhibition (LICI) (Kujirai et al., 1993). In general, a ppTMS study begins with establishing the motor threshold (MT) in order to calculate the CS and TS intensities for each participant. Determining the MT is, however, not a trivial task considering that the MEP amplitude at a given suprathreshold stimulus intensity is highly variable (Kiers et al., 1993). It is noteworthy (and relevant to the present study) that in the recently published guidelines for clinical and research applications of TMS (Rossini et al., 2015), adaptive methods were recommended for faster and more accurate MT determination (Mishory et al., 2004; Silbert et al., 2013).

The result of such an adaptive MT determination session is the statistical estimate of the TMS intensity required to generate MEPs of an amplitude of about 50–100 μV. With an increasing number of stimuli, more information (MEP data) will be available for the statistical estimation model which, in turn, will deliver an increasingly more confident final MT estimate. This is for example the main feature of the ML-PEST (maximum likelihood model using

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parameter estimation by sequential testing) strategy (Pentland, 1980; Lieberman and Pentland, 1982) used for psychophysical tests and further developed by Awiszus and collaborators (Awiszus, 2003) for TMS research.

The high trial-to-trial variability of the MEP response observed during MT estimation is also a problem when performing ppTMS studies in which each CS-TS pair of stimuli must be repeated many times to obtain a reliable result of the conditioning effect. Obviously, this may limit the possibility of combining different types of ppTMS excitability tests in a study due to time constraints and/or discomfort from the stimuli. In line with adaptive methods being used to obtain faster and more accurate MT estimation, a few previous studies have explored the possibilities of using adaptive methods in ppTMS-studies (Awiszus et al., 1999; Fisher et al., 2002; Vucic et al., 2006). In these studies the fixed stimulus strength of the CS was followed by a TS with a stimulation intensity that was determined by a 'threshold hunting' algorithm (Bostock, 1998) aiming for intensities that produce MEP responses of a predefined amplitude (Awiszus et al., 1999; Fisher et al., 2002). The adaptive threshold hunting technique used for ppTMS seems to overcome some methodological limitations (further described in DISCUSSION) of the more commonly used fixed-stimulus ppTMS in which the TMS intensity for the TS is kept constant. However, the above described threshold hunting technique used for ppTMS does not make use of the powerful statistical approach provided by the ML-PEST model for determining the TS intensities. Therefore, in a previous ppTMS study we (Axelson et al., 2014) decided, to use a ML-PEST algorithm (best-PEST, Pentland, 1980, Zuberbuhler, 2002) to not only determine the resting MT but also to set the TS intensity for ppTMS. In simplified terms, the model estimates the TS intensity required to obtain a 500 μV MEP following a CS suitable for SICI, ICF and LICI. The main difference between this adaptive ppTMS (A-ppTMS) approach and the 'conventional' fixed-stimulus ppTMS (FS-ppTMS) method is how the TS intensity is established, i.e., adaptive during the trial instead of pre-set before the trial.

In this study we examine how the excitability indices (SICI, ICF, LICI) obtained from A-ppTMS correlate to those acquired by the more commonly used FS-ppTMS in healthy subjects. This comparison was made with the aim to further validate the A-ppTMS method before implementing the technique in future studies.

2. Methods

2.1. Subjects

Twenty healthy subjects (age 32 ± 13 years, mean ± SD) recruited among medical students and laboratory staff participated in the study. Nine of them were women. Two subjects were left-handed. All volunteers were given thorough information about the experimental procedure. Exclusion criteria were a history of neurological or psychiatric disorder, a family history of seizure disorder, a history of seizures or unclear seizure-like episodes, facial pain, previous head trauma, pregnancy, pacemaker or other implanted stimulators, or metal objects in the head region. Two subjects were on oral contraceptives and one used antihistamine regularly. Possible side-effects related to TMS were specifically asked for before and after the investigation. The subjects gave their oral and written informed consent. The study was approved by the Uppsala University regional ethical review board (DNR 2012/72). The subjects participated in a subsequent study later the same day (Axelson et al., 2014).

2.2. Experimental setup

The subjects were seated in a comfortable chair with head support. A pillow was placed in their lap to support the upper extremities. They were carefully instructed to remain awake with their eyes open during the experiments. Earplugs were used throughout the session. The first dorsal interosseus (FDI) muscle of the dominant hand (as reported by the subject) was the TMS target muscle. Disposable adhesive surface electrodes (Blue Sensor N, Ambu, Ballerup, Denmark) were used for recordings with the active electrode placed over the mid part of the FDI muscle belly and the reference electrode on the proximal phalanx of digit II. A ground surface electrode was placed over the dorsum of the hand. In order to confirm proper placement of the active electrode, the compound muscle action potential (CMAP) from the FDI muscle was elicited by ulnar nerve electrical stimulation (Keypoint Workstation, Natus Medical Inc, IL, USA). If the CMAP peak-to-peak amplitude was below 10 mV, the active electrode was slightly repositioned for a higher amplitude. All MEP and CMAP signals were recorded and filtered (20–1000 Hz bandwidth) using a multipurpose acquisition system (Powerlab, AD Instruments Ltd, Hastings, UK). The free-running EMG was displayed continuously online to assure muscle relaxation during measurements.

2.3. TMS protocol and MEP recording

TMS was delivered through a 70 mm figure-of-eight coil (P/N 3190–00 coil, BiStim^2 Stimulator, Magstim Company Ltd, Whitland, UK) held tangential to the scalp at 45° to the anterior-posterior line with the handle pointing backwards. The single pulse mode of the stimulator was used for cortical mapping and MT determination. Following cortical mapping (as described below), a software application (Excel VBA script, Microsoft, Redmond, USA) developed at our institution was launched to control the BiStim^2 stimulator parameters and receive and analyze MEP data from the acquisition system during ppTMS. In this way, the main part of the experiments was completely automatized except for coil positioning and triggering of the TMS pulse which was handled by the examiner. The time interval between each single stimuli or pairs of stimuli exceeded 6 s throughout the study. A TMS navigation system (Visor 2, ANT, Enschede, Netherland) was used to track the position of the subject's head and the stimulating TMS coil in relation to a 3D rendered standard brain MRI to ensure accurate and consistent TMS coil positioning throughout the experiment. A curve linear surface of the 3D brain MRI served as a background image for TMS stimulation markers that were automatically added to the image. The markers represented the centre position of the coil at the time of stimulation and were colour coded based on the amplitude of the obtained motor evoked potentials (MEP). The 'hotspot' for the FDI muscle in the primary motor cortex was established by visually determining the 'centre of gravitation' of those markers that represented MEPs with the highest amplitude from the muscle. In accordance with the navigation software workflow for targeting TMS, the hotspot was marked on the MRI image by a single TMS pulse in order to get a defined target coil position for all subsequent TMS pulses in the study. The operator was provided with online visual feedback of targeting accuracy throughout the experiments.

2.4. Resting motor threshold (rMT)

The rMT was obtained before each ppTMS session (i.e., FS-ppTMS and A-ppTMS) using a well-established adaptive (best-PEST) method (Pentland, 1980) in which the final result represented the stimulus strength needed to produce a 100 μV MEP response after 20 sequential trials, i.e., the adaptive resting MT.

2.5. Fixed stimulus and adaptive ppTMS

This study compared within-subject measures of SICI, ICF and LICI from FS-ppTMS to those obtained from A-ppTMS. In all sub-

jects, FS-ppTMS was performed first, followed by A-ppTMS after approximately 5 min of rest. For both tests, the excitability measures (SICI, ICF and LICI) and the unconditioned control stimuli (ucTS) were examined in a randomized order and the CS was set to 80% of the resting MT except for LICI which was 120% of the MT. The interstimulus intervals (ISI) between CS and TS were 3 ms for SICI, 13 ms for ICF and 150 ms for LICI. The FS-ppTMS results were based on 10 conditioned test stimuli per excitability measure (i.e. SICI, ICF and LICI) and 10 unconditioned test stimuli (ucTS) as previously recommended (Rossini et al., 2015). The main difference between the A-ppTMS and the FS-ppTMS protocol was that in the latter the TS intensity was set to 120% of the MT and in the former the TS intensity was determined by the same ML-PEST algorithm used for MT determination. The target TS amplitude for A-ppTMS was set to 500 μV, which was presumed to be within the range where the stimulus-response relationship is approximately linear on a log scale (Kukke et al., 2014). The ML-PEST model used for the rMT and A-ppTMS is similar to the best-PEST algorithm described by Pentland (1980) and explained in detail by Zuberbuhler (2002). In brief, a sigmoid shaped logistic function i.e., the core function for the ML-PEST model, describes the increasing probability of obtaining a positive response (e.g. a MEP response above 100 μV) with increasing stimulation strength.

$$\text{Logistic core function for the best-PEST}: \varphi(\phi) = \frac{1}{(1 + e^{r_i 4\beta(\theta-\phi)})}$$

φ probability; r_i response at i-th trial; ϕ stimulus intensity; β slope of the sigmoid curve ; θ threshold.

The main objective of the ML-PEST procedure is to find the stimulation strength that statistically generates positive responses with a probability of 50% based on previous stimulation results according to:

$$\hat{\theta}_N = \max_{\phi\in(0,100)} \sum_{i=1}^{N-1} \frac{\log 1}{(1 + e^{r_i 4\beta(\hat{\theta}_i-\phi)})}$$

The threshold $\hat{\theta}_N$ after N-1 number of stimulations is estimated by a function in which ϕ is a range of TMS intensities passed to the logistic kernel function. The stimulation response (r_i) is the MEP response from the i-th trial from TMS. The TMS intensity is given by threshold estimate from the last trial (θ_{N-1}). The response is +1 if the MEP amplitude is above a predefined value (e.g., above 100 μV) and otherwise −1. The β represents the slope of the sigmoid function and was set to 4 which seemed to be a suitable choice for adaptive MT estimation according to a concurrent study on cortical excitability changes from trigeminal nerve stimulation (Axelson et al., 2014). The final TS estimates for each type of test (ucTS, SICI, ICF and LICI) was based on 15 trials which is within the range of recommended number of trials for adaptive MT using a similar statistical model (Awiszus, 2011). An important feature of the ML-PEST/best-PEST algorithm used in our study, is the *a priori* assumption that 100% TMS machine output (MO) will generate a positive response (at $i = 1$) and a subsequent ($i = 2$) stimulation at 0% MO will result in a negative response. Based on this information the first real trial stimulus intensity ($i = 3$) provided by the best-PEST algorithm is always 50% MO and consequently this was the initial stimulation strength for rMT assessment in both types of tests (FS-ppTMS and A-ppTMS) and the TS for SICI, ICF and LICI in A-ppTMS.

2.6. Statistical analysis

TMS intensities are given in MO% within a 0–100% stimulation range. FS-ppTMS measures (SICI, ICF and LICI) were calculated as a ratio between the average conditioned MEP amplitude divided with the average unconditioned amplitude expressed in percentage. The FS-ppTMS data was presented as median with the full range of values. A-ppTMS data was presented as mean ± 2SD (for further details on how these data were processed and normalized, see the Results section). Correlation between different variables such as FS-ppTMS and A-ppTMS excitability indices were analysed with two-sided Pearson correlation test and Pearson's r is given together with p-values. Paired sample T-test was used to compare differences (mean + 2SD) in rMT between the two tests. The significance level was set to $p < 0.05$. All data were collected in Excel (Microsoft, Redmond, USA) and statistical calculations were made using SPSS (v19, IBM, New York, USA).

3. Results

The ppTMS sessions were well-tolerated by all subjects. There were no technical errors other than that one subject was re-examined due to hardware failure. The FDI CMAP amplitude ranged from 12.3–28.8 mV (mean 20 mV). SICI, ICF and LICI were obtained using FS-ppTMS and A-ppTMS in all subjects. In one individual, the maximal machine output of 100% was reached in a SICI A-ppTMS test.

3.1. Resting motor thresholds and ppTMS measurements

There was no significant difference in rMT (Table 1) between the two tests (Δ 0.20 ± 4.20 MO% FS-ppTMS vs. A-ppTMS, p = 0.67). As shown in Fig. 1, the rMT obtained by the ML-PEST procedure converges towards the final threshold estimate and after 15 stimulations there is only ~1% MO difference between the rMT estimate at this point and the final estimate after 20 stimulations. The FS-ppTMS session produced the expected excitability measures of inhibition for SICI and LICI and facilitation for ICF (Table 1). Fig. 2 displays the results of the following A-ppTMS session from one subject and illustrates how the best-PEST algorithm generates new TS intensity estimates (MO%) based on preceding stimulation results (MEP amplitudes). As shown in Fig. 2, inhibition (SICI and LICI) is represented by final TS estimates that are above the unconditioned TS (ucTS) estimate indicating an inhibitory influence from the CS (in turn necessitating a higher subsequent TS to produce the same target response). In contrast, less TS strength is required to generate MEPs of the same amplitude when measuring ICF. Consequently, the results from the A-ppTMS test can be expressed as the difference between the conditioned (SICI, ICF and LICI) and the unconditioned (ucTS) final TS estimates (i.e., ΔSICI, ΔICF and ΔLICI).

3.2. Normalized A-ppTMS data

Fig. 3 shows an overall trend where ΔSICI and ΔICF (but not ΔLICI) tend to correlate to the unconditioned TS estimate (ucTS) for each individual although only significantly for ICF (ICF: p = 0.03, $r = -0.72$ ΔICF vs. ucTS, Fig. 3B; SICI: p = 0.06, $r = 0.43$ ΔSICI vs ucTS, Fig. 3A; LICI: p = 0.62, $r = 0.12$ ΔLICI vs. ucTS, Fig. 3C). Thus, the inhibitory and facilitatory effects seem to be

Table 1
Excitability data for fixed stimulus and adaptive ppTMS. Resting motor thresholds (rMT) are given in machine output %. Fixed stimulus paired pulse TMS (FS-ppTMS) excitability indices are presented as median and full range of values. Adaptive-ppTMS (A-ppTMS) data are presented as mean ± 2 SD.

	FS-ppTMS	A-ppTMS
rMT	42.9 ± 13.5%	42.7 ± 13.1%
SICI	24% (7–169%)	25.3 ± 43.4%
ICF	143% (75–338%)	−2.5 ± 16.8%
LICI	22% (2–100%)	16.2% ± 25.4%

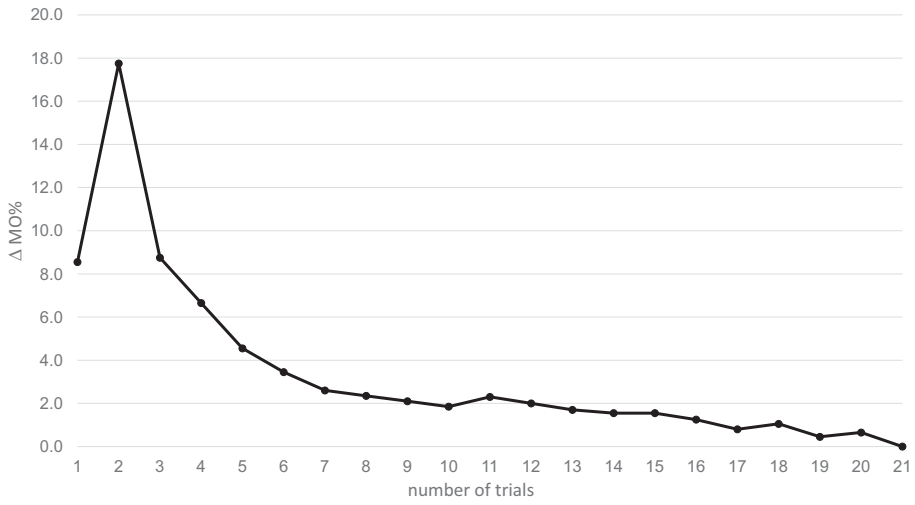

Fig. 1. Resting motor threshold estimation by ML-PEST. The initial deflection of the curve represents a large change in TMS MO% followed by an exponential-like convergence of the rMT estimate towards the final estimate after 20 trials i.e., the TMS MO% for trial number 21. The Y-axis represents the difference (ΔMO%; given in absolute values) between the rMT estimate for each trial and the final estimate after 20 trials. The plot is based on rMT estimation data from all 20 subjects obtained before FS-ppTMS.

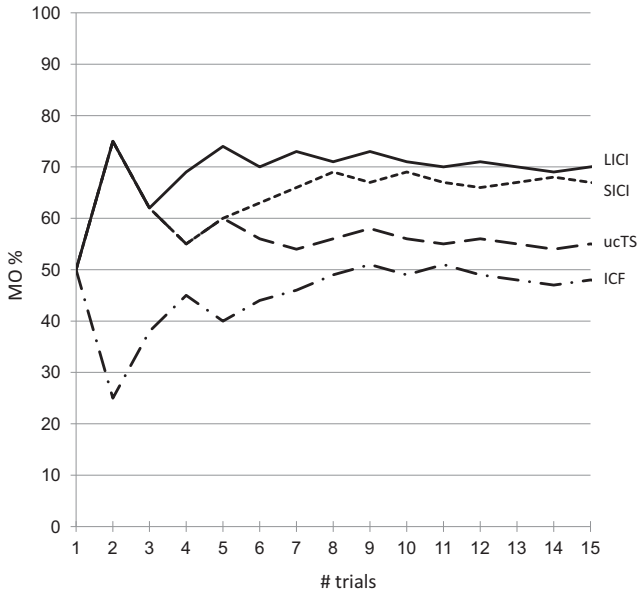

Fig. 2. Adaptive ppTMS (A-ppTMS) with unconditioned (ucTS) and conditioned (SICI, ICF and LICI) test stimulus estimates in one subject. After each stimulation, the statistical algorithm (ML-PEST) presents a new and more confident estimate of the TS intensity required to produce MEPs of 0.5 mV for ucTS, SICI, ICF and LICI. The final estimates after 15 trials are shown rightmost in the figure where SICI, ICF and LICI effects are demonstrated as differences in MO% compared to ucTS. The plots for ucTS, SICI and LICI are overlapping for the first 2–3 stimuli (the first stimulus was always set to 50% MO), see text for details.

more pronounced in those individuals with higher unconditioned test pulse intensities (ucTS). In addition, ΔICF was also correlated to the rMT indicating a smaller ICF effect in subjects with lower rMT (data not shown). Based on the above observations, the conditioning effects (ΔSICI, ΔICF and ΔLICI) were normalized in relation to the ucTS estimate (e.g. (ΔSICI/ucTS) * 100). These normalized indices (Table 1) were then used in the subsequent analysis (below).

3.3. Correlation between excitability indices from A-ppTMS and FS-ppTMS

Fig. 4 demonstrates how the normalized SICI, ICF and LICI excitability data from A-ppTMS correlate to those obtained by FS-ppTMS for each subject. There were significant ($p < 0.05$) correlations for all excitability measures between the two methods (SICI: $p = 0.02$, $r = -0.52$; ICF: $p = 0.02$, $r = -0.53$ and LICI: $p = 0.04$, $r = -0.46$). As shown (Fig. 4A and C), FS-ppTMS SICI and LICI data tend to cluster at near maximal inhibition whereas the A-ppTMS indices are more evenly scattered between the extreme values. Also shown (Fig. 4B as well as Fig. 3B) is that the expected facilitation effect for ICF is only evident in 45% of the cases for A-ppTMS and 70% for FS-ppTMS (no significant difference, $p = 0.11$, 2-sample z-test). By contrast, the expected inhibition effects for SICI and LICI were established in the majority (>90%) of subjects for both types of tests.

3.4. Differences in test stimulus intensities for A-ppTMS and FS-ppTMS

Since the two methods (FS-ppTMS and A-ppTMS) differed in how the TS was set for each CS-TS pair of stimulations, the FS-ppTMS TS intensity (pre-set) was compared to the A-ppTMS TS (given by the final ML-PEST estimate) in relation to the rMT. For the whole group of subjects, the A-ppTMS TS was significantly higher for SICI ($p = 0.02$, 137 ± 60%) and lower for ICF ($p < 0.01$, 105% ± 13%) compared to the 120% rMT in FS-ppTMS. There was also a trend towards higher LICI TS intensities ($p = 0.05$, 125 ± 24%). Taken together, the main differences between the two methods compared (FS-ppTMS and A-ppTMS) were the strategy to set the TS (fixed vs. adaptive), the actual TS intensities (as presented above) and the number of trials for each subject and test (10 vs. 15).

4. Discussion

The present study clearly demonstrated that the adaptive ppTMS (A-ppTMS) technique was able to detect changes in cortical excitability from SICI, ICF and LICI and that the adaptive ppTMS

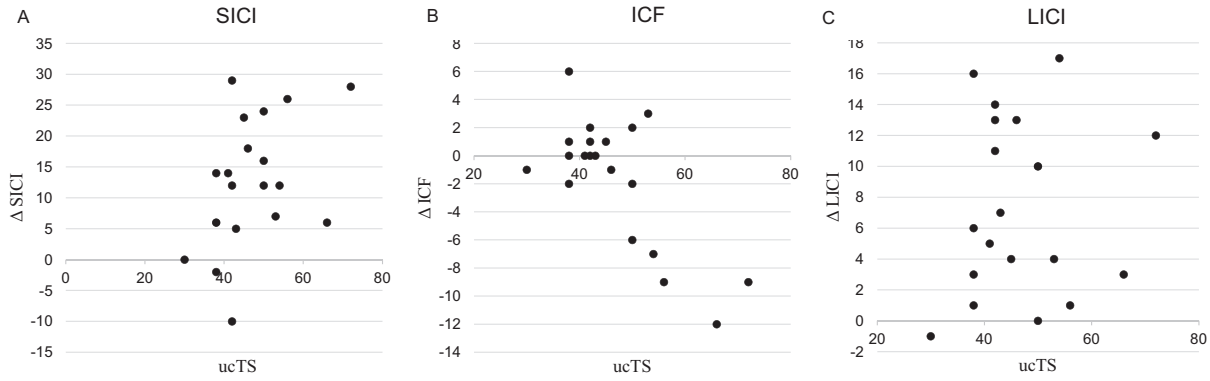

Fig. 3. Excitability effects (ΔSICI, ΔICF and Δ LICI) in relation to unconditioned test stimulus intensities (ucTS). Data from twenty subjects. The conditioning effect (expressed as the difference (Δ) between the conditioned and unconditioned TS intensities) plotted against ucTS intensities for SICI (A), ICF (B) and LICI (C). Positive values on the y-axis indicate inhibition and negative values indicate facilitation. Eleven out of the 20 subjects did not demonstrate ICF (B). All values on X and Y-axes represent TMS machine output %.

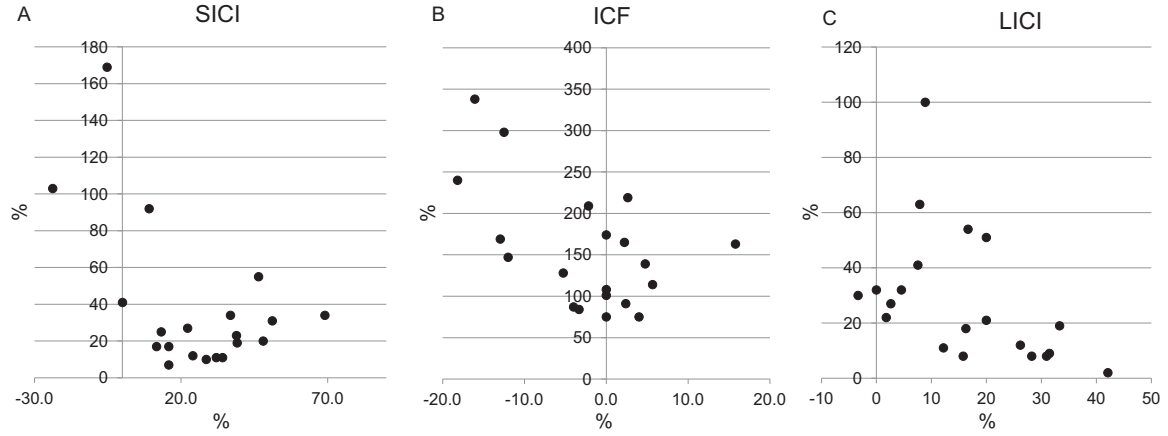

Fig. 4. Correlation between fixed stimulus ppTMS and adaptive ppTMS indices for SICI (A), ICF (B) and LICI (C). Fixed stimulus ppTMS data are presented on the Y-axis where values below 100% indicate inhibition. Normalised adaptive ppTMS indices are presented on the X-axis where values above 0% indicate inhibition.

measures correlate to those obtained from the more conventional fixed stimulus (FS-ppTMS) method. A possible advantage of the adaptive technique is that the excitability measures (expressed as either normalized values or absolute machine output) do not exhibit 'floor/ceiling' effects with clustering of SICI and LICI measures at near-maximal level which may preclude detection of further increased inhibition induced by an experimental intervention. A-ppTMS may thus be more sensitive in detecting differences in SICI and LICI as compared to FS-ppTMS since it is not limited to a particular range of MEP amplitude reduction following the conditioning stimulus (Awiszus et al., 1999; Boroojerdi et al., 2001; Fisher et al., 2002; Wassermann, 2002). There was, however, a similar phenomenon for the adaptive ICF measures in the sense that ~50% of the subjects did not exhibit facilitation, with a tendency for the data to gather around the zero effect level as shown in Fig. 3b. It is of interest to note that Vucic et al. (2011), using an adaptive 'threshold-hunting' technique (Bostock, 1998), had similar difficulties (as judged by their data) to detect the ICF effect in normal subjects. We hypothesise that, at least in our study, the lack of ICF effect could be explained by the relatively low (105% rMT) average TS intensity for A-ppTMS compared to the commonly recommended 120% rMT for FS-ppTMS. In a study (Garry and Thomson, 2009) elucidating the effect of TS intensity on SICI, it was shown that SICI was not observed when the TS was less than 110% of the resting MT. It was suggested that TS intensities at this level and below do not generate late I-waves sensitive to intracortical inhibition (Di Lazzaro et al., 2008). It can then be speculated that a similar mechanism explains the limited ICF response among the subjects, i.e., a lack of facilitatory influence on late I-waves elicited by the relatively low-intensive adaptive TS.

A potential limitation of the study was that the TMS-MEP recruitment curve for the FDI muscle in each subject was not examined in order to choose the proper MEP threshold for the A-ppTMS procedure (Kukke et al., 2014). Establishing the recruitment curve may, for instance, have resulted in higher TS intensities for the adaptive ICF and thus possibly demonstrating ICF in more subjects. Another possible explanation for the limited ICF effect in the A-ppTMS trials was the strength of the conditioning stimuli which was set to 80% MT. Increasing the CS intensity somewhat above this level seems to be more favourable for ICF studies (Kossev et al., 2003; Du et al., 2015). In the TMS guidelines by Rossini et al. (2015) it was pointed out that in individuals with a pathological change in MT, it may be inadequate to only try a single CS intensity (e.g., 80% MT) but rather use a range of CS intensities in order to demonstrate inhibiting and facilitating effects. In this context we may also add that in our study, the subjects with low rMT

to a lesser extent demonstrated the expected facilitatory effect from ICF and also exhibited a less pronounced inhibition from SICI (Fig. 3a and b) which may be due to a similar problem with the CS not being sufficiently strong to produce the desired conditioning effect on the neural network. Similarly, an ideal experimental set-up should have included different inter-stimulus intervals for SICI, ICF and LICI in order to demonstrate excitability changes from ppTMS to a larger extent than observed in the present study. For example, including a 2 ms inter-stimulus interval for SICI is likely to be more optimal for measurement of inhibition than the chosen 3 ms interval (Peurala et al., 2008).

Finally, it is important to point out that although correlations were established between the excitability measures from A-ppTMs and FS-ppTMS, this does not necessarily implicate a commonality of probed intracortical networks since the TS intensity significantly differed between the two methods and thus possibly recruited different networks.

As shown by Vucic and collaborators (e.g., Vucic et al., 2006; Vucic et al., 2011), threshold-tracking techniques offer unique opportunities to detect certain measures of cortical excitability by overcoming MEP amplitude variability (Vucic et al., 2006) and the 'floor/ceiling effect' of SICI and LICI mentioned above. This, in turn, has proven valuable in a clinical setting where threshold-tracking TMS techniques (particularly SICI measurements) may provide earlier diagnosis of ALS, reliably distinguish ALS from mimic disorders (Vucic et al., 2011; Menon et al., 2015a), and identify important features of ALS pathogenesis (Geevasinga et al., 2015; Menon et al., 2015b). To make this available and useful to the individual patient outside a research laboratory setting, however, optimization of the adaptive techniques are warranted to make them more efficient and manageable. In ppTMS studies an accurate estimate of the individual threshold is usually not available in advance. As stated by Lieberman and Pentland (1982), the best-PEST algorithm is preferable in this case to other tracking procedures such as the conventional staircase procedure and Wetherill tracking. Best-PEST is considered relatively insensitive to the initial choice of step size and starting point and generally reaches a certain level of accuracy in fewer trials. It is noteworthy that the original implementation of the best-PEST model was for psychophysical discrimination tasks (Pentland, 1980) where stimulus responses are often classified as either "yes" or "no". The corresponding "yes" response for TMS applications is whether the MEP response is above a predetermined amplitude (e.g., 500 μV) and the model as such does not make any further use of information concerning the actual MEP amplitude. The core function of the ML-PEST/best-PEST model could be any sigmoid function describing the probability of obtaining a "yes" (or MEP response above a certain amplitude) with increasing stimulus strength. Whether it would be preferable to use a different slope (β, see Methods) of the logistic function or to choose another sigmoid "psychometric" core function for the adaptive model seems to be unexplored.

The software application used in the present study for the best-PEST calculation also automates randomization of the different measures which reduces the potential influence of fluctuations in cortical excitability during testing. Applying the ML-PEST algorithm to excitability studies may thus be a more efficient way to reach an accurate threshold estimate, i.e. a measure of cortical excitability, while keeping the advantages gained from adaptive techniques when compared to the conventional fixed-stimulus ppTMS. Whether A-ppTMS indeed offers more confident data in a shorter time remains, however, to be studied. Further evaluation of e.g. test-retest variability, termination criteria (e.g., the number of stimuli, intra-rater/inter-rater variability) need to be performed. If such studies show satisfactory results, it is not unlikely that adaptive techniques for ppTMS studies will gradually replace some of the more well-established fixed-stimulus methods in TMS research.

Conflict of interest

The authors have no conflicts of interest.

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