Brain Stimulation xxx (2014) 1–7

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.brainstimjrnl.com

Original Research

Inter- and Intra-individual Variability Following Intermittent Theta Burst Stimulation: Implications for Rehabilitation and Recovery

Mark R. Hinder [a,*,1], Emily L. Goss [a,1], Hakuei Fujiyama [a,b], Alison J. Canty [c], Michael I. Garry [a], Jennifer Rodger [d], Jeffery J. Summers [a,e]

[a] Human Motor Control Laboratory, School of Medicine, University of Tasmania, Private Bag 30, Hobart, Tasmania 7001, Australia
[b] Movement Control and Neuroplasticity Research Group, Department of Kinesiology, KU Leuven, Belgium
[c] School of Medicine, University of Tasmania, Australia
[d] Experimental and Regenerative Neuroscience, School of Animal Biology, University of Western Australia, Australia
[e] Research Institute for Exercise and Sport Sciences, Liverpool John Moores University, UK

ARTICLE INFO

Article history:
Received 19 August 2013
Received in revised form
3 January 2014
Accepted 4 January 2014
Available online xxx

Keywords:
TMS
Theta burst stimulation
Inter- and intra-individual variability
Cortical plasticity

ABSTRACT

Background: The continued refinement of non-invasive brain stimulation (NBS) techniques is indicative of promising clinical and rehabilitative interventions that are able to modulate cortical excitability. Intermittent theta burst stimulation (iTBS) is one such technique that can increase cortical excitability, purportedly via LTP-like mechanisms. While iTBS may have the capacity to promote recovery after neurological injury, and to combat cognitive and motor decline, recent reports observed highly variable effects across individuals, questioning the efficacy of iTBS as a clinical tool.
Objective: The aim of this study was to examine intra-individual reliability and inter-individual variability in responses to iTBS.
Methods: Thirty healthy participants completed two experimental sessions of the iTBS protocol 1–3 weeks apart. Motor evoked potentials in response to single pulse TMS were used to assess corticospinal excitability prior to, and up to 36 min following, iTBS.
Results: At the group level, iTBS evoked statistically significant increases in motor cortical excitability across both sessions ($P < 0.001$), with 22 out of 30 participants exhibiting increases in excitability in both sessions. A strong intraclass correlation demonstrated that both the direction, and magnitude of the plastic changes were reliable at the individual level.
Conclusions: Overall, our results suggest that iTBS is capable of inducing relatively robust and consistent effects within and between young individuals. As such, the capacity for iTBS to be exploited in clinical and rehabilitative interventions should continue to be explored.

Introduction

The human brain has the capacity to undergo adaptive modification to external environmental change and to reorganize itself in response to physiological degeneration or damage [1,2]. The development of techniques that are capable of augmenting cortical plasticity therefore have the potential to help combat motor and cognitive decline associated with normal aging [3]. Furthermore, such techniques may play a critical role in promoting recovery of function after brain injury [4]. Accordingly, the number of studies utilizing non-invasive brain stimulation (NBS) techniques to induce cortical plasticity within the human motor cortex has dramatically increased [5,6].

One type of NBS that has been purported to result in robust changes in cortical excitability is theta burst stimulation (TBS) [5]. TBS is a variant of repetitive transcranial magnetic stimulation (rTMS) that uses high frequency, sub-threshold bursts of stimulation. TBS is an appealing technique for application in clinical populations as it requires less stimulation time and lower stimulation intensity than traditional rTMS protocols [5]. Huang et al. [5] reported that intermittent TBS (iTBS) elicited an increase in the amplitude of motor evoked potentials (MEPs) – indicative of increases in cortical excitability – that persisted beyond the period of stimulation, while continuous TBS (cTBS) was found to significantly

This work was supported by an NHMRC project grant (JJS, AJC, MRH, MIG and JR) and an ARC DECRA fellowship (MRH).
* Corresponding author. Tel.: +61 (03) 6226 2243.
E-mail address: Mark.Hinder@utas.edu.au (M.R. Hinder).
[1] These authors contributed equally to the work.

1935-861X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.brs.2014.01.004

depress MEP size following stimulation. The authors described the effects, observed in a group of nine healthy individuals, as controllable, consistent, long-lasting and powerful. As the effects of TBS appeared to be larger, and somewhat less variable, than standard rTMS protocols [7], it was inferred that TBS may be a promising alternative for eliciting plastic change within the corticospinal network. A number of studies have since replicated Huang and colleague's results by demonstrating that, averaged across a group of participants, iTBS results in long-term potentiation (LTP)-like plasticity and increases MEP amplitude, while cTBS causes long-term depression (LTD)-like plasticity [6,8–12].

However the belief that such techniques are able to induce consistent, robust and relatively long-lasting changes in cortical excitability has recently been brought into question by a number of studies that have failed to observe the anticipated plasticity-inducing effects (across a group of participants) for both cTBS [13,14] and iTBS [14]. Furthermore, in the first of the experiments reported within Todd et al. [15] cTBS was observed to have a modulatory effect on excitability; however in another series of experiments within the same paper cTBS and iTBS did not significantly modulate excitability.

It seems plausible that the relatively small sample sizes (generally $n < 15$) common across the TMS literature, combined with an emphasis on statistical significance [16–18], rather than interpretation of the magnitude of effects, has contributed to these somewhat disparate reports. Cumming [19] noted that interpretation of confidence intervals, rather than *P*-values, appears to be a better way of interpreting results across studies. Indeed, with small sample sizes confidence intervals are generally wide (low precision) such that two studies can have substantially different *P*-values, one 'significant' and the other 'non-significant,' yet their results may be entirely consistent as reflected by a large overlap of 95% confidence intervals [19]. From this perspective apparent discrepancies among studies may be more illusory than real. Another possibility, not mutually exclusive from the above scenario, is that intrinsic variability within the technique for assessing change in plasticity (i.e., single pulse TMS) may result in some disparity between different studies' conclusions with regard to the efficacy of TBS for inducing plastic changes.

Alongside the aforementioned inconsistency of TBS group effects, recent studies have also reported highly variable responses within groups of participants to both TBS [14,15] and other NBS protocols (e.g. paired-associative stimulation, PAS [20]). Hamada et al. [14] reported that responses to both iTBS and cTBS were highly variable across a group of 52 individuals and, at the group level, neither form of stimulation elicited significant changes in corticospinal excitability. Moreover, a high proportion of the individuals exhibited excitability changes in the opposite direction to those that would be expected according to our current understanding of LTP- and LTD-like plasticity inducing protocols of iTBS and cTBS, respectively [5].

Such variability between individuals can be seen as problematic in regard to the potential efficacy of a TBS paradigm for inducing plastic changes, e.g. as an intervention to improve motor or cognitive function across a population of stroke survivors or those recovering from traumatic injury that has resulted in loss of muscle strength or coordination. Nevertheless, if the TBS-induced changes are reliable within an individual, one could predict after a single session whether an individual is likely to benefit from that particular (plasticity-inducing) intervention. In this case, TBS may still prove beneficial for individuals who exhibit a substantial response to the intervention. Thus, the issues of inter- and intra-individual variability in TBS appear to be intrinsically linked, with inadequate information existing in regard to both types of variability (for further discussion on inter- and intra-individual variability of NBS, particularly in regard to potential clinical applications, see Hinder et al. [21]).

Vernet et al. [22] made a first step in assessing intra-individual reliability in responses to cTBS, thought to induce LTD-like plasticity. They reported some degree of reliability of responses across two sessions (conducted an average of 100 days apart), but only tested ten participants with a very large age range. Given that age can affect plastic responses to non-invasive brain stimulation [23] it is difficult to ascertain the degree to which the relatively small sample of participants with vastly varying ages may have affected their findings.

To date there is no literature that systematically investigates whether individual responses to iTBS are robust and reliable such that someone who exhibits a large response to iTBS on one day would also exhibit similar changes in a subsequent session. The present study was therefore conducted on thirty individuals (18–44 years) who received iTBS in two sessions one to three weeks apart. While cTBS has been reported to induce more robust and reliable aftereffects than iTBS [24] (but also see Ref. [22]) the current study focused on iTBS due to its potential in rehabilitation via the induction or promotion of LTP-like effects (e.g. increasing the excitability of pathways that have been down-regulated following injury). Our analyses focused on inter- and intra-individual variability of iTBS-induced changes in corticospinal excitability.

Materials and methods

Participants

Thirty healthy volunteers (11 males) aged between 18 and 44 years (Mean (M) = 25.3, standard deviation (SD) = 8.7) participated in this study which was approved by the Tasmanian Human Research Ethics Committee Network and followed the international safety guidelines and recommendations for TMS [25]. All participants gave written informed consent, and completed a medical history questionnaire which confirmed the absence of any known neurological and neuromuscular dysfunction and any contraindications to TMS. Twenty-seven participants were right handed (laterality quotient, LQ, = 86.7, SD = 12.6, Range = 65–100), two were left handed (LQ = −42.5, SD = 3.5, Range = −45 to −40), and one was within the mid range (LQ = −25) [26]. All participants completed two separate sessions of the iTBS protocol (see TBS technique section, below, for details), at least one week apart (Range = 7–21 days). For each participant, both sessions were conducted at the same time of day to account for any diurnal effects on corticospinal plasticity [27].

Experimental procedure

The study was designed to assess intra- and inter-individual variability in responses to iTBS. Corticospinal excitability of projections from the left motor cortex was assessed by recording evoked potentials in the right index finger in response to single pulse TMS (see Electromyography and Transcranial magnetic stimulation sections below). Baseline corticospinal excitability was assessed in two separate blocks of TMS stimulations 3 min apart, after which iTBS was administered (see TBS technique section). Following iTBS, excitability was reassessed every 3 min for 36 min (i.e., 13 post-iTBS time points: post0, post3…, post36).

Electromyography

Participants were seated in a comfortable chair with both forearms quiescent. Electromyographic (EMG) activity was recorded

from the right first dorsal interosseous (FDI) using a pair of Ag/AgCl disposable electrodes arranged in a belly-tendon montage at 5 kHz (CED Power1401, Cambridge, United Kingdom), amplified (×1000), before being band-pass filtered (20–1000 Hz) and stored for offline analysis. Visual feedback of EMG activity allowed muscle relaxation to be monitored, and participants were instructed to relax their hand when necessary.

Transcranial magnetic stimulation

Single-pulse TMS was performed using a Magstim 200^2 stimulator (Magstim Company, UK) and a figure-of-eight coil (internal wing diameter 70 mm). The coil was positioned tangentially to the skull with the handle pointing backwards and laterally at 45° to the sagittal plane resulting in posterior–anterior (p–a) current flow in the brain. Standard procedures were used to locate the motor 'hotspot.' Resting motor threshold (RMT) was defined as the lowest stimulator intensity required to elicit three out of five MEPs of ≥50 μV amplitude in the resting right FDI [28,29]. Corticospinal excitability was assessed by eliciting MEPs in the right FDI using 15 single pulse MEPs, delivered at 130% RMT with an inter-stimulus-interval (ISI) of 6 s ± 20% (i.e., 4.8–7.2 s). A neuronavigation device (Visor & Xensor TMS Neuronavigation, eemagine Medical Imaging Solutions GmbH, Berlin, Germany) was used to ensure consistent coil placement within each experimental session (for both single pulse TMS, and iTBS, see below).

Active motor threshold (AMT) was determined using a Magstim Super Rapid^2 stimulator (Magstim Company, UK) during voluntary contraction of the right FDI muscle at 10% of their individual maximum voluntary contraction (MVC). To determine MVC, participants were asked to isometrically abduct their right index finger as hard as possible against a force transducer for three times for 2 s with ~10 s rest between each contraction. The averaged peak value across the three contractions was defined as MVC. 10% MVC was maintained by the participant using visual feedback. AMT was defined as the minimum stimulator intensity required to evoke MEPs of ≥200 μV in three out of five trials [5]. The coil was held at 45° to the sagittal plane resulting in p–a/a–p current flow in the 400 μs wide biphasic pulse.

Please refer to Ancillary information for methods, analysis and results investigating the purported relationship linking the latency difference between MEPs measured in a–p versus lateral–medial (l–m) coil orientations and response to iTBS [14].

TBS technique

iTBS intensity was set for each participant at 80% AMT and was applied over the hotspot using the Magstim Super Rapid^2 stimulator. iTBS involved 2 s trains of TBS (3 pulses at 50 Hz repeated at 200 ms intervals) that occurred every 10 s for a total of 190 s (600 pulses) [5]. The current flow (in the brain tissue) in the 400 μs wide biphasic pulse was p–a/a–p. During iTBS participants kept their arms relaxed and looked passively forwards.

Data analysis and statistical procedures

All inferential statistical procedures were undertaken using IBM Statistics SPSS (Version 21). RMT and AMT were compared between sessions using paired *t*-tests. Trials in which rms (root mean square) EMG exceeded 0.015 mV in a 40 ms time window immediately prior to TMS stimulation were excluded from analysis due to the effect that muscle activity has on measures of excitability. Corticospinal excitability was then determined as the peak-to-peak MEP amplitude in the right FDI muscle in a time window 20–80 ms following single pulse TMS at each time point (two baseline and 13 post-iTBS measures). The two baseline measures in each session were compared using two-way (block × session) analysis of variance (ANOVA). Corticospinal excitability at each time following iTBS was normalized to the average corticospinal excitability prior to iTBS (i.e., the average of the two baseline measures). Normalized MEPs (nMEP) were then log-transformed prior to analysis [30] to reduce skewness of data that is otherwise inherent in ratios. Log-transformed ratios >0 represent post-iTBS facilitation, whereas values <0 represent inhibition. Two-way repeated measures ANOVA with the factors session (S1, S2) and time (13 post-iTBS time points) was then undertaken; the grand mean was assessed to determine whether, averaged over all variables (i.e., both sessions and all time points), there was a significant potentiation of MEPs. Specifically, we assessed whether the grand mean of the log-transformed normalized MEPs was significantly greater than 0. Significant main effects and interactions were further explored using Bonferroni-adjusted post-hoc comparisons. The a-priori alpha level was set at 0.05. Huynh–Feldt corrections were applied if the assumption of sphericity was violated ($\varepsilon < 0.7$). Partial eta-squared (ANOVA) and Cohen's *d* (paired *t*-tests) are presented as a measure of effect size and used to aid, along with 95% confidence intervals, in the interpretation of the tests of significance. Furthermore, specific determination of eta-squared values within the omnibus ANOVA permit us to directly compare and contrast the proportion of the variance accounted for by intra- and inter-individual effects.

Linear regression and intraclass correlations (ICCs) were used to investigate the reliability of RMT, AMT baseline MEPs and iTBS-induced changes in corticospinal excitability for each individual across the two sessions. Linear regression indicates the degree of association between two variables (which may differ in absolute size), while ICC also takes in account the relative magnitude of each variable. For correlations pertaining to iTBS-induced changes in excitability, we determined the average log-transformed MEP ratio across all 13 post-iTBS time points in each session and using the first 8 time points (i.e., post0–post21). Furthermore, we conducted similar regressions/ICC at the individual time point where (at the group level) the greatest excitability change was observed, and the point where a quadratic function fit representing post-iTBS excitability change exhibited its peak value. The correlation using the first 8 time points was undertaken to replicate the duration of the post-stimulation time epoch studied in the original paper using iTBS in humans [5], while the single time points measures were examined to determine whether, despite the intrinsic variability of responses to single pulse TMS, 15 MEPs in a single time epoch can be used as an indication of the reliability of a plasticity-inducing protocol, i.e., iTBS. Finally, to determine the temporal reliability of corticospinal excitability, we conducted one-way ANOVA, linear regression and ICC on the time epoch (e.g. post15, post18) where the maximum change in corticospinal excitability occurred in each session for each individual.

Results

All participants completed both experimental sessions without any reported side effects or negative events. Results are reported as mean ± 95% confidence intervals (CI).

Motor threshold values and baseline MEPs

Expressed as a percentage of maximum stimulator output, RMT (S1: 39.9 ± 2.4%; S2: 39.7 ± 2.4%) and AMT (S1: 47.7 ± 2.1%; S2: 48.1 ± 2.2%) did not differ significantly between the two sessions

(paired t-test: AMT: $t(58) = 0.08$, $P = 0.94$, $d = 0.029$; RMT: $t(58) = 0.26$, $P = 0.80$, $d = 0.067$). The higher values for AMT (relative to RMT) are due to the less powerful biphasic rapid stimulator used for AMT assessment and subsequent iTBS. We observed very high intra-individual reliability of the values obtained in the two sessions, for both RMT ($r_{ICC} = 0.965$; $P < 0.001$) and AMT ($r_{ICC} = 0.909$; $P < 0.001$).

Baseline MEPs did not vary significantly between the two baseline blocks within each session ($F(1,29) = 0.193$, $P = 0.664$, $\eta_p^2 = 0.007$) or as a function of session ($F(1,29) = 2.833$, $P = 0.103$, $\eta_p^2 = 0.089$) [S1 block 1: 1.75 ± 0.41 mV; S1 block 2: 1.66 ± 0.42 mV; S2 block 1: 1.97 ± 0.54 mV; S2 block 2: 1.99 ± 0.50 mV]. The interaction of session and block was not statistically significant ($F(1,29) = 0.403$, $P = 0.531$, $\eta_p^2 = 0.014$).

Furthermore, there was a high degree of intra-individual reliability of the two measures of baseline excitability obtained in each session (S1: $r_{ICC} = 0.873$; $P < 0.001$; S2: $r_{ICC} = 0.869$; $P < 0.001$). For all subsequent analyses, the measure of baseline excitability in each session was taken as the average MEP across the two baseline (pre-iTBS) blocks. These averaged baseline values exhibited a high intraclass correlation between sessions ($r_{ICC} = 0.744$; $P < 0.001$).

iTBS-induced plasticity

Figure 1 (presented for qualitative interpretation only) illustrates iTBS-induced increases in cortical excitability (based in raw MEPs in mV) in both sessions.

Log-transformed normalized MEPs (see Materials and Methods section) are presented in Fig. 2. Over both sessions and all time points the average increase in excitability following iTBS was 30.2% (95% CI: +17.1% to +44.8%) which was statistically significant and associated with a large effect size ($F(1,29) = 25.762$, $P < 0.001$, $\eta_p^2 = 0.470$).[2] The main effect of session was not significant and was associated with a very small effect size ($F(1,29) = 0.659$, $P = 0.457$, $\eta_p^2 = 0.019$), indicating the effect of iTBS on the group did not vary significantly between the two sessions. The main effect of time was statistically significant ($F(12,348) = 8.414$, $P < 0.001$, $\eta_p^2 = 0.225$). To further elucidate the nature of this time main effect we assessed the contrasts which yielded significant linear ($P < 0.001$, $\eta_p^2 = 0.495$) and quadratic ($P = 0.001$, $\eta_p^2 = 0.299$) components. With reference to Fig. 2, the linear and quadratic components indicate a gradual (linear) increase in the magnitude of the excitability change. Peak excitability increase was observed 30 min following stimulation followed by a small reduction in the extent of the excitability gain in the final two time epochs. Averaged across both sessions, the excitability increase at post0 was not statistically significant; however there was a statistically significant increase in excitability at all post3–post36 time points, with the largest ln nMEP value occurring 30 min post-stimulation. The peak excitability gain in the least-squares fit quadratic function best representing the data occurred at the post27 time point. The interaction between session and time was not statistically significant ($F(12,348) = 1.077$, $P = 0.378$, $\eta_p^2 = 0.036$). Finally, a comparison of the eta-squared values from within the omnibus ANOVA reveal that 41.4% of the total variance was accounted for by inter-individual variability (i.e., $\eta^2 = 0.414$), while intra-individual variability accounted for a substantially lower percentage (12.6%) of the total variance (i.e., $\eta^2 = 0.126$).

[2] The average excitability increase and associated confidence intervals are calculated by back-transforming the grand-mean log ratio. The average excitability increase reported is thus equivalent to the geometric mean of the normalized MEP values.

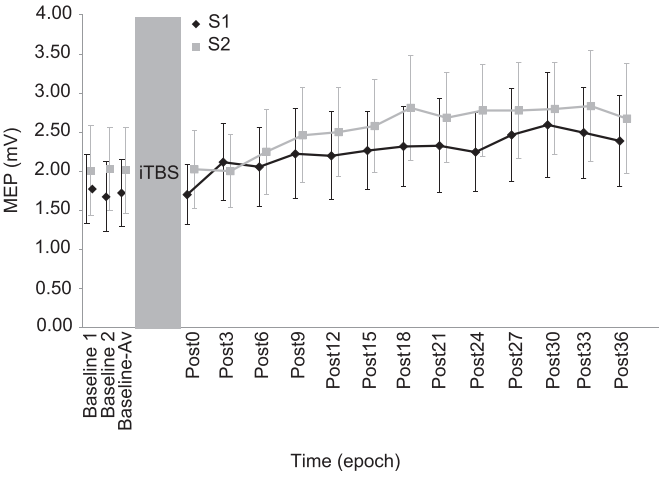

Figure 1. Right FDI MEP amplitudes (group average, $N = 30$) for session 1 (black line) and session 2 (gray line) conducted ≥ 1 week apart. Error bars represent 95% CI.

Inter- and intra-individual effects

To assess whether the effect of iTBS observed at the group level was observed for all individuals, and whether each individual exhibited a similar magnitude change in excitability in both sessions, we assessed data at the individual participant level. Figure 3A shows the average change in excitability across all post-stimulation time points (expressed as a log-transformed ratio relative to pre-stimulation excitability) for each individual in session 1 (abscissa) and session 2 (ordinate). The majority of participants (22 out of 30) exhibited an increase in corticospinal excitability in response to iTBS in both sessions. Five participants displayed this excitability increase in only one session (S1 or S2) and the opposite response in the other session, while three participants exhibited the opposing response (i.e., a decrease in excitability) in both sessions. Linear regression revealed a strong correlation between the excitability change in each session ($r = 0.55$, $P = 0.001$). Intraclass correlation also yielded a strong correlation ($r_{ICC} = 0.534$; $P = 0.001$), indicating good reliability (in terms of direction and magnitude of responses) of each individual's response to iTBS across sessions. Further regression

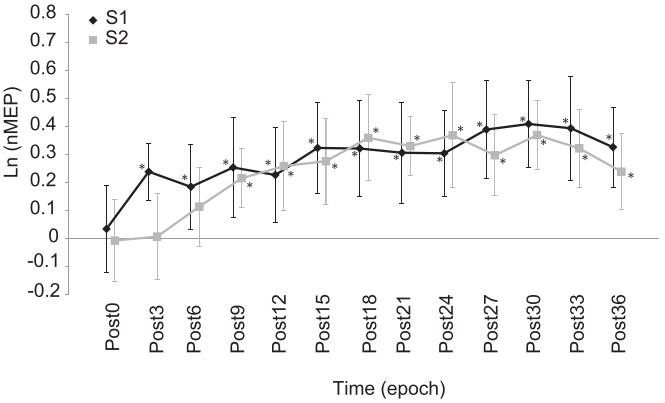

Figure 2. Log-transformed (Ln) normalized MEP amplitudes following iTBS (group averages) in session 1 (S1) and session 2 (S2). Ln(1) = 0 such that a ratio of 1 between pre- and post-stimulation excitability is depicted as 0. Error bars represent 95% CI as such error bars that lie completely above zero represent a statistically significant increase in excitability relative to baseline. * indicates significant ($P < 0.05$) increase in excitability relative to baseline.

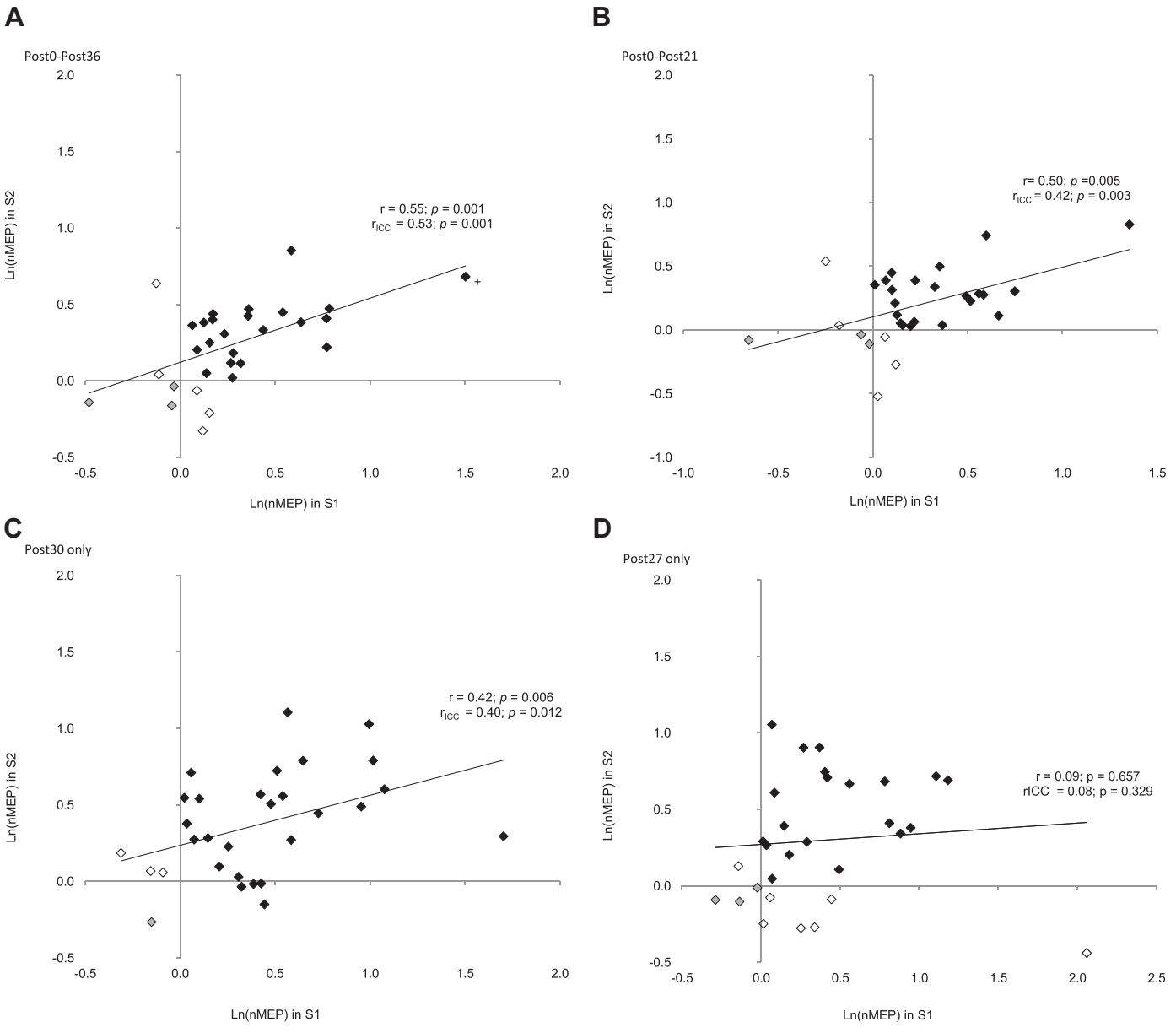

Figure 3. Individual participants' responses to iTBS in session 1 (S1: x-axis) and session 2 (S2: y-axis). A) Data averaged across all post-stimulation time points. The majority of participants (22/30, solid black diamonds) exhibited excitability increases in both sessions, 5/30 participants (open diamonds) showed excitability increases in 1 of the 2 sessions (and excitability decreases in the other session) while 3/30 participants (solid gray diamonds) exhibited excitability decreases in both sessions. We note that the participant who had a substantial excitability change in both sessions (denoted by the + symbol) does not overly affect the overall regression i.e., with this participant excluded from the analyses, a significant group effect in both sessions are still observed, together with significant linear ($r = 0.49$) and intraclass ($r_{ICC} = 0.49$) correlations. B) Data averaged over the post0–post21 period (as per Huang et al., 2005 [5]). C) Data for the post30 time point only. D) Data for the post27 time point only.

analyses were undertaken on parameters reflecting the average excitability change over the first 8 time points (post0–post21; Fig. 3B), the excitability change at the time point where (at the group level) the greatest excitability change was observed (post30; Fig. 3C), and at the peak of the fitted quadratic function of the time main effect (post27; Fig. 3D). Averaging over the first 8 time points yielded a similar result to that obtained using all 13 post-stimulation time points, i.e., a statistically significant linear relationship ($r = 0.503$, $P = 0.005$) and intraclass correlation ($r_{ICC} = 0.486$; $P = 0.003$). While linear regression ($r = 0.418$, $P = 0.006$) and intraclass correlation ($r_{ICC} = 0.406$; $P = 0.012$) conducted on the post30 time point yielded statistically significant correlations, regressions conducted on the post27 time point ($r = 0.085$, $P = 0.657$; $r_{ICC} = 0.083$; $P = 0.329$) were not statistically significant and were associated with low correlation coefficients, despite the fact that the excitability increase across the group was very similar in both sessions at both these time points (as shown in Fig. 2).

Reliability of temporal aspects of excitability change

The preceding analyses do not consider the time frame following stimulation in which the greatest changes in excitability were observed. To determine whether this time was consistent across the two sessions we first determined the epoch (e.g. post18, post30) of maximum excitability change for each individual in both sessions. One-way ANOVA and intraclass correlation were then undertaken on these values. ANOVA revealed that the temporal

location of peak excitability change (S1: 22.2 min; S2: 21.8 min) did not differ significantly as a function of session, $F(1,29) = 0.026$, $P = 0.874$, $\eta_p^2 = 0.001$). This corresponds relatively well to the group-average post-stimulation excitability traces (Fig. 2) where the largest change (in the group average trace) was seen to occur at time points between 18 and 33 min post-stimulation), with the actual peak in both sessions occurring at the post30 time point. However, intraclass correlation revealed an extremely low correlation ($r_{ICC} = 0.025$; $P = 0.446$) between the two sessions, suggesting that the temporal response for each individual was not reliable between the two sessions.

Discussion

The present study examined the intra- and inter-individual variability of responses to iTBS across two sessions. Across the group of thirty participants the target muscle (right index finger abductor, FDI) exhibited a statistically significant increase in corticospinal excitability following iTBS, the magnitude of which was not statistically different across the two sessions. Averaged over a 36 min post-stimulation period in both sessions, MEPs were potentiated by 30% relative to pre-stimulation values; this increase was associated with a large effect size ($\eta_p^2 = 0.470$) suggesting a substantial effect of the intervention at the group level. Examination of individuals' data revealed that the 22 out of 30 (73%) participants exhibited an increase in excitability following iTBS in both sessions. If one were to set a 10% increase as a nominal cut-off for potentially clinically-relevant changes in cortical excitability, 18/30 (60%) participants are observed to meet this criterion in both sessions (Fig. 3).

Five participants showed an expected increase in excitability in response to iTBS in only one session (either session 1 or 2) and an unexpected response (i.e., a decrease in corticospinal excitability) in the other. Only three participants exhibited decreases in excitability in both sessions; however only one of these participants exhibited decreases greater than 10% in both sessions (Fig. 3). Thus, it could be surmised that only one participant exhibited a clinically-relevant change in excitability in the *opposite* direction to that which iTBS is expected to elicit. More research is certainly warranted to determine what magnitude change in cortical excitability may lead to clinically-relevant improvement in outcome measures such as motor or cognitive function. Only then can it be determined the suitability of an individual for therapies involving stimulation methods such as iTBS.

Across all individuals linear regression ($r = 0.55$) and intraclass correlation ($r_{ICC} = 0.53$) revealed a relatively strong correlation between each individual's plasticity response (averaged over all 13 post-stimulation time points) in the two sessions. Specifically, the fact that linear regression and ICC yielded very similar correlation coefficients indicates that not only can an individual's response in a second session be predicted from that in the first session, but that the magnitude of increased excitability is not dissimilar between sessions. The ICC result, indicating that there is a relatively high degree of intra-individual reliability, is consistent with the eta-squared values obtained from the omnibus ANOVA (comparable to variance component analysis [31]), which revealed that there was a significantly lower proportion of the total variance accounted for by intra-individual (12.6%) compared to inter-individual effects (41.4%).

Similarly robust findings were observed when we conducted regression over the first 21 min (8 time points), a post-stimulation period equivalent in duration to that studied in previous work [5]. However, when we restricted our analysis to a single time epoch, inconsistent results were observed. Specifically, statistically non-significant linear regression and intraclass correlation were observed 27 min post-iTBS, whereas these regressions were statistically significant when undertaken at the adjacent time point (30 min post-iTBS). This apparent contradiction in results occurred despite the fact that overtly similar changes were observed in excitability at the group level at these time points in both sessions (Fig. 2). Overall, the present findings therefore provide support for the notion that, in the majority of participants, iTBS is capable of inducing changes in corticospinal excitability which are reliable across different sessions, i.e., good inter- and intra-individual reliability. However, it is apparent that the intrinsic variability of responses to single pulse TMS may be an important underlying factor in permitting reliability to be observed. As such, the measure of plastic response should be determined over a substantial number of TMS stimuli to permit the true reliability of the plasticity-inducing protocol to be accurately assessed.

Our findings (indicating an increase in excitability in response to iTBS at the group level) are consistent with the group-effect conclusions drawn from a number of previous studies [5,6,8–12]; importantly, however, by testing 30 individuals in two separate sessions this study provides a much more comprehensive test of inter- and intra-individual reliability of iTBS. On the basis of our results, it appears that iTBS provides a promising tool for reliably inducing increases in cortical plasticity which, pending further research may have the potential to be beneficial in clinical and rehabilitative settings for ~60% of the population.

In contrast to the current results, and other published findings [5], Hamada et al. [14] reported that neither iTBS nor cTBS elicited significant changes in excitability averaged across their participant cohort. Furthermore, they also observed considerable inter-individual variability in responses to both TBS protocols (see also Todd et al. [15]). Hamada and colleagues concluded that much of the inter-individual variation in response to TBS could be accounted for by differences in the populations of neurons (interneuron networks) targeted by each single pulse of TMS during assessment of excitability – rather than the intrinsic amount of plasticity in cortical neurons. To form this conclusion they measured the latency of MEPs evoked by single pulses of TMS with anterior(a)–posterior(p) and lateral(l)–medial(m) current directions. The latency difference between the two current directions (which preferentially recruit different interneurons) for each individual correlated with the extent of TBS-induced plasticity. In the present study, we found very little association between the a–p/l–m latency difference and excitability change (see Ancillary information). It is apparent that our latency analysis was conducted after iTBS, at a time when cortical excitability was still likely above baseline levels; however there is no evidence to suggest that this increased excitability affects MEP latency [32], and as such our latency analysis remains valid. Our range of latency differences (generally 0–4.5 ms using the figure of eight coil) was somewhat more restricted than the 0–6.5 ms latency difference range reported by Hamada et al. [14]; however the results of the present study do not support the hypothesis that a latency difference of >4 ms can identify an individual who will exhibit the expected changes to iTBS (as per Ref. [14]) because ~75% of those participants in the current study who exhibited reliable increases in excitability following iTBS exhibited latency differences of less than 4 ms.

With respect to the timing of the induced changes in excitability, we observed the greatest excitability changes between 19 and 33 min post-stimulation (Fig. 2), somewhat later than reported previously (largest changes 1–10 min post-stimulation) [5]. The time of peak excitability change (S1: 22.2 min; S2: 21.8 min) averaged across all individuals did not differ significantly between the two sessions. However intraclass correlation revealed a very low ($r_{ICC} = 0.025$) correlation between the two sessions for individual participants, indicating that there was a lack of replication of

temporal response profiles for each individual. This suggests that assessments of excitability following plasticity-inducing NBS techniques should be conducted with sufficient temporal resolution to chart the excitability changes for each participant (see Ref. [22]). Whether this intra-individual temporal variability reflects physiological differences in the manner in which the motor system responds to iTBS, or whether it rather represents natural variability of single pulse TMS to measure corticospinal excitability remains to be determined.

Summary

The present data confirm that iTBS has the potential to be used as a non-invasive therapeutic tool in a clinical or rehabilitative setting. We show significant inter- and intra-individual reliability of responses with the majority of participants exhibiting the 'expected' increase in excitability changes in response to the stimulation protocol. iTBS should, therefore, continue to be explored as a potential neuromodulatory technique to increase corticospinal excitability in a clinical setting, for example following stroke, or to assist in motor/cognitive learning, where reduced cortical excitability may affect behavior. It should be noted, however, that while the current data indicate a reliable response to iTBS in healthy younger adults, it is not necessarily the case that such reliability would be observed in older adults, or in a patient population. Follow-up studies should therefore target these specific populations. Further investigation is also warranted to explore the intra-individual variation in temporal response to iTBS, such that a robust measure of neuroplasticity can be attained, which may in future be able to predict an individual's response to interventions such as motor or cognitive training.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.brs.2014.01.004.

References

[1] Pascual-Leone A, Amedi A, Fregni F, Merabet LB. The plastic human brain cortex. Annu Rev Neurosci 2005;28:377–401.
[2] Cramer SC, Sur M, Dobkin BH, O'Brien C, Sanger TD, Trojanowski JQ, et al. Harnessing neuroplasticity for clinical applications. Brain 2011;134:1591–609.
[3] Hummel FC, Heise K, Celnik P, Floel A, Gerloff C, Cohen LG. Facilitating skilled right hand motor function in older subjects by anodal polarization over the left primary motor cortex. Neurobiol Aging 2010;31:2160–8.
[4] Ridding MC, Rothwell JC. Perspectives - Opinion - Is there a future for therapeutic use of transcranial magnetic stimulation? Nat Rev Neurosci 2007;8:559–67.
[5] Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–6.
[6] Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, et al. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol 2008;586:3871–9.
[7] Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A. Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. Exp Brain Res 2000;133:425–30.
[8] Doeltgen SH, Ridding MC. Modulation of cortical motor networks following primed theta burst transcranial magnetic stimulation. Exp Brain Res 2011;215:199–206.
[9] Mastroeni C, Bergmann TO, Rizzo V, Ritter C, Klein C, Pohlmann I, et al. Brain-Derived Neurotrophic Factor - a major player in stimulation-induced homeostatic metaplasticity of human motor cortex? PLoS One 2013;8:e57957.
[10] Murakami T, Muller-Dahlhaus F, Lu MK, Ziemann U. Homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory neural circuits in human motor cortex. J Physiol 2012;590:5765–81.
[11] Talelli P, Cheeran BJ, Teo JTH, Rothwell JC. Pattern-specific role of the current orientation used to deliver Theta Burst Stimulation. Clin Neurophysiol 2007;118:1815–23.
[12] Teo JTH, Swayne OB, Rothwell JC. Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. Clin Neurophysiol 2007;118:1649–51.
[13] Goldsworthy MR, Pitcher JB, Ridding MC. The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. Eur J Neurosci 2012;35:125–34.
[14] Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. The role of interneuron networks in driving human motor cortical plasticity. Cereb Cortex 2013;23:1593–605.
[15] Todd G, Flavel S, Ridding M. Priming theta-burst repetitive transcranial magnetic stimulation with low- and high-frequency stimulation. Exp Brain Res 2009;195:307–15.
[16] Button KS, Ioannidis JPA, Mokrysz C, Nosek BA, Flint J, Robinson ESJ, et al. Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci 2013;14:365–76.
[17] Altman DG, Bland JM. Absence of evidence is not evidence of absence. Br Med J 1995;311:485.
[18] Sterne JA, Davey Smith G. Sifting the evidence-what's wrong with significance tests? Br Med J 2001;322:226–31.
[19] Cumming G. Replication and p intervals p values predict the future only vaguely, but confidence intervals do much better. Perspect Psychol Sci 2008;3:286–300.
[20] Muller-Dahlhaus JFM, Orekhov Y, Liu Y, Ziemann U. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp Brain Res 2008;187:467–75.
[21] Hinder MR, Reissig P, Fujiyama H. Noninvasive brain stimulation can elucidate and interact with the mechanisms underlying motor learning and retention: implications for rehabilitation. J Neurophysiol 2014. http://dx.doi.org/10.1152/jn.00766.2013.
[22] Vernet M, Bashir S, Yoo WK, Oberman L, Mizrahi I, Ifert-Miller F, et al. Reproducibility of the effects of theta burst stimulation on motor cortical plasticity in healthy participants. Clin Neurophysiol 2014;125:320–6.
[23] Todd G, Kimber TE, Ridding MC, Semmler JG. Reduced motor cortex plasticity following inhibitory rTMS in older adults. Clin Neurophysiol 2010;121:441–7.
[24] Cardenas-Morales L, Nowak DA, Kammer T, Wolf RC, Schonfeldt-Lecuona C. Mechanisms and applications of theta-burst rTMS on the human motor cortex. Brain Topogr 2010;22:294–306.
[25] Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008–39.
[26] Oldfield RC. The Assessment and Analysis of Handedness: the Edinburgh Inventory. Neuropsychologia 1971;9:97–113.
[27] Sale MV, Ridding MC, Nordstrom MA. Cortisol inhibits neuroplasticity induction in human motor cortex. J Neurosci 2008;28:8285–93.
[28] Carroll TJ, Barry B, Riek S, Carson RG. Resistance training enhances the stability of sensorimotor coordination. Proc Biol Sci 2001;268:221–7.
[29] Hinder MR, Schmidt MW, Garry MI, Summers JJ. The effect of ballistic thumb contractions on the excitability of the ipsilateral motor cortex. Exp Brain Res 2010;201:229–38.
[30] Sinclair C, Hammond GR. Excitatory and inhibitory processes in primary motor cortex during the foreperiod of a warned reaction time task are unrelated to response expectancy. Exp Brain Res 2009;194:103–13.
[31] Sommer M, Wu T, Tergau F, Paulus W. Intra- and interindividual variability of motor responses to repetitive transcranial magnetic stimulation. Clin Neurophysiol 2002;113:265–9.
[32] Di Lazzaro V, Pilato F, Dileone M, Profice P, Capone A, Ranieri F, et al. Modulating cortical excitability in acute stroke: a repetitive TMS study. Clin Neurophysiol 2008;119:715–23.