Inter- and intra-subject variability of motor cortex plasticity following continuous theta-burst stimulation.

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Running title: Reproducibility of cTBS-induced neuroplasticity.

Abstract

Background: The potential of non-invasive brain stimulation (NIBS) for studying, and inducing, functionally relevant neuroplasticity is dependent on protocols that can induce lasting, robust and reliable effects. A current limiting factor is the large inter- and intra-subject variability in NIBS-induced neuroplastic responses. There has been some study of inter-subject response variability and factors that contribute to it; however, intra-subject response variability has, so far, received little investigation.

Objectives: By testing participants on multiple occasions we aimed to (1) compare inter- and intra-subject variability of neuroplastic responses induced by continuous theta-burst stimulation (cTBS); (2) determine whether the transcranial magnetic stimulation (TMS) intensity used to measure cTBS-induced neuroplastic responses contributes to response variability; (3) determine whether assessment of factors known to influence response variability can be used to explain some of the variability in cTBS-induced neuroplastic responses across experimental sessions.

Methods: In three separate experimental sessions, motor evoked potential (MEP) input-output (IO) curves were obtained before and after cTBS, and questionnaire-based assessments of physical activity and perceived stress were obtained.

Results: cTBS-induced MEP suppression was greatest at the upper end of the IO curve (150–180% resting motor threshold; RMT) and most consistent across subjects and across experimental sessions when assessed with a TMS intensity of 150% RMT. The magnitude of cTBS-induced MEP suppression evoked at 150% RMT correlated with self-reported perceived stress, but not with self-reported physical activity.

Conclusions: The most reliable TMS intensity to probe cTBS-induced long-term depression-like neuroplastic responses is 150% RMT. This is unlikely to simply be a ceiling effect and, we suggest, may be due to changes in the descending volley evoked at higher stimulus

intensities. The perceived stress scale appears to be sufficiently sensitive to measure the influence of subject stress on long-term depression-like neuroplastic responses.

Non-invasive brain stimulation (NIBS) protocols that induce short-lasting neuroplasticity in the human cortex have attracted considerable and growing interest for use in both basic science and clinical settings. For the past 15 years, repetitive transcranial magnetic stimulation (rTMS) protocols have been used to induce neuroplasticity; single-pulse TMS is used to measure rTMS-induced changes in corticospinal excitability, providing a marker of synaptic plasticity. Commonly used rTMS protocols can induce bidirectional changes in cortical excitability, and there is evidence that the increases and decreases in excitability reflect changes in synaptic efficacy brought about via long-term potentiation- (LTP) and long-term depression- (LTD) like processes respectively (Cooke and Bliss 2006; Hoogendam et al. 2010). While many studies report significant changes in corticospinal excitability following application of rTMS (e.g. Di Lazzaro et al. 2011; Huang et al. 2007; Huang et al. 2005; Stefan et al. 2000) a number of others do not (Clow et al. 2014; Goldsworthy et al. 2012a; Hamada et al. 2013; McAllister et al. 2013; McAllister et al. 2011), highlighting that the response to rTMS is rather variable. Large inter- and intra-subject NIBS response variability is a limiting factor in both basic and applied research (Vallence and Ridding 2013).

A growing body of literature describes factors that can influence rTMS-induced neuroplastic responses (see Ridding and Ziemann 2010). A number of these identified factors contribute to inter-subject response variability, such as age (Fathi et al. 2010; Muller-Dahlhaus et al. 2008; Tecchio et al. 2008; Todd et al. 2010), genetics (Cheeran et al. 2008), and motor cortical physiology (Hamada et al. 2013). However, several factors have been identified that could contribute to both inter- and intra-subject response variability, such as an individual's history of physical activity (Cirillo et al. 2009) and levels of the stress hormone cortisol (Clow et al. 2014; Sale et al. 2008).

Neuroplastic responses to rTMS are most commonly characterised using a single test TMS intensity to elicit MEPs, and examining the change in MEP amplitude after rTMS application; typically, this test TMS intensity elicits MEPs with peak-to-peak amplitudes of ~1 mV at baseline. Given the inter-subject variability evident in MEP input-output (IO) curves, this somewhat arbitrary peak-to-peak MEP amplitude occurs at different points on the IO curve for different individuals (Burke and Pierrot-Deseilligny 2010). Therefore, in addition to the factors outlined above, the TMS intensity used to measure rTMS-induced plasticity could contribute to response variability.

Despite the importance of understanding intra-subject response variability for both investigating mechanisms of neuroplasticity induction and the therapeutic application of rTMS (which most often involves application over repeated sessions), there are very few data describing intra-subject variability in rTMS-induced neuroplastic responses. The current study had three aims. First, we wanted to investigate and compare inter- and intra-subject variability of neuroplastic responses induced using continuous theta-burst stimulation (cTBS), an rTMS protocol shown to reduce cortical excitability via LTD-like processes (Huang et al. 2005; Huang et al. 2008). We measured cTBS-induced neuroplastic responses, indexed as a change in MEP amplitude, in the same individuals on multiple testing days. Second, we wanted to determine whether the TMS intensity used to measure cTBS-induced neuroplastic responses contributes to response variability; we measured MEP IO curves to fully characterise cTBS-induced neuroplastic responses across testing days. Third, in light of the evidence showing that a history of exercise and the stress-related hormone cortisol influence neuroplasticity induction, we wanted to investigate whether an easily applied, questionnaire-based assessment of these factors can explain some of the variability in

neuroplastic responses; therefore, we assessed physical activity levels and perceived stress before application of cTBS in the same individuals on multiple testing days using well-established questionnaires.

2. Experimental Procedures

2.1 Subjects

Eighteen subjects (mean ± SD: 23.1 ± 4.0 years; 10 females) participated in three experimental sessions. All sessions were conducted in the afternoon to minimise time-of-day influences (Sale et al. 2007) and sessions were separated by ≥2 days (Goldsworthy et al. 2012b; Hamada et al. 2013; Vallence et al. 2013).

2.2 Transcranial Magnetic Stimulation

Electromyographic (EMG) activity was recorded from the relaxed right first dorsal interosseous (FDI) using surface electrodes (muscle belly-tendon configuration). The EMG signal was amplified (x1000; CED 1902 amplifier), band pass filtered (20-1000 Hz) and digitized at a sampling rate of 5 kHz (CED 1401 interface). A Magstim-200 stimulator generated single-pulse stimuli, delivered through a figure-of-eight coil (90 mm) placed tangentially to the scalp with the handle pointing backward, 45° away from the midline. Suprathreshold pulses were delivered over the left primary motor cortex (M1) to identify the optimal site for consistently evoking MEPs in the relaxed contralateral FDI, and this site was marked on the scalp to ensure accurate coil placement throughout the experimental session. Resting motor threshold (RMT) was determined at the beginning of each experimental session; RMT was defined as the minimum intensity (as a percentage of maximal stimulator output; MSO) required to elicit MEPs in the relaxed FDI ≥50 μV in at least 5/10 consecutive trials.

Input-output curves. IO curves were constructed from blocks of 80 single TMS pulses (monophasic pulse waveform) of different stimulus intensities (90, 100, 110, 120, 130, 140, 150, 160, 170, 180% RMT). In each of the three experimental sessions, the intensities used for IO curves were determined at baseline and were not changed throughout the experiment. In each block, eight single TMS pulses at each of the ten intensities were presented in a pseudorandom order with an inter-stimulus interval of 5 s (± 10% variance). The time taken to obtain a complete IO curve was ~7 min. A total of five IO curves were obtained in each experimental session: two at baseline i.e. before cTBS (BL_1, BL_2), and three following cTBS at 0–7, 15–22, and 30–37 min post-cTBS (P_1, P_2, and P_3 respectively).

Continuous theta-burst stimulation. cTBS was delivered using a Double-Cooled-Coil-System coil (70 mm, Magstim). Short bursts of three pulses were delivered at 50 Hz every 200 ms for 40 s (Huang et al. 2005). cTBS intensity was set to 70% of RMT (Gentner et al. 2008; Goldsworthy et al. 2014; Goldsworthy et al. 2012c), determined immediately before cTBS application using the Double-Cooled-Coil-System coil (biphasic pulse waveform).

2.3 Behaviour Assessments

Subjects were required to complete physical activity and perceived stress questionnaires at the beginning of each experimental session. The short version of the international physical activity questionnaire (IPAQ) consists of 4 self-report items regarding time spent engaging in moderate physical activity, vigorous physical activity, walking, and sitting over the previous 7-day period (Craig et al. 2003). The perceived stress scale consists of 10 self-report items regarding the amount of stress associated with various life situations over the past month (Cohen et al. 1983).

2.4 Data Analysis

To test for differences in RMT (%MSO) across the experimental sessions, a one-way repeated-measures analysis of variance (RM-ANOVA) with within-subject factor of SESSION (S_1, S_2, S_3) was performed.

All MEP trials were examined at high gain and individual trials were excluded if EMG activity was present in the 100 ms immediately prior to TMS. The peak-to-peak MEP amplitude (mV) was calculated for each TMS trial.

To test for differences between baseline IO curves, two-way RM-ANOVAs with within-subject factors of BASELINE CURVE (BL_1, BL_2) and INTENSITY (10 levels: 90, 100, 110, 120, 130, 140, 150, 160, 170, 180% RMT) were performed on raw mean MEP amplitudes (separate analyses for each of the three experimental sessions). No significant differences were observed between baseline curves in any of the three sessions (see Results); therefore, the two baseline IO curves were averaged. The maximum mean MEP amplitude (MEP_{max}) of the average baseline IO curve was determined for each subject, and was defined as the largest recorded mean MEP amplitude at any stimulus intensity after averaging between baseline curves. The average baseline IO curve was normalised to this baseline MEP_{max} value (Muellbacher et al. 2000; Pitcher et al. ; Ridding and Rothwell 1997).

To test for differences between the post-cTBS IO curves (P_1, P_2, and P_3), separate two-way RM-ANOVAs with within-subject factors of TIME (BL, Post) and INTENSITY (90-180% RMT inclusive) were performed on the average normalised IO curves for each experimental session. No significant differences were observed between post-cTBS IO curves in any of the

experimental sessions (see Results); therefore, for each experimental session, the three post-cTBS IO curves were normalised to baseline maximum mean MEP amplitude (as per the baseline IO curves), and were then averaged ($Post_{AVERAGE}$). All subsequent analyses were performed on the average normalised IO curves.

For the main analysis examining plasticity responses to cTBS across experimental sessions, a three-way RM-ANOVA with within-subject factors of TIME (2 levels: BL, POST), INTENSITY (10 levels: 90-180% RMT inclusive), and SESSION (3 levels: sessions 1-3) was performed on the average normalised IO curves. To examine neuroplastic responses within each experimental session, two-way RM-ANOVAs with within-subject factors of TIME and INTENSITY were performed separately for each experimental session and, conditional on a significant interaction between factors, *post hoc* comparisons between baseline and post-cTBS MEP amplitudes were conducted for each stimulus intensity, where appropriate.

cTBS-induced neuroplasticity was quantified at four target stimulus intensities:

1. SI_{1mV}: defined as the intensity between 110–180% RMT (inclusive) at which average baseline MEP amplitudes were closest to 1 mV; the standard TMS intensity used to probe NIBS-induced plasticity.
2. SI_{50}: defined as the intensity at which average baseline MEP amplitudes were closest to 50% MEP_{MAX}; this reflects the point on the IO curve at which the MEP amplitude is 50% of the maximum, with equal opportunity for an increase or decrease in excitability.
3. 150% RMT; this reflects a point on the IO curve at which late I-wave recruitment is near-maximal (Di Lazzaro et al. 2004).

4. 180% RMT; the highest stimulus intensity tested, evoking maximal (or near-maximal) MEPs.

To quantify neuroplastic responses, each subject's mean MEP amplitude at each target stimulus intensity from the $Post_{AVERAGE}$ IO curve was expressed as a ratio of that subject's mean MEP amplitude at each target stimulus intensity from the $BL_{AVERAGE}$ IO curve. Therefore, a 'plasticity ratio' was calculated for each subject for each of the four target stimulus intensities; ratios <1.0 indicating MEP suppression following cTBS (i.e. the expected response to cTBS) and ratios >1.0 indicating MEP facilitation following cTBS (i.e. opposite to the expected response to cTBS). While we found no significant difference in MEP amplitudes across the three post-cTBS time points when analysing the full I/O curve data, we performed additional analyses to examine a potential effect of time on cTBS-induced MEP suppression at specific test MEP intensities; separate two-way repeated measures ANOVAs were performed with the within-subject factors of SESSION (3 levels: S_1, S_2, S_3) and TIME (4 levels: BL, Post-0 min, Post-15 min, Post-30 min) at each of the four target test TMS intensities (SI_{1mV}, SI_{50}, 150% RMT, and 180% RMT).

To examine the reproducibility of neuroplastic responses across experimental sessions, the intra-class coefficient was calculated for plasticity ratios at each of the four target stimulus intensities. In addition correlational analyses were performed between neuroplastic responses probed at 150% RMT across the three experiments sessions: S_1-S_2; S_1-S_3; and S_2-S_3. Finally, to compare the proportion of total variance explained by inter- and intra-subject variance, eta-squared values were calculated for plasticity ratios calculated based on mean MEP amplitudes (i) averaged across stimulus intensities, (ii) at SI_{1mV}, and (iii) at 150% RMT.

To examine a potential ceiling effect (i.e. bias whereby the capacity for a decrease in excitability (expected change) is greater than the capacity for an increase in excitability at the upper-end of the IO curve), correlational analyses were performed to determine the relationship between neuroplastic responses probed at 150% RMT and the likelihood that baseline MEP amplitudes elicited at the upper-end of the IO curve were near the ceiling of the testable range (i.e. near MEP_{max}). Ceiling assessment was based on the slope of the linear regression line fitted to the average baseline mean MEP amplitudes (normalised to average baseline MEP_{max}) between 150–180 RMT; a value close to 0 indicates that the baseline MEPs elicited using these intensities were near the ceiling of the testable range.

To examine the influence of physical activity and stress on neuroplastic responses, correlational analyses were performed between plasticity ratios at each of the four target stimulus intensities and (i) IPAQ score and (ii) perceived stress scale score (data from all three experimental sessions); separate analyses were performed for plasticity ratios at each of the four target stimulus intensities (SI_{1mV}, SI_{50}, 150% RMT, 180% RMT). Additional correlational analyses were performed between plasticity ratios at each of the four target stimulus intensities averaged across experimental sessions (i.e. mean plasticity ratios S_1, S_2, S_3) and (i) average IPAQ score (i.e. mean S_1, S_2, S_3) and (ii) average perceived stress scale score (i.e. mean S_1, S_2, S_3).

For all analyses, assumption testing was performed prior to analysis and Huynh-Feldt corrections were used for analyses in which the assumption of sphericity was violated (Mauchly's test of sphericity; for simplicity, uncorrected degrees of freedom are reported). The Pearson correlation coefficient was used for all correlations analyses, except where

assumptions of normality were violated, in which case Spearman's rho was used. Two-tailed tests were used for all analyses. Statistical significance was accepted for $P < 0.05$.

Figures show standard error of the mean (SEM).

3 Results

The number of days between experimental sessions ranged from 2 to 37 days. The average number of days between session 1 and 2 was 11.6 ± 9.7 (range 2 - 37 days; median 7 days) and the average number of days between session 2 and 3 was 10.7 ± 9.4 (range 2 – 35 days; median 7 days). There was no difference in the day-intervals between sessions 1–2 and sessions 2–3 (paired-samples *t*-test; $t_{(17)} = 0.28$, $P = 0.786$).

3.1 Resting motor threshold

The mean RMT (determined using a monophasic pulse waveform) was 39.1% (± 4.2), 40.0% (± 4.6), and 40.1% (± 5.0) MSO for sessions 1, 2, and 3 respectively. A one-way RM-ANOVA showed a significant main effect of SESSION for RMT ($F_{(2,34)} = 4.21$, $P = 0.023$). Paired-samples *t*-tests showed that RMT was lower in session 1 compared with both session 2 ($t_{(17)} = 3.07$, $P = 0.007$) and session 3 ($t_{(17)} = 2.38$, $P = 0.029$) but not different between session 2 and session 3 ($t_{(17)} = 0.13$, $P = 0.897$). For RMT determined using a biphasic pulse waveform, the mean RMT was 48.1% (± 5.7), 49.1% (± 5.5), and 49.4% (± 5.8) MSO for Sessions 1, 2, and 3 respectively. A one-way RM-ANOVA showed no main effect of SESSION ($F_{(2,34)} = 2.53$, $P = 0.109$).

3.2 Baseline IO curves

Separate two-way RM-ANOVAs performed to test for differences between the two baseline IO curves (BL_1, BL_2) obtained in each experimental session showed a main effect of

INTENSITY (S1: $F_{(9,153)} = 29.75$, $P < 0.001$; S2: $F_{(9,153)} = 27.75$, $P < 0.001$; S3: $F_{(9,153)} = 30.66$, $P < 0.001$) but no main effect of BASELINE CURVE (S1: $F_{(1,17)} = 1.89$, $P = 0.188$; S2: $F_{(1,17)} = 1.21$, $P = 0.288$; S3: $F_{(1,17)} = 0.45$, $P = 0.513$) and no INTENSITY*BASELINE CURVE interaction (S1: $F_{(9,153)} = 0.95$, $P = 0.462$; S2: $F_{(9,153)} = 0.30$, $P = 0.950$; S3: $F_{(9,153)} = 0.96$, $P = 0.445$). Therefore, all further analyses were performed on the normalised average baseline curves ($BL_{AVERAGE}$).

A two-way RM-ANOVA performed to test for differences in baseline IO curves across sessions showed a main effect of INTENSITY ($F_{(9,153)} = 501.55$, $P < 0.001$) but no main effect of SESSION ($F_{(2,34)} = 0.13$, $P = 0.881$) and no INTENSITY*SESSION interaction ($F_{(18,306)} = 1.53$, $P = 0.114$).

3.3 Post-cTBS IO curves

Separate two-way RM-ANOVAs performed to test for differences between the three post-cTBS IO curves (P_1, P_2, P_3) obtained in each experimental session showed a main effect of INTENSITY (S1: $F_{(9,153)} = 30.52$, $P < 0.001$; S2: $F_{(9,153)} = 45.28$, $P < 0.001$; S3: $F_{(9,153)} = 32.31$, $P < 0.001$) but no main effect of POST CURVE (S1: $F_{(2,34)} = 0.25$, $P = 0.782$; S2: $F_{(2,34)} = 0.05$, $P = 0.966$; S3: $F_{(2,34)} = 0.66$, $P = 0.524$) and no INTENSITY*POST CURVE interaction (S1: $F_{(18,306)} = 1.00$, $P = 0.463$; S2: $F_{(18,306)} = 0.91$, $P = 0.488$; S3: $F_{(18,306)} = 1.20$, $P = 0.312$). Therefore, all further analyses were performed on the normalised average post-cTBS curves ($POST_{AVERAGE}$).

3.4 cTBS-induced neuroplastic responses

The mean TMS intensity for cTBS was 33.7% (± 4.0), 34.4% (± 3.9), and 34.6% (± 4.1) MSO for sessions 1, 2, and 3 respectively. Figure 1 shows $BL_{AVERAGE}$ and $POST_{AVERAGE}$ IO

curves for each of the three experimental sessions. The three-way RM-ANOVA showed main effects of TIME ($F_{(1,17)}$ = 6.32 P = 0.022) and INTENSITY ($F_{(9,153)}$ = 519.40, P < 0.001), and a TIME*INTENSITY interaction ($F_{(9,153)}$ = 3.40, P = 0.005). It is clear from Figure 1 that the TIME*INTENSITY interaction is driven by smaller MEPs post-cTBS than at baseline at high stimulus intensities, i.e. the upper end of the IO curve. (Post-hoc analyses reported below.) There was no main effect of SESSION ($F_{(2,34)}$ = 0.51, P = 0.607), no SESSION*TIME interaction ($F_{(2,34)}$= 0.37, P = 0.695) or SESSION*INTENSITY interaction ($F_{(18,306)}$ = 1.13, P = 0.325), and no three-way interaction of SESSION*TIME*INTENSITY ($F_{(18,306)}$ = 1.07, P = 0.381).

To further examine the TIME*INTENSITY interaction, separate two-way RM-ANOVAs (TIME, INTENSITY) were performed to examine neuroplastic responses to cTBS within each experimental session. For session 1, there was a main effect of TIME ($F_{(1,17)}$ = 5.84, P = 0.027), a main effect of INTENSITY ($F_{(9,153)}$ = 330.33, P < 0.001), and a TIME*INTENSITY interaction ($F_{(9,153)}$ = 4.38, P = 0.002). Post hoc analysis showed that this was due to reduced post-cTBS MEP amplitudes at 150–180% RMT (all $t_{(17)}$ > 2.22, all P < 0.040). For session 2, there was a main effect of INTENSITY ($F_{(9,153)}$ = 300.96, P < 0.001), however, the main effect of TIME and the TIME*INTENSITY interaction failed to reach statistical significance ($F_{(1,17)}$ = 3.60, P = 0.075; $F_{(9,153)}$ = 2.08, P = 0.059). For session 3, there was a main effect of INTENSITY ($F_{(9,153)}$ = 355.61, P < 0.001) but no main effect of TIME ($F_{(1,17)}$ = 1.93, P = 0.183) and no TIME*INTENSITY interaction ($F_{(9,153)}$ = 0.68, P = 0.720).

Figure 2 shows MEP amplitude at baseline and post-cTBS at each of the four target stimulus intensities: SI_{1mV}, a standard TMS intensity used to probe NIBS-induced plasticity; SI_{50}, the

point on the IO curve at which the MEP is 50% of maximal MEP and there is an equal opportunity for MEP amplitude to increase or decrease; 150% RMT, the point on the IO curve at which late I-wave recruitment is likely to be near-maximal (Di Lazzaro et al. 2004); 180% RMT, the stimulus intensity eliciting maximal (or near-maximal) MEPs. At 150% RMT, MEP suppression was observed following cTBS in all three experimental sessions. At 180% RMT, MEP suppression was only observed following cTBS in session 1 and, at SI_{1mV}, MEP suppression was only observed following cTBS in session 3. No MEP suppression was observed at SI_{50} in any of the experimental sessions.

To further examine a potential effect of time on cTBS-induced MEP suppression, separate two-way ANOVAs (TIME, SESSION) were performed at each of the four target test intensities. At SI_{1mV}, there was no main effect of TIME ($F_{(3,51)}$ = 2.14, P = 0.107) or SESSION ($F_{(2,34)}$ = 0.03, P = 0.971), and no TIME*SESSION interaction ($F_{(6,102)}$ = 0.14, P = 0.990). At SI_{50}, there was no main effect of TIME ($F_{(3,51)}$ = 0.93, P = 0.434) or SESSION ($F_{(2,34)}$ = 0.98, P = 0.386), and no TIME*SESSION interaction ($F_{(6,102)}$ = 0.59, P = 0.671). At 150% RMT, there was a main effect of TIME ($F_{(3,51)}$ = 4.04, P = 0.012), but no main effect of SESSION ($F_{(2,34)}$ = 1.13, P = 0.321) and no TIME*SESSION interaction ($F_{(6,102)}$ = 0.37, P = 0.899). Post-hoc analysis at 150% RMT showed significant post-cTBS MEP suppression at post-15 min (t_{17} = 3.65, P = 0.002) and at post-30 min (t_{17} = 2.28, P = 0.036) in Session 1, and at post-0 min (t_{17} = 2.16, P = 0.046) and at post-30 min (t_{17} = 2.89, P = 0.010) in Session 2; MEP suppression at other post-cTBS time points in Session 1 and 2, and all post-cTBS time points in Session 3 failed to reach statistical significance. At 180% RMT, there was a main effect of TIME ($F_{(3,51)}$ = 3.01, P = 0.039), but no main effect of SESSION ($F_{(2,34)}$ = 0.29, P = 0.748) and no TIME*SESSION interaction ($F_{(6,102)}$ = 1.62, P = 0.148). Post-hoc analysis at 180% RMT showed significant post-cTBS MEP suppression at post-15 min (t_{17} = 3.69, P =

0.002) and at post-30 min (t_{17} = 2.60, P = 0.019) in Session 1, and at post-0 min (t_{17} = 2.21, P = 0.041) in Session 2; MEP suppression at other post-cTBS time points in Session 1 and 2, and all post-cTBS time points in Session 3 failed to reach statistical significance.

Figure 3 shows the change in MEP amplitude from baseline to post-cTBS for each subject at each of the target intensities across sessions. Consistent with the MEP suppression observed at 150% RMT, the largest number of subjects showing the expected MEP amplitude suppression was observed at 150% RMT: 14/18 (78%) in session 1; 14/18 (78%) in session 2; 11/18 (61%) in session 3.

Intra-class coefficients calculated for the plasticity ratios at each of the four target stimulus intensities are presented in Table 1. Significant intra-class coefficients indicate strong correlations between MEP suppression following cTBS across sessions at stimulus intensities of 150 and 180% RMT, while non-significant intra-class coefficients indicate no correlation between MEP suppression following cTBS across sessions using SI_{1mV} and SI_{50}. Scatter plots in Figure 4 show relationships between neuroplastic responses probed at 150% RMT across experimental sessions. While there were no significant correlations, a trend towards a positive linear relationship was observed for neuroplastic responses in session 1 and 2, and in session 1 and 3 (S_1-S_2: r = 0.42; 95% confidence limits: -0.06, 0.74; S_1-S_3: r = 0.44; 95% confidence limits: -0.03, 0.75). Finally, eta-squared values show a greater proportion of the total variance was explained by inter-subject variability than intra-subject variability. Eta-squared values calculated from neuroplastic responses across all stimulus intensities showed that inter-subject variability explained 16% (i.e. η^2 = 0.16) and intra-subject variability explained 5% (i.e. η^2 = 0.05) of total variance; at 150% RMT, inter-subject variability explained 51% (i.e. η^2 = 0.51) and intra-subject variability explained 2% (i.e. η^2 = 0.02) of total variance; at

SI_{1mV}, inter-subject variability explained 26% (i.e. $\eta^2 = 0.26$) and intra-subject variability explained 4% (i.e. $\eta^2 = 0.04$) of total variance.

To examine the possibility that a ceiling effect contributed to the greater MEP suppression observed at higher test stimulus intensities, correlational analyses were performed between the plasticity response probed at 150% RMT and (i) slope between 150–180% RMT and (ii) baseline 150% RMT MEP amplitude normalised to baseline MEP_{max}. If the cTBS-induced MEP suppression evident at 150% RMT was due to a ceiling effect, we would expect significant linear relationships between cTBS-induced MEP suppression at 150% RMT and the likelihood that baseline MEP amplitudes elicited at the upper-end of the IO curve were near the ceiling of the testable range (i.e. near MEP_{max}); that is, greater cTBS-induced MEP suppression at 150% RMT in those subjects for whom baseline MEP amplitudes at 150–180% RMT were near MEP_{max}. Figure 5 shows no significant relationship between cTBS-induced MEP suppression probed at 150% RMT and either baseline slope between 150–180 %RMT or baseline 150% RMT MEP amplitude normalised to baseline MEP_{max}.

3.5 Factors influencing cTBS-induced plasticity

The average total score from the short IPAQ, quantified as MET-minutes (a measure that takes into account the energy requirements of different activity types), was 3428 ± 2907 for session 1, 3706 ± 2928 for session 2, and 4104 ± 4295 for session 3; a one-way repeated measures ANOVA showed no main effect of SESSION ($F_{(2,34)} = 0.35$, $P = 0.707$). The average perceived stress scale score was 12.7 ± 6.5 for session 1, 11.3 ± 5.4 for session 2, and 10.9 ± 5.1 for session 3; a one-way repeated measures ANOVA showed no main effect of SESSION ($F_{(2,34)} = 3.19$, $P = 0.076$).

Figure 6 shows the relationships between each subject's plasticity ratios at the four target stimulus intensities and their (i) IPAQ score and (ii) perceived stress scale score. Perceived stress scale score and plasticity ratio were positively correlated at 150% RMT (r = 0.27; 95% confidence limits: 0.01, 0.50), with a larger perceived stress scale score associated with a larger plasticity response. There were no relationships between plasticity ratios at SI_{1mV}, SI_{50}, or 180% RMT and perceived stress scale score or IPAQ score, and no relationships between plasticity ratios at 150% RMT and IPAQ score. Figure 7 shows the relationships between each subject's *average* plasticity ratios (i.e. mean across the three experimental sessions) at each of the four target stimulus intensities and their (1) average IPAQ score (mean across the three experimental sessions) and (2) average perceived stress scale score (mean across the three sessions). No significant relationships were evident; however, a linear trend was observed between average perceived stress scale scores and average plasticity ratios at 150% RMT, with a larger perceived stress scale score associated with a larger plasticity response (r = 0.42; 95% confidence limits: -0.06, 0.74).

4 Discussion

The main novel finding reported here is that cTBS-induced MEP suppression is observed at the upper end of the IO curve. Specifically, cTBS-induced LTD-like neuroplastic responses are most consistent between subjects and across experimental sessions when assessed with a TMS intensity of 150% RMT.

To our knowledge, this is the first study to examine neuroplastic responses induced by cTBS across the full MEP IO curve, although a few have examined neuroplastic responses induced by regular frequency rTMS paradigms across the full IO curve. These studies all report

significant MEP suppression at the upper end of the IO curve (150 – 170% RMT) following low frequency rTMS (1 Hz)(Gangitano et al. 2002; Hortobagyi et al. 2009; Muellbacher et al. 2000). Pharmacological studies show that both cTBS and 1 Hz rTMS induce NMDA receptor-dependant LTD-like plasticity (Chen et al. 1997; Huang et al. 2007), albeit via the modulation of different cortical circuits; studies recording descending volleys from the epidural space show that cTBS primarily suppresses the I1 wave (Di Lazzaro et al. 2005), while 1 Hz rTMS primarily suppresses late I waves (Hill et al. 1996; Makowiecki et al. 2014). The current results suggest that the upper end of the MEP IO curve is optimal for probing cTBS-induced LTD-like plasticity.

This raises the question: why is cTBS induced LTD-like plasticity most obvious at the upper end of the IO curve? It is possible that the cTBS-induced neuroplastic response observed at the upper end of the curve reflects a ceiling effect whereby the significant MEP suppression could be due to a bias where the capacity for a decrease in excitability (i.e. a change in the expected direction) is greater than the capacity for an increase in excitability at the upper end of the IO curve. However, if the results simply reflect a ceiling effect, we would expect that the cTBS-induced MEP suppression at the highest stimulus intensity of the IO curves (180% RMT) would be *at least* as reproducible as the cTBS-induced MEP supression at 150% RMT. This did not occur, with MEP suppression evident in all three experimental sessions when probed at 150% RMT, but only evident in Session 1 when probed at 180% RMT. Furthermore, if the results simply reflect a ceiling effect, we would also expect that those subjects for whom baseline MEP amplitude at 150% RMT was near-maximal would show a greater cTBS-induced MEP suppression than those subjects for whom baseline MEP amplitude at 150% RMT was far from their maximal MEP amplitude. This is not the case: our results show no relationship between cTBS-induced MEP suppression at 150% RMT and

baseline MEP amplitude at 150% RMT normalised to baseline MEP_{max}. Therefore, while it is possible that the near-maximal MEPs evoked at the upper end of the curve might increase the opportunity to observe MEP suppression following cTBS, this evidence shows that there are other factors that contribute to the reliability of the cTBS-induced MEP suppression at the upper-end of the IO curve.

One possible explanation is that at higher stimulus intensities, that is, 150% RMT, late I wave recruitment is near maximal, and therefore less variable than at lower intensities. This may allow more reliable measurement of cTBS-induced suppression of the earlier I1 waves. The evidence for this comes from studies recording descending volleys from the epidural space. These studies have separately shown greater recruitment of late I-waves at the upper end of the IO curve (i.e. when a larger population of neurons is being recruited), and a reduction in the amplitude of I1 following cTBS (Di Lazzaro et al. 2004; Di Lazzaro et al. 2005). Since the MEP is a compound potential reflecting both late and early I waves, less variability in the contribution from the late I waves may increase the likelihood of I1 suppression being detected in the MEP. MEP suppression following cTBS was more reliable across expeirmental sessions at 150% RMT than 180% RMT. At first glance this finding appears inconsistent with the suggestion that the capacity to detect a change in MEP amplitude following cTBS is greatest when late I-wave recrutiment is near-maximal. However, the MEP elicited by a stimulus intensity of 180% RMT most likely reflects early and late I-wave recruitment as well as some D-wave contribution (Di Lazzaro et al. 2004). It is possible that inconsistent recruitment of the D-wave at 180% RMT increases variability in the MEP and, in turn, decreases the liklihood of the cTBS-induced I1 suppression being reliably detected. Therefore, the contribution of a D-wave to the descending volley at 180% but not 150% RMT

might make the measurement of cTBS-induced MEP suppression less reliable at 180% than 150% RMT.

When probed at 150% RMT, mean MEP amplitude following cTBS (i.e. average of all three post-cTBS time points) was suppressed compared to baseline in all three experimental sessions. When we examined MEP suppression following cTBS separately at each of the three post-cTBS time points (0-, 15-, 30-mins) results were inconistent; there was no systematic effect of time post-cTBS on MEP suppression across experimental sessions. This is consistent with recent studies that show neuroplasticity induced by intermittent theta-burst stimulation is most reliable when MEP amplitudes were averaged across all post-stimulation time points (Hinder et al. 2012).

The current data show that inter-subject variability explain a greater proportion of total variance than intra-subject response variability. In the NIBS literature, individuals are increasingly being categorised as 'responders' or 'non-responders', depending upon whether they do or do not show the expected response to NIBS protocols respectively. Here, the stimulus intensity used to probe neuroplastic responses affected the proportion of responders categorised based on the expected response (MEP suppression post-cTBS). When cTBS-induced neuroplastic responses were probed at 150% RMT, the percentage of responders was 78, 78, and 61% for sessions 1, 2, and 3 respectively; when cTBS-induced neuroplastic responses were probed at SI_{1mV}, the stimulus intensity typically used in NIBS studies, the percentage of responders was 66, 66, and 72% for sessions 1, 2, and 3 respectively. This variable nature of the responder/non-responder categorisation, together with the knowledge that we have only indirect measures of LTP-/LTD-like processes, highlights the importance of interpreting such categorised results with caution. Indeed, these data suggest that whether

an individual responds to NIBS protocols is dependent, at least in part, on the stimulation paramaters used.

Strong evidence shows that stress influences learning and memory (Lupien et al. 2007), and we have shown that fluctuations in the circulating levels of the stress hormone cortisol can influence rTMS response variability (Clow et al. 2014; Sale et al. 2008). We have also shown that the physical activity history of an individual can influence rTMS-induced neuroplastic responses (Cirillo et al. 2009). Here, we were interested in examining whether simple questionnaire-based assessments of physical activity or stress (surrogate for cortisol) could be used to explain some of the variability in neuroplastic responses. We found no evidence of a relationship between short IPAQ scores and cTBS-induced plasticity. This finding is somewhat surprising given our previous study demonstrating enhanced rTMS-induced plasticity in physically active adults compared to sedentary adults (Cirillo et al. 2009). However, this previous research selectively studied subjects of extremely high and extremely low physical activity levels based on physical activity over several years (Cirillo et al. 2009), thereby maximising the opportunity to demonstrate differences in plasticity across groups. Here, the sample consisted of healthy young adults with moderate levels of physical activity, and, as such, was not sufficient to differentiate changes in cTBS-induced neuroplastic responses. It is also possible that the short IPAQ does not provide a sufficiently sensitive measure of physical activity levels in moderately active individuals to identify the influence of neuroplasticity. It remains unknown whether a more comprehensive assessment of physical activity, such as the full IPAQ or data from wrist accelerometers worn by subjects, would be more useful for identifying physical activity influences on rTMS-induced neuroplastic responses.

Neuroplastic responses probed at 150% RMT were associated with higher levels of perceived stress. This relationship is most likely to be mediated by the stress hormone cortisol which has been shown to influence rTMS-induced plasticity (Clow et al. 2014; Pitcher et al. 2012; Sale et al. 2008). While we did not directly measure cortisol, the relationship between cTBS-induced neuroplasticity and stress is consistent with animal studies showing that high corticosterone release facilitates LTD (Chaouloff et al. 2008; Yang et al. 2004), and one human study which similarly showed that higher levels of salivary cortisol are strongly associated with a greater response to cTBS (Pitcher et al. 2012). The current finding shows that the perceived stress scale (Cohen et al. 1983) is associated with cTBS-induced MEP suppression, and could be used to explain some of the variability in LTD-like rTMS-induced neuroplastic responses. It is important to note that the perceived stress scale score only accounted for 7–18% of the variability in cTBS-induced neuroplastic responses in our sample; further studies are required to determine whether this questionnaire-based assessment of stress can be used as a predictive marker of rTMS-induced LTD-like neuroplasticity. In addition, it remains unknown whether the perceived stress scale would be sufficiently sensitive to show the relationship between cortisol and rTMS-induced LTP-like neuroplasticity. Animal studies have shown a complex inverted-U-shaped relationship between the magnitude of LTP induced and corticosterone levels (Diamond et al. 1992; Rey et al. 1994) but the corollary has not yet been shown in humans for rTMS-induced LTP-like neuroplasticity. Finally, it is important to note that the correlations between perceived stress and cTBS-induced neuroplastic responses (and indeed between cTBS-induced neuroplastic responses across sessions) are weak and their significance is unclear. Further investigation of these possible relationships is warranted.

Surprisingly, an increase in RMT was observed from session 1 to session 2. Previous work shows that RMT is unchanged immediately following a single application of cTBS (Goldsworthy et al. 2013; Munneke et al. 2013). To our knowledge, no previous study has examined changes in RMT in the days following a single application of cTBS. Although the change in RMT between session 1 and sessions 2 and 3 in the current study was statistically significant, the magnitude of the change was only 1% of MSO. The physiological significance of this (if any) is unclear and, in light of the evidence to show no change in RMT immediately following cTBS, we do not believe it would be meaningful to interpret this result.

Conclusions

This study provides the first comprehensive investigation of the reproducibility of cTBS-induced neuroplastic responses. The current data show that cTBS-induced LTD-like neuroplastic responses are most consistent when measured using a TMS intensity of 150% RMT, and we suggest that this intensity provides the most reliable probe for cTBS-induced neuroplastic responses. Further, greater cTBS-induced neuroplastic responses (at 150% RMT) were associated with higher perceived stress scale scores, suggesting that the perceived stress scale can be used to explain some of the variability in LTD-like rTMS-induced neuroplastic responses. These results show that the stimulus intensity used to probe changes in corticospinal excitability following non-invasive brain stimulation is an important factor determining the magnitude and consistency of the induced plasticity response. This information is important for the development of non-invasive brain stimulation protocols for inducing less variable, robust neuroplastic responses that can be used therapeutically.

Acknowledgements

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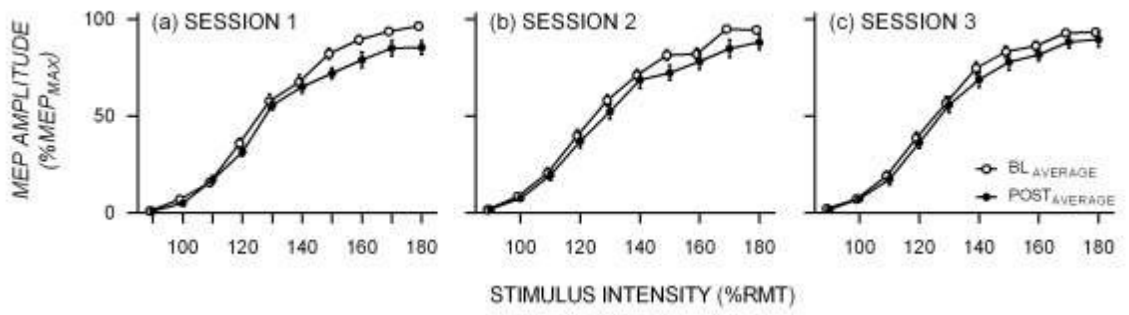

Figure 1. Figure shows averaged IO curves recorded before and following cTBS in each of the three experimental sessions (a: Session 1; b: Session 2; c: Session 3). cTBS-induced MEP suppression was evident at the upper-end of the input output (IO) curve. (All data represent group means ± SEM. Note: data offset to improve clarity.)

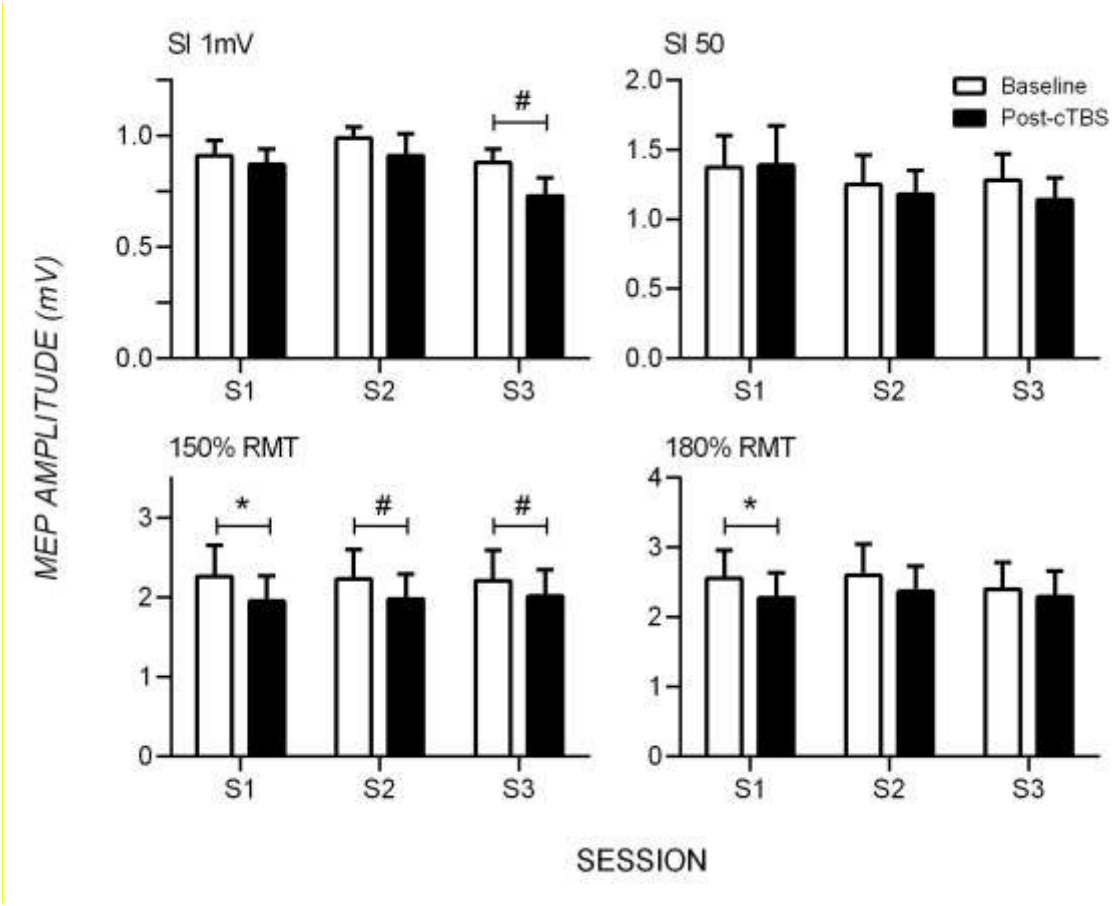

Figure 2. Figure shows the influence of cTBS on raw MEP amplitudes evoked using stimulus intensities SI_{1mV} (top left), SI_{50} (top right), 150% RMT (bottom left), and 180% RMT (bottom right). cTBS-induced MEP suppression was most consistent across sessions at 150% RMT. (* and # denote a significant difference between baseline (average of BL_1 and BL_2) and post-cTBS (average of P_1, P_2, and P_3) MEP amplitudes at $P < 0.012$ and $P < 0.05$ respectively. Data represent group means ± SEM. Note the different scales on the Y axes.)

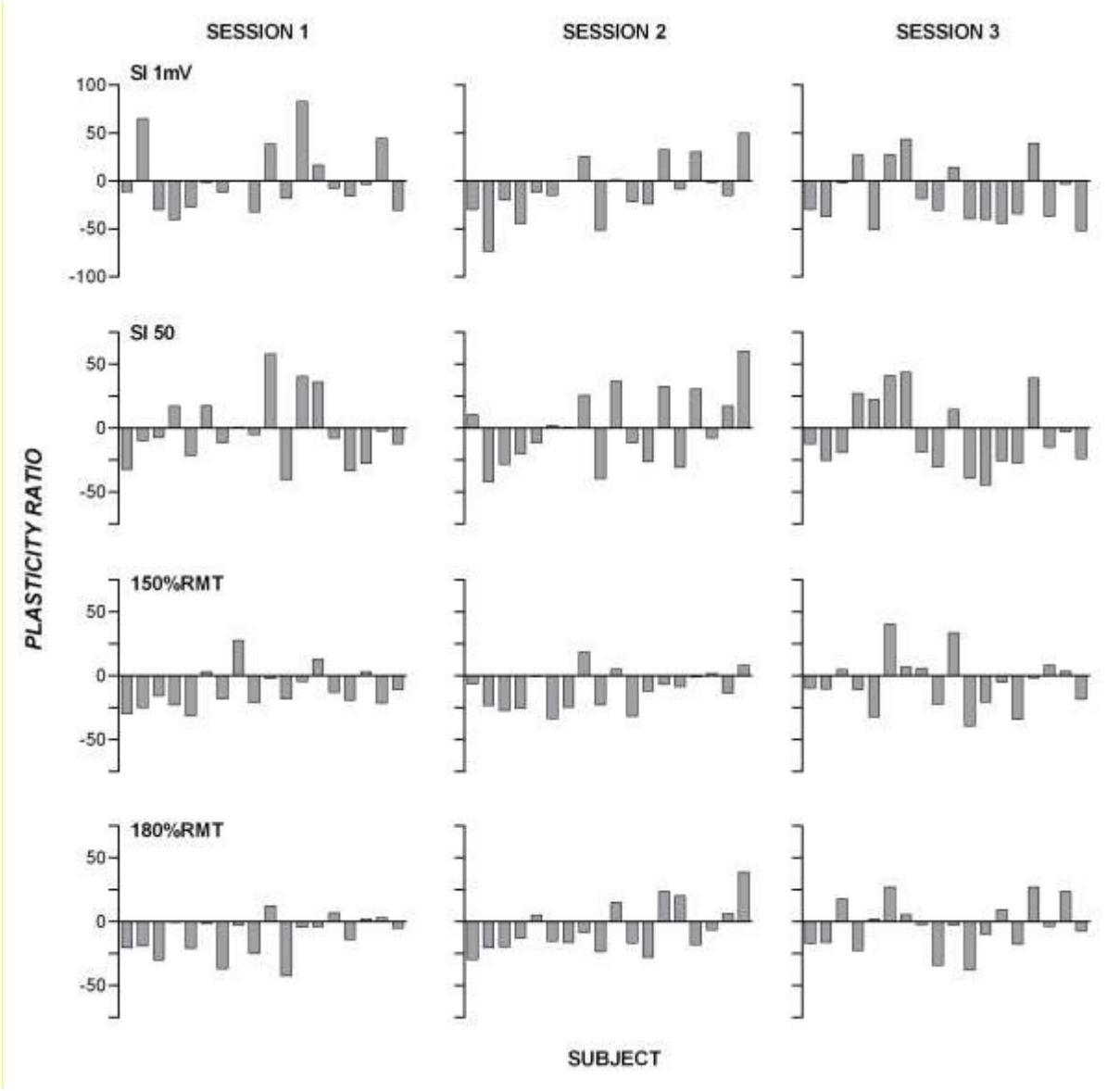

Figure 3. Figure shows inter-subject response variability to cTBS across experimental sessions probed using stimulus intensities SI_{1mV} (top row), SI_{50}, (second row), 150% RMT (third row), and 180% RMT (bottom row). cTBS-induced MEP suppression was evident in the greatest number of participants at 150% RMT. (Data represent mean percentage change of post-cTBS MEP amplitudes from baseline, with positive and negative values indicating an increase and decrease in MEP amplitudes following cTBS respectively.)

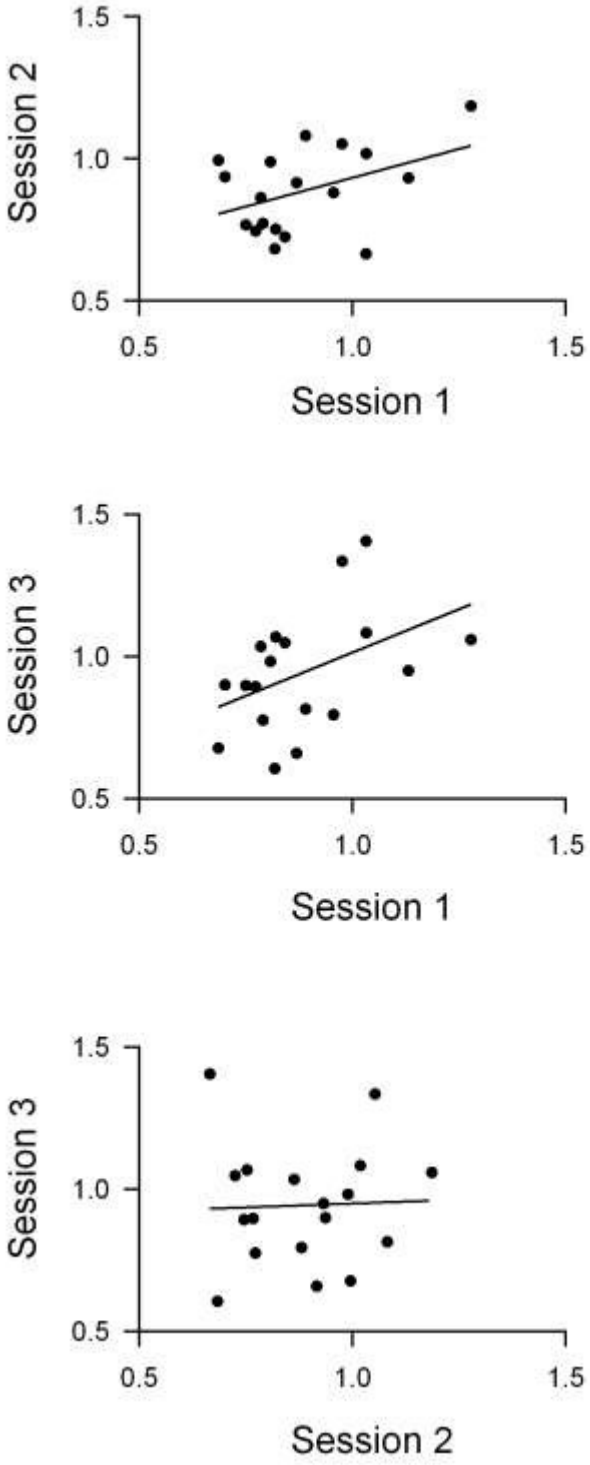

Figure 4. Figure shows scatter diagrams between neuroplastic responses (change in MEP amplitude) following cTBS probed using the stimulus intensity 150% RMT across experimental sessions. Trends toward positive linear relationships are observed in neuroplastic responses between session1 and session 2 and 3 (top and middle).

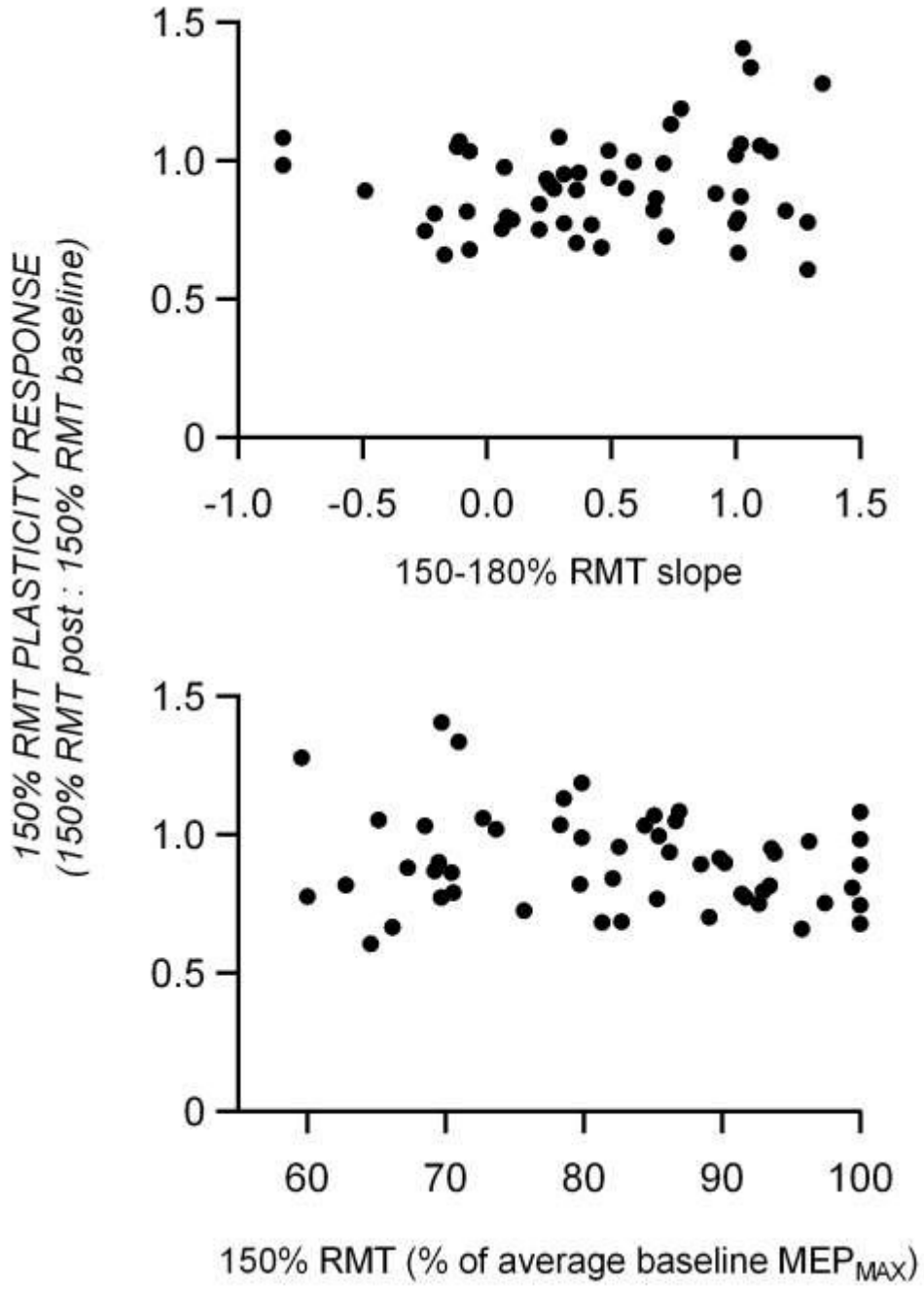

Figure 5. Figure shows scatter diagrams between plasticity response (change in MEP amplitude) following cTBS probed using the stimulus intensity 150% RMT and (i) baseline slope at the upper-end of the IO curve (150-180% RMT) and (ii) baseline MEP amplitude at 150% RMT normalised to baseline MEP_{max}. Smaller slope values indicate average baseline MEP amplitudes were near to the ceiling of the testable range (i.e. near to MEP_{MAX}). There is

no relationship between cTBS-induced :MEP suppression at 150% *RMT* and baseline :MEP amplitude at the upper-end of the IO curve.

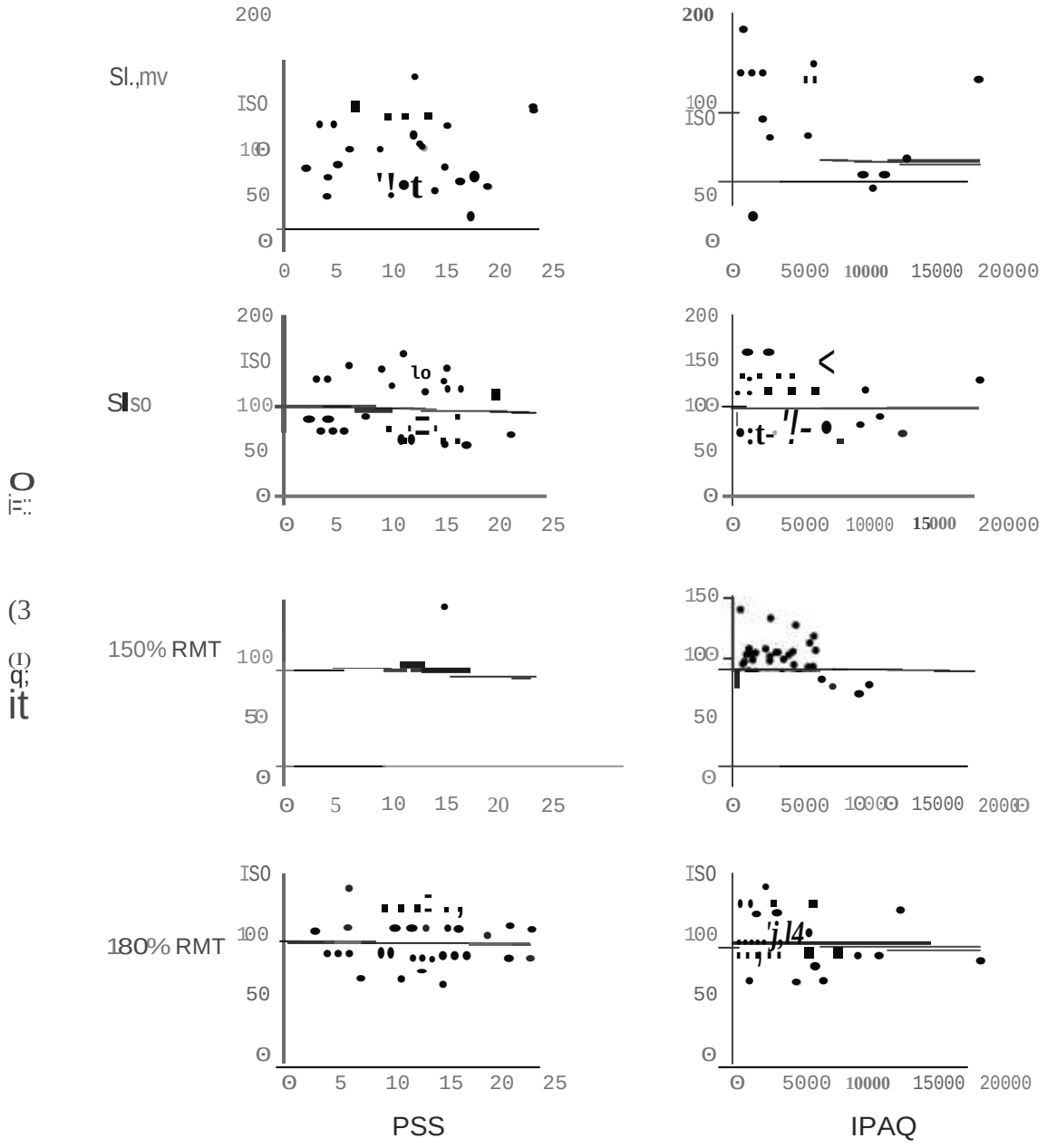

Figure 6. Figures shows scatter diagrams showing relationships between perceived stress (left) and physical activity (right) and plasticity responses (change in :MEP amplitude) following cTBS probed using stimulus intensities SI1mV (top row), Siso, (second row), 150%

RMT (third row), and 180% RMT (bottom row). At 150% RMT, larger neuroplastic responses were associated with larger perceived stress scores.

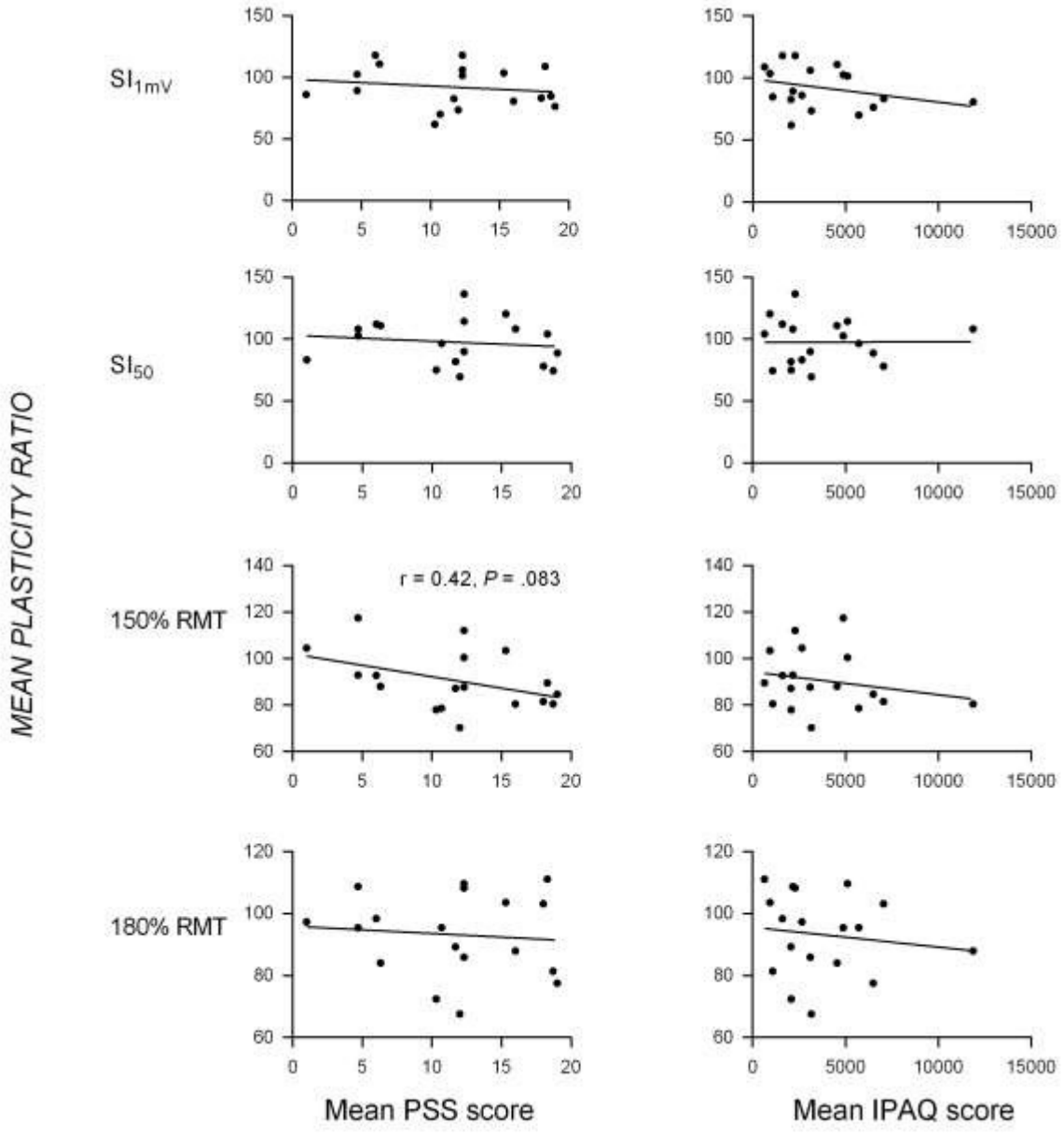

Figure 7. Relationships between plasticity responses and (i) perceived stress and (ii) physical activity *averaged* across the three experimental sessions. Scatter diagrams show relationships between mean perceived stress (left) and mean physical activity (right) and mean plasticity

responses (change in MEP amplitude) following cTBS probed using stimulus intensities SI_{1mV} (top row), SI_{50}, (second row), 150% RMT (third row), and 180% RMT (bottom row).

Table 1. Intra-class coefficients for plasticity ratios calculated at each of the target stimulus intensities. Significant ICCs indicate strong correlations between MEP suppression following cTBS across sessions at stimulus intensities of 150 and 180% RMT.

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