Does Continuous Theta Burst Stimulation to the Human Motor Cortex Modulate Neural Noise?

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Abstract

Brain activity as measured by electroencephalography (EEG) is composed of periodic oscillations and aperiodic signals called neural noise. The physiological relevance of neural noise had been largely ignored until recently, when researchers discovered that neural noise changes with different task states and arousal levels. While the origins of neural noise are not well understood, there is converging evidence that neural noise reflects excitation/inhibition balance in the brain. No study has before investigated the impact of an excitation-/inhibition-inducing paradigm such as continuous theta burst stimulation (cTBS) on neural noise. Here, we used EEG to compare changes in neural noise before and after cTBS to the human primary motor cortex. Eighteen healthy adults (23.7±6.0 years, 8 males) attended one real and one sham cTBS session, with 14 participants (24.9±6.9 years, 8 males) returning for another real session to assess test-retest reliability. Data from the 3-30 Hz frequency range indicated that cTBS did not significantly alter neural noise at the group level (p>0.05). However, considerable inter-individual variability was noted in the direction and magnitude of neural noise changes following cTBS, and these changes showed moderate reproducibility at mid-cTBS (r=0.50, p=0.03) and post-cTBS (r=0.53, p=0.02) timepoints within individuals across real sessions. Our results thus suggest that cTBS may alter neural noise in some individuals, although the direction of change differs between individuals. Overall, this study adds to the growing body of literature demonstrating high variability in cTBS outcomes and underscores the need to personalise stimulation paradigms at the individual level.

Introduction

Brain activity is composed of periodic oscillations as well as aperiodic, non-rhythmic signals called neural noise. For over 80 years, electroencephalography (EEG) has been used to analyse periodic oscillations while largely ignoring the neural noise signal. Recent research, however, has proposed that neural noise may also contain physiologically relevant information. When EEG recordings are in the log-log space frequency domain, periodic oscillations appear as peaks while neural noise takes the form of an underlying, negatively linear 1/f slope[1]. Emerging studies have found that the 1/f slope of neural noise changes with different task states and arousal levels independently from oscillations[2,3,4]. In this way, the 1/f slope presents as an increasingly promising index for measuring changes in neuronal activity.

The exact physiological mechanisms underpinning neural noise are not well understood, but there is converging evidence that neural noise reflects excitation/inhibition (E:I) balance in the brain. In a computer-simulated model, it was found that increasing the ratio of inhibitory input to a population of neurons caused the 1/f slope to decay more rapidly[5]. This finding was replicated in an animal model, where changes in the 1/f slope correlated with the ratio of excitatory and inhibitory synapses across the depth of the rat hippocampus[5]. To support this hypothesis in humans, the 1/f slope has been shown to diverge in conditions thought to affect E:I balance[6,7,8] and become steeper following administration of an inhibitory γ-aminobutyric acid (GABA)-mediated anaesthesia[4].

Given the importance of E:I balance for healthy neural function[5,9], an important question is whether E:I balance can be altered in humans. One method capable of non-invasively interacting with excitatory and inhibitory circuits is transcranial magnetic stimulation (TMS), which involves the use of electromagnetic induction to stimulate targeted neuronal regions. When stimulation is applied in repetitive patterns, known as repetitive TMS (rTMS), TMS harnesses the brain's neuroplasticity to reorganise local circuits[10]. rTMS-induced plasticity can be quantified in the

primary motor cortex (M1) via a muscle response in the hand, known as a motor-evoked potential (MEP). For example, continuous theta burst stimulation (cTBS) is a form of rTMS where 50 Hz trains of TMS pulses are repeated at 5 Hz intervals for 40 seconds. Huang et al.[11] found that applying cTBS to M1 reduced MEP amplitude for up to 20 minutes post-cTBS, suggesting a decrease in excitability of the corticospinal system. Corroborating this finding, cTBS similarly suppressed intracortical facilitation (ICF) in the hand M1 area using paired-pulse TMS[12], again indicating that cTBS reduces cortical excitability[11].

Alongside the recognised impact of cTBS on cortical excitation, several new lines of evidence indicate that cTBS may also influence inhibitory circuitry. Using paired-pulse TMS[12], cTBS was shown to decrease short-interval cortical inhibition (SICI), implying a reduction in the small-scale synaptic effects of GABA[13]. In contrast, however, cTBS was also demonstrated to increase large-scale GABA concentrations as measured via magnetic resonance spectroscopy, suggesting an increase in overall inhibitory neurotransmission[14,15]. The perceived inhibitory effects of cTBS thus appear to differ depending on the metric by which inhibition is measured, representing a major controversy in the broader field.

A further controversy in the field is that, while the intra-individual reliability of rTMS in recent research is modest[16], many rTMS paradigms continue to suffer from high inter-individual response variability[17]. For example, in a study of 52 individuals, 42% showed the expected reduction in corticospinal excitability following cTBS while 58% showed the opposite response[18]. Two approaches have emerged in the literature for mitigating inter-individual variability. First, researchers such as Chung et al.[19] have adopted a personalised approach, wherein rTMS parameters such as stimulation frequency are adjusted to optimise the effects of rTMS within an individual. Second, researchers such as Goldsworthy et al.[13] have sought to harness the principles of metaplasticity. Metaplasticity is a complex and non-linear phenomenon in which the plasticity

observed following rTMS is contingent upon the history of the synapses being targeted[20]. In this way, applying a second block of the same rTMS paradigm to the same site may either strengthen or abolish the effects of the first block[21]. The plasticity observed appears to depend on the interval between stimulation blocks, where an interval of 10 minutes is thought to result in a more robust and stable reduction in cortical excitability and SICI[13,22], while an interval of five minutes may reverse the direction of plasticity[23]. Research into the relationship between rTMS and metaplasticity continues to expand, however no study has yet analysed the impact of paired cTBS on the EEG spectrum.

With mounting evidence that cTBS interacts with both excitatory and inhibitory circuits, cTBS presents as an excellent candidate for altering the E:I balance that potentially underpins neural noise. Many studies have used EEG to examine how cTBS interacts with oscillations[24,25,26], however no study to date has explored the impact of cTBS on neural noise. For example, Rocchi et al.[27] employed EEG to determine that cTBS caused a significant decrease in the power of delta frequency oscillations without considering how cTBS might also be interacting with the underlying 1/f slope of neural noise. On this basis, it is unclear whether reported cTBS findings in the literature reflect true changes in oscillations or a change in the 1/f slope gradient.

The aim of this study was to determine whether cTBS alters the 1/f slope of neural noise in the human M1. To assess this, we compared changes in the 1/f slope exponent before and after cTBS in two real conditions and one sham condition. Computational/animal modelling and human pharmacological studies have shown that increasing the ratio of inhibition causes a steepening of the 1/f slope[4,5]. As cTBS has been shown to decrease cortical excitability and ICF and increase large-scale inhibitory GABA concentrations[11,14,15], we hypothesise that the 1/f slope will be steeper following single and paired cTBS in the real condition. Furthermore, we hypothesise that the 1/f slope post-cTBS will be steeper in the real condition than in the sham condition. Finally, in light of

current cTBS reproducibility findings in the literature[16], we hypothesise that the change in 1/f slope post-cTBS will be moderately reproducible within individuals across two real cTBS sessions.

Materials and Methods

Data used in this study were collected in 2016 for another project investigating the impact of cTBS on functional connectivity. The following describes the methods pertaining to this study alone.

Participants

A total of 18 healthy, young adults were involved in the study, aged 18-42 (M=23.7, SD=6.0 years, 8 males). Participants were eligible for inclusion if they were aged 18-50 years, had no history of neurological or psychiatric disease, had no neurological or musculoskeletal impairments of the upper limb, were not taking any medications known to alter the central nervous system, and had no contraindications to TMS[28]. All participants gave informed written consent prior to commencement and ethics approval was granted by the University of Adelaide Human Research Ethics Committee.

Experimental Protocol

Participants attended a real cTBS session counterbalanced with a sham cTBS session, separated by a minimum of seven days. Fourteen participants (M=24.9, SD=6.9 years, 8 males) then returned for a further real cTBS session after another minimum of seven days to investigate intra-individual variability in response to cTBS. Participants were blinded to the real/sham conditions. Sessions were held in the afternoon to mitigate the effects of diurnal cortisol level variations on neuroplasticity[29]. All procedures were conducted in a quiet room with participants seated in a recliner chair. The experimental protocol for a real or sham cTBS session is described in Figure 1. Each EEG recording was taken for three minutes at resting state with eyes open.

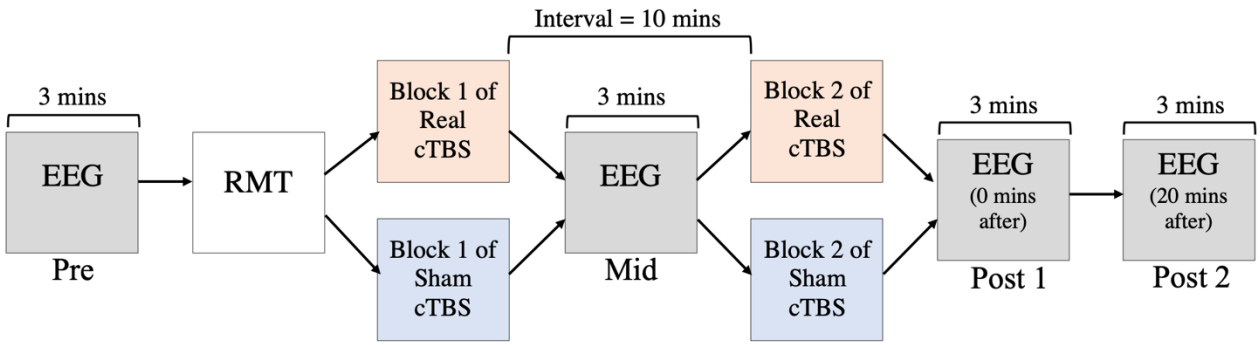

Figure 1: Experimental protocol for a real/sham cTBS session showing EEG recordings at Pre, Mid, Post 1, and Post 2 timepoints. RMT=resting motor threshold.

EEG

EEG data were recorded using an Asa-Lab EEG system with a Waveguard 64-electrode cap that had sintered Ag-AgCl electrodes in standard 10-10 positions (Advanced Neuro Technology, B.V., Enschede, The Netherlands). Conductive gel was inserted into each electrode to reduce impedance to <5 kΩ. The ground electrode was located at AFz. Signals were sampled at 2048 Hz, amplified 20x, online filtered (high-pass DC, low-pass 553 Hz), and online referenced to the average of all electrodes. Participants were instructed to refrain from speaking or moving and to maintain eye gaze toward a point at eye level.

Electromyography

For RMT measurements, surface electromyography was used to record MEPs from the right first dorsal interosseous (FDI) muscle. Two Ag-AgCl electrodes were placed over the muscle in a belly-tendon montage, with a grounding strap around the wrist. Signals were sampled at 5 kHz (Cambridge Electronic Design 1401, Cambridge, UK), amplified 1000x, and bandpass filtered (20-1000 Hz; Cambridge Electronic Design 1902 amplifier, Cambridge, UK).

TMS

All TMS was delivered via a Magstim figure-of-eight coil connected to a Magstim Rapid stimulator (Magstim Company, Dyfed, United Kingdom) using a biphasic pulse waveform (anterior-posterior/posterior-anterior current flow). The coil was positioned tangentially over the hand representation in the left M1 and the handle was rotated posterior-laterally at 45° to the sagittal plane. To identify the optimal position for eliciting MEPs in the relaxed right FDI muscle, the coil was shifted systematically. Once the optimal position was located, it was marked on the scalp for reference and checked continually. RMT was then determined as the minimum stimulation intensity required in the M1 to elicit a MEP with peak-to-peak amplitude >50 μV for at least five of 10 consecutive trials. cTBS was applied to the marked position and each block of cTBS consisted of 600 pulses, delivered in bursts of three pulses at 50 Hz repeated at 5 Hz for 40 seconds[11]. Two blocks of spaced, paired cTBS were used with an interval of 10 minutes as this method has been shown to provide a more robust and stable reduction in cortical excitability and SICI as measured by MEPs[13,22]. In the sham condition, a sham TMS coil was used that mimicked the auditory feedback associated with cTBS.

Data Pre-Processing

EEG data were analysed using EEGLAB[30], FieldTrip[31], and custom scripts using MATLAB (R2013a, The Mathworks, USA). First, unused electrodes were removed and data were bandpass filtered between 1-100 Hz and bandstop filtered at 48-52 Hz to remove contaminating line noise. Data were then epoched into two second, non-overlapping windows and concatenated across time points within each condition to avoid bias in artefact correction. Epochs and channels were visually inspected and removed if contaminating artefacts were present. Independent component analysis (ICA) was then run using the FastICA algorithm[32] to remove remaining artefacts such as scalp/facial muscle activity and eyeblinks, which was manually checked using TESA[33] component

selection. Finally, missing electrodes were replaced using linear interpolation and data were re-referenced to the average of all electrodes.

Extracting 1/f

Data were converted into the frequency domain using Welch's Method. The FOOOF[1] toolbox was then used to extract the 1/f slope exponent for all recordings via Python 3.0[34]. The 1/f slope (L) was modelled as per Equation 1:

(1) $$L = b - \log(k + F^X),$$

where b is the broadband offset, x is the exponent, k is the 'knee' parameter to control for the bend in the aperiodic slope (k=0 in log-log space), and F is the vector of input frequencies[1]. Two sets of input frequencies were applied to the data (3-30 Hz and 20-40 Hz) to explore the impact of cTBS on the 1/f slope at different ranges of the frequency spectrum. The 3-30 Hz range was selected as this is a standard frequency range assessed in EEG data as recommended by the FOOOF manual[1]. The 20-40 Hz range was then also selected as modelling data has suggested that higher frequency ranges are optimally sensitive to changes in E:I balance[5].

Statistical Analysis

Calculating the 1/f slope exponent is a relatively new analysis, and thus we first established the intra-individual reliability of the 1/f slope at baseline via intraclass correlation coefficients (ICC). Data were grouped into a priori regions of interest (ROIs; frontal, left central, right central, and parietal). The 1/f slope exponent was averaged across electrodes in each ROI and reliability was then assessed with a two-way mixed, single score, consistency ICC model. An r value of 0.50=moderate reliability, 0.75=good reliability, and 0.90=excellent reliability[35].

Next, cluster-based permutation statistics were used to assess change in 1/f slope exponent following cTBS. The cluster-based approach accounts for the multiple comparisons problem that

occurs when analysing mass univariate EEG data[36]. Clusters were defined by 2 or more neighbouring electrodes with *t*-statistics reaching significance threshold of $p<0.05$. Monte Carlo *p*-values were calculated from 5,000 randomisations of data labels. To test our first hypothesis, cluster-based permutation statistics (two-tailed *t*-tests) were conducted to compare the 1/f slope exponent at Mid, Post 1, and Post 2 timepoints to baseline in the real condition. For our second hypothesis, 1/f slope exponents at Mid, Post 1, and Post 2 timepoints were subtracted from baseline and then cluster-based permutation statistics (two-tailed *t*-tests) were used to compare change in 1/f slope exponent in the real versus sham condition at all timepoints.

To assess our third hypothesis, changes in 1/f slope exponent at Mid, Post 1, and Post 2 timepoints were compared across the two real conditions using ICC. For all analyses, $p<0.05$ was considered significant.

Results

cTBS was tolerated by all participants with no adverse events reported. The average time between experimental sessions one and two was 25.7 days (SD=37.8 days), and between sessions two and three was 44.3 days (SD=43.0 days). Mean RMT for each session is shown in Table 1.

Table 1: Mean RMT for each session.

	Real Session 1	Sham Session	Real Session 2
Sample size (n)	18	18	14
RMT (mean±SD; %MSO^{+})	71.9±9.2	71.6±10.1	68.1±10.5

$^{+}$MSO = maximal stimulator output

Reliability of 1/f Slope at Baseline

ICC was used to assess intra-individual reliability of the 1/f slope exponent at baseline. ICC found that the 1/f slope exponent in the 3-30 Hz range was highly reliable at baseline, with *r* values equal to or approaching excellent reliability in the frontal (r=0.91, p=3.5x10^{-8}), left central (r=0.88, p=3.2x10^{-7}), and right central (r=0.89, p=2.2x10^{-7}) ROIs (Figure 2A). In the 20-40 Hz range, poor baseline reliability of the 1/f slope exponent was observed in all ROIs except for the parietal region (r=0.76, p=8.9x10^{-5}), which showed good reliability (Figure 2B).

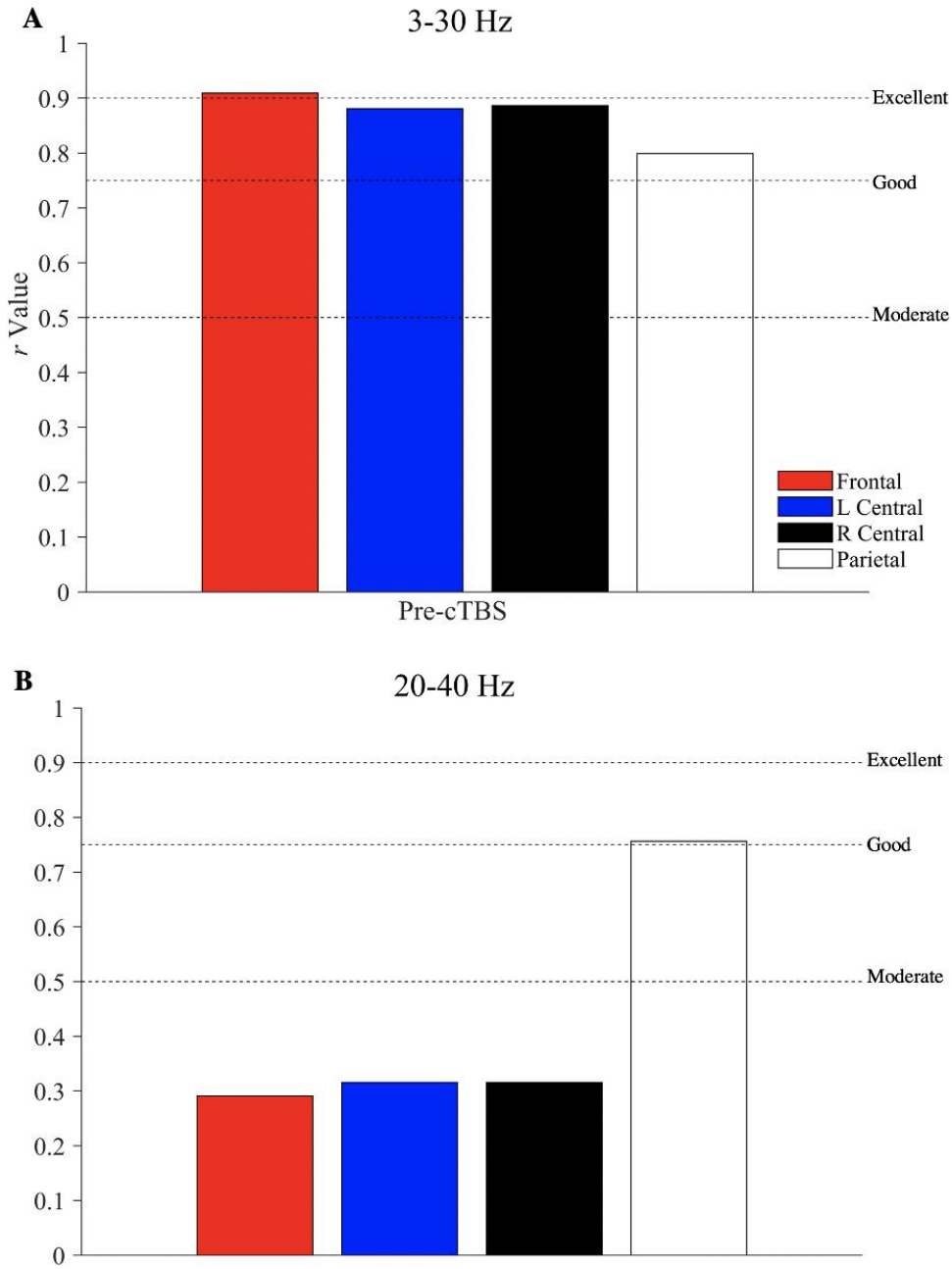

Figure 2: ICC results showing reliability of the 1/f slope exponent at baseline across the real and sham cTBS sessions in frequency ranges (A) 3-30 Hz and (B) 20-40 Hz. Reliability thresholds are indicated by dashed horizontal lines.

Change in 1/f slope

3-30 Hz

Next, we assessed whether the 1/f slope was altered in the 3-30 Hz range following cTBS via cluster-based permutation statistics. Figure 3 shows EEG power spectra at each timepoint in each condition from a representative individual. In the real condition, the 1/f slope exponent tended to decrease at the Mid and Post 2 timepoints as compared to baseline (Figure 4A), indicating a flattening of the 1/f slope. These changes did not reach the threshold for statistical significance (Post 2 p=0.094). In the sham condition, the 1/f slope became steeper at the Post 1 timepoint, however this was not statistically significant (p=0.124; Figure 4B).

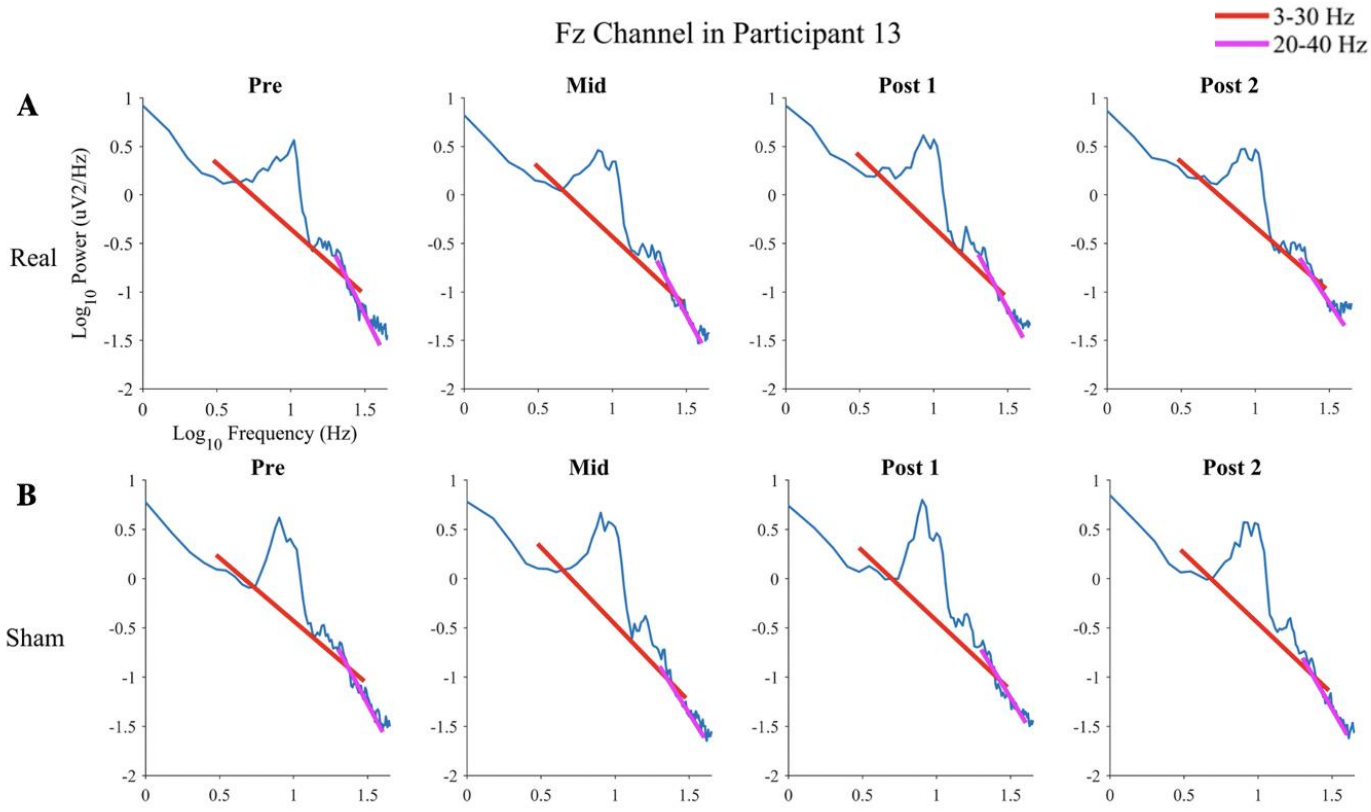

Figure 3: EEG power spectra at each timepoint from a representative individual showing the 1/f slope across two frequency ranges (3-30 Hz and 20-40 Hz) for each timepoint in the (A) real and (B) sham condition.

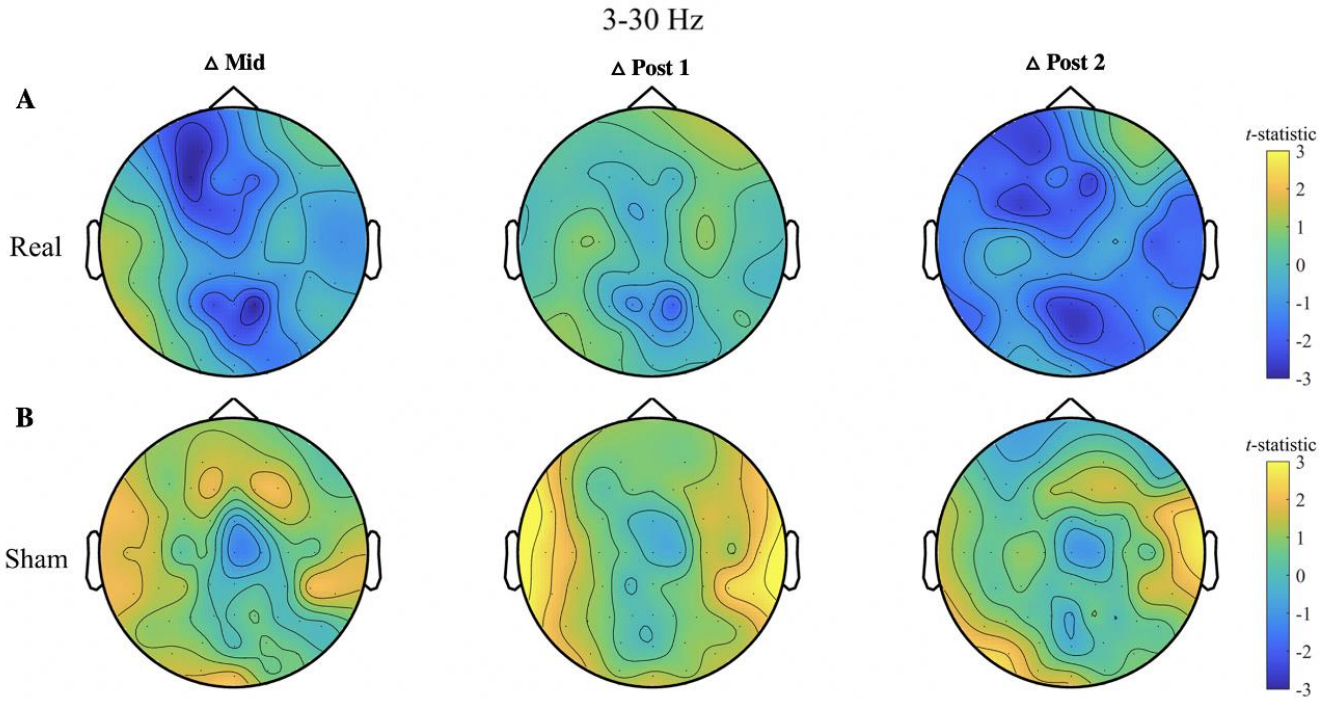

Figure 4: Topographic representation of *t*-statistics comparing change in 1/f slope following cTBS to baseline within the 3-30 Hz range in the (A) real and (B) sham condition. Blue regions=flattening of 1/f slope, yellow=steepening*, and green=no change. *=hypothesised direction of outcome.

<u>20-40 Hz</u>

As above, the change in 1/f slope exponent in the 20-40 Hz range was assessed via cluster-based permutation statistics. In the real condition, the 1/f slope exponent significantly increased at the Mid timepoint, with this difference most pronounced over the frontoparietal sensors (p=0.010; Figure 5). The 1/f slope exponent also significantly increased at the Post 1 timepoint, most prominent over the left central sensors (p=0.048). In the sham condition, the 1/f slope exponent significantly increased at the Post 1 timepoint, with the difference most pronounced over the left (p=0.008) and right (p=0.012) frontocentral sensors. The 1/f slope exponent also significantly increased at the sham Post 2 timepoint over the right central sensors (p=0.028).

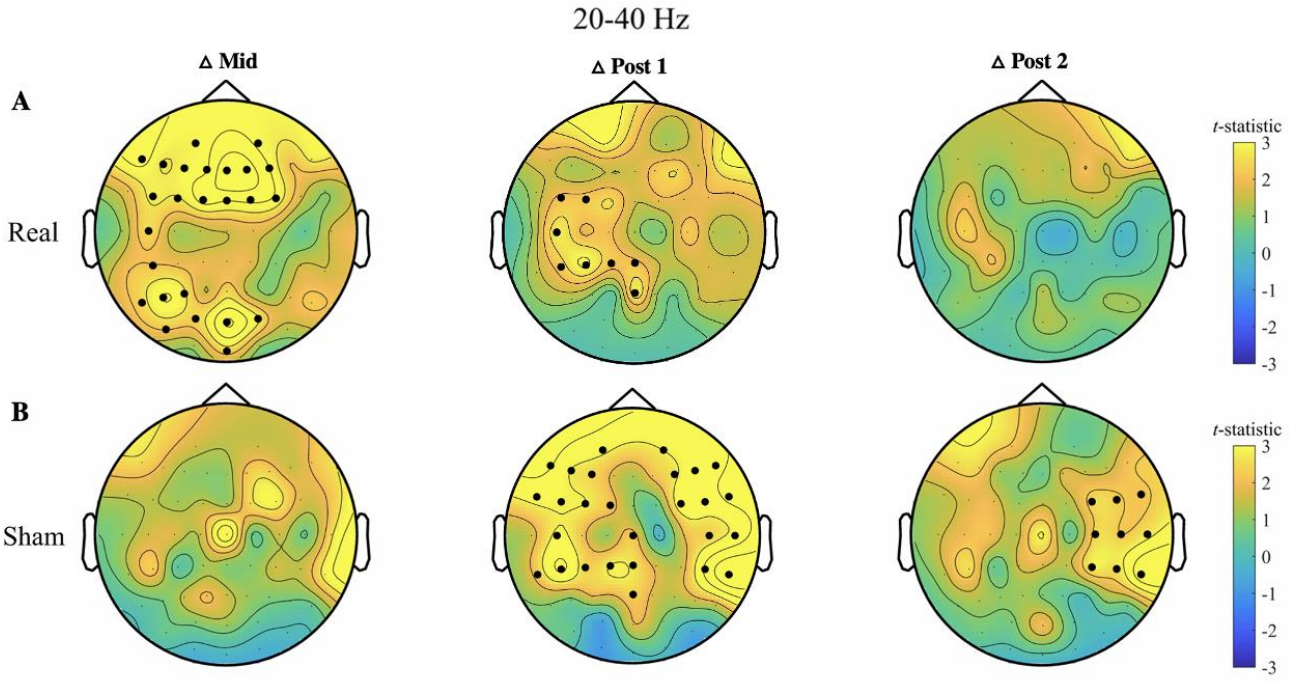

Figure 5: Topographic representation of *t*-statistics comparing change in 1/f slope following cTBS to baseline within the 20-40 Hz range in the (A) real and (B) sham condition. Significant clusters are marked by black circles.

Real versus sham

Cluster-based analysis was also conducted to compare the effects of real versus sham stimulation on the 1/f slope exponent. For 3-30 Hz, no significant difference was found between the change in 1/f slope exponent following real versus sham cTBS in either the 3-30 Hz or 20-40 Hz range (Figure 6). To assess the variability in response to cTBS, changes in 1/f slope from baseline were plotted for each participant in an example electrode (Fz; Figure 7). Considerable inter-individual variability in both the direction and magnitude of 1/f slope change was observed in Fz electrode at all timepoints. Table 2 shows the number of participants showing an increase/decrease in 1/f slope exponent as compared to baseline for this electrode.

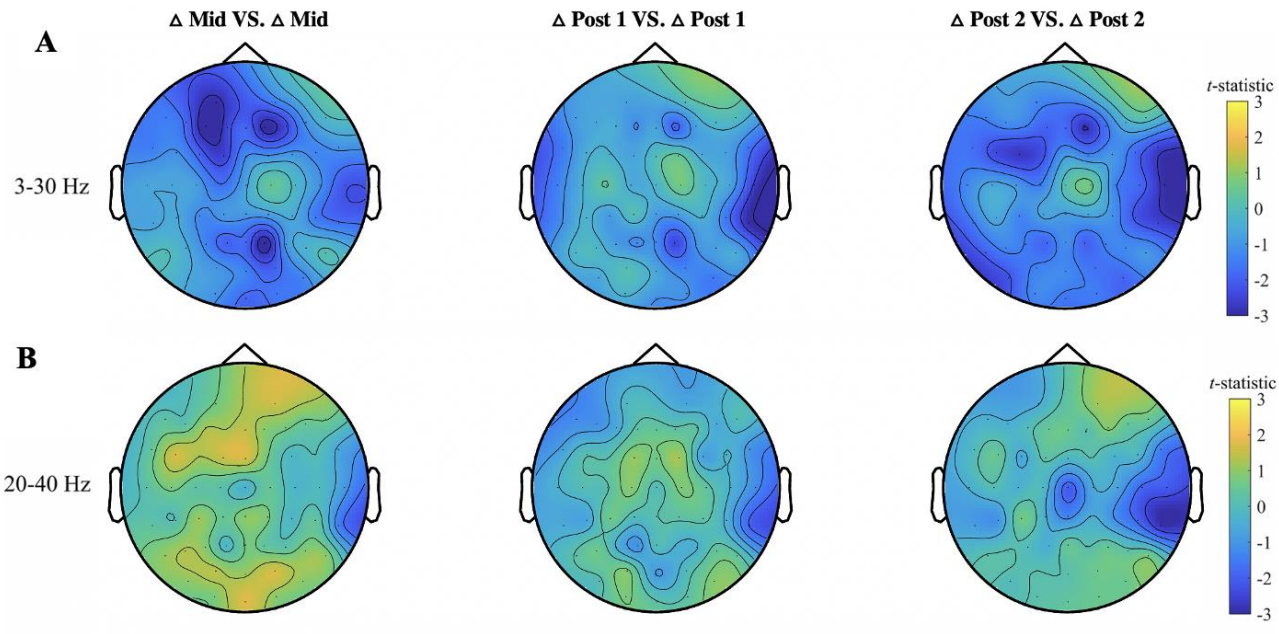

Figure 6: Topographic representation of *t*-statistics comparing change in the 1/f slope in the real versus sham condition within frequency ranges (A) 3-30 Hz and (B) 20-40 Hz. Blue regions=real 1/f slope was flatter than sham, yellow=real 1/f slope was steeper than sham*, green=no difference. *=hypothesised direction of outcome.

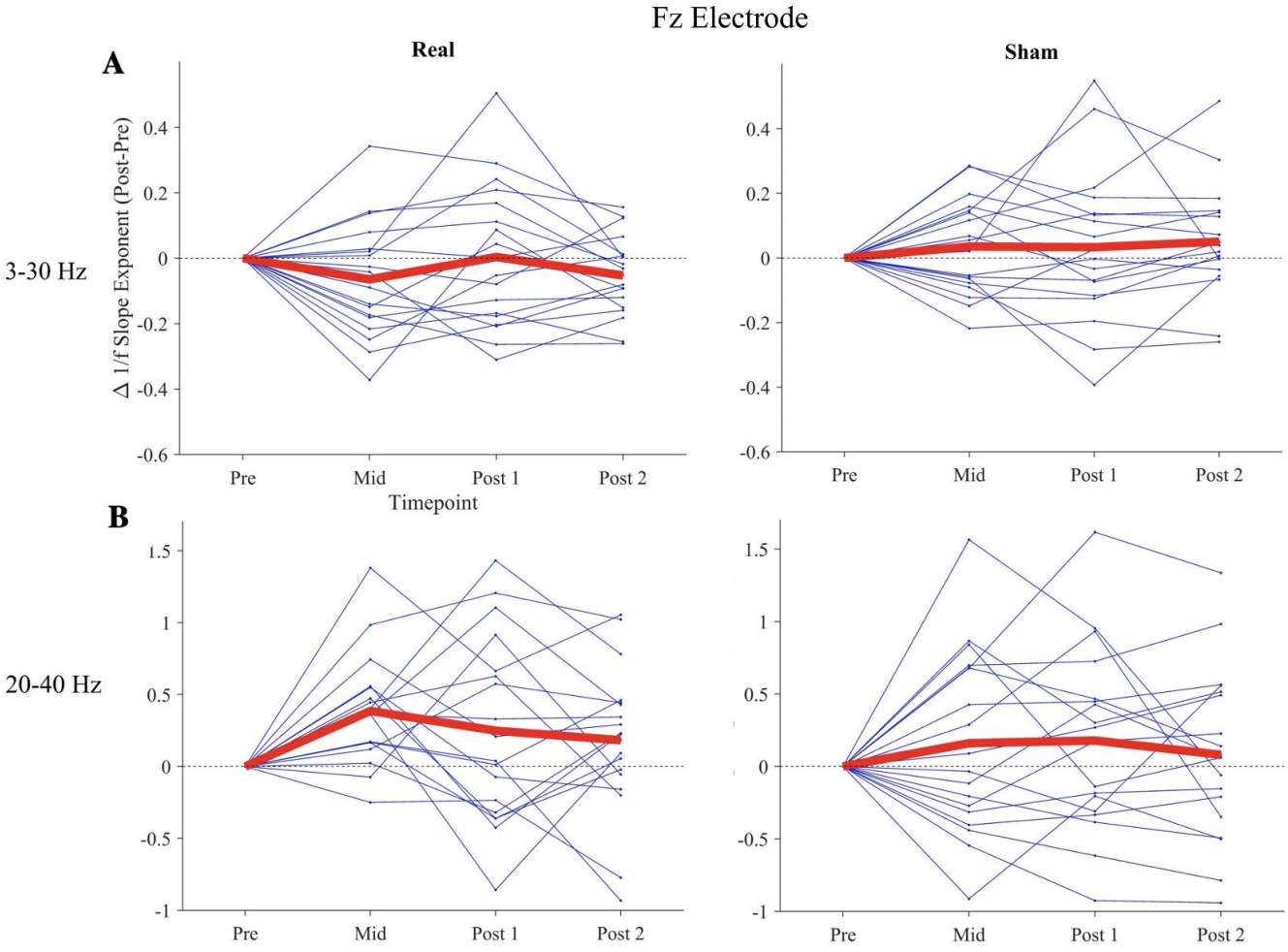

Figure 7: Change in 1/f slope exponent from baseline for each participant (blue lines) across time in frequency ranges (A) 3-30 Hz and (B) 20-40 Hz. Red line represents group mean. Data are taken from Fz electrode.

Table 2: Change in 1/f slope exponent direction as compared to baseline in Fz electrode.

		Δ Mid	Δ Post 1	Δ Post 2
3-30 Hz	Real	7/11	9/9	7/11
	Sham	10/8	9/9	11/7
20-40 Hz	Real	16/2	11/7	12/6
	Sham	9/9	10/8	10/8

Presented as n of 18 total participants showing increase/decrease

Reliability of cTBS response

Finally, ICC analysis was used to determine the intra-individual reproducibility of cTBS effects across two real cTBS sessions. In the 3-30 Hz range, moderate reliability was observed at the Mid timepoint in the parietal region (r=0.50, p=0.03; Figure 8) and at the Post 2 timepoint in the left (r=0.53, p=0.02) and right (r=0.52, p=0.02) central ROIs. In the 20-40 Hz range, poor reliability was observed at all timepoints.

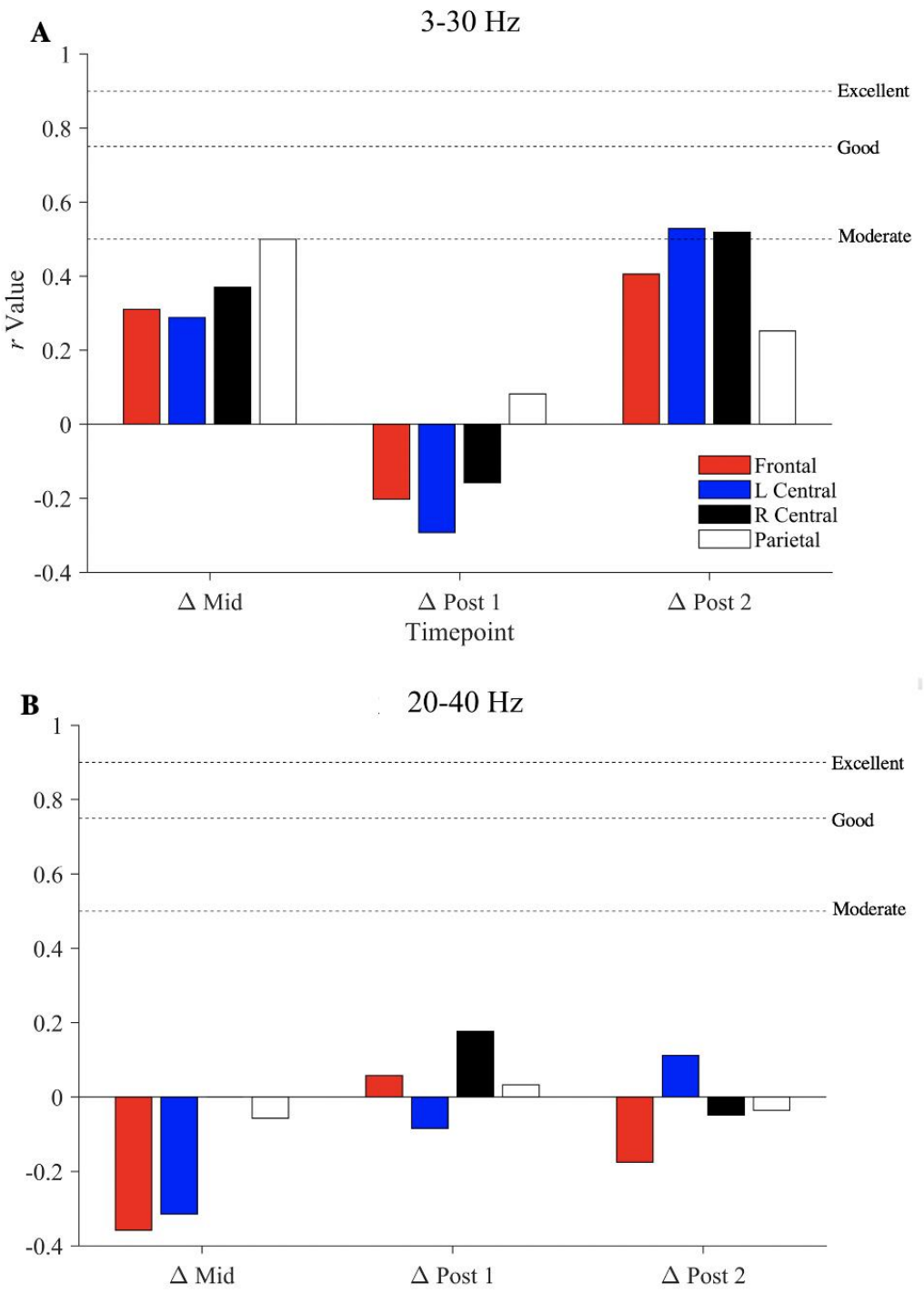

Figure 8: ICC results showing reliability of cTBS response across two real cTBS sessions in frequency ranges (A) 3-30 Hz and (B) 20-40 Hz. Note the value for right central at the Mid timepoint is -0.001 and is thus not visible below the x-axis.

Discussion

The current study investigated whether cTBS to the human M1 modulates the 1/f slope of neural noise. In the 3-30 Hz range, we found no evidence that either single or paired blocks of cTBS significantly altered the 1/f slope at group level. Similarly, we could not find evidence of a significant difference between the change in 1/f slope following real versus sham cTBS at group level. However, considerable inter-individual variability in the magnitude and directionality of 1/f slope changes was observed following cTBS, with these changes showing moderate reproducibility within individuals in the 3-30 Hz range. Our results thus tentatively suggest that cTBS may alter the 1/f slope of neural noise in the 3-30 Hz range in some individuals, although the direction of change differs between individuals. Contrastingly, the 1/f slope in the 20-40 Hz range became significantly steeper in the sham condition and showed poor reproducibility across sessions at baseline and following cTBS. As such, this frequency range appears unreliable for assessing 1/f slope characteristics in humans.

Changes in the 1/f Slope in the 3-30 Hz Range

Data in the 3-30 Hz range suggest that cTBS did not significantly alter the 1/f slope of neural noise at group level. This lack of net change may be attributed to the inter-individual response variability noted in this frequency range, as illustrated in Fz electrode where 61% of participants showed a flattening of the 1/f slope at Mid and Post 2 timepoints while 39% showed a steepening. Inter-individual variability following cTBS has been well documented throughout MEP literature[17,27], with numerous sources of variability posited. For example, Hamada et al.[18] proposed that cTBS response variation is influenced by the interneurons recruited by each pulse[37]. As the 1/f slope may be sensitive to changes in E:I balance, which is governed by excitatory and inhibitory interneurons, it is possible that a similar mechanism may impact changes in the 1/f slope following cTBS. Alongside this, a number of other factors not controlled for in this study may have contributed to inter-individual variability, including genetics, M1 physiology, and history of physical activity[38].

Although subject to considerable inter-individual variability, 1/f slope changes in the 3-30 Hz range following cTBS were moderately reproducible within individuals across two real sessions. This finding is consistent with current cTBS literature on intra-individual variability, where Vallence et al.[16] showed that cTBS at intensities of 150% and 180% of RMT caused a suppression of MEPs that was also moderately reproducible across three sessions. Given this moderate level of intra-individual reliability, it is possible that 1/f slope changes in the 3-30 Hz range may represent a true neurophysiological outcome of stimulation. On this basis, our results highlight the need to personalise rTMS protocols to drive change consistently on an individual level, as opposed to the current 'one-size-fits-all' approach employed at the group level. This complements work by Chung et al.[19], who sought to individualise rTMS protocols to optimise stimulation effects within an individual. Future work should adopt a similar individualised approach to determine whether cTBS reliably induces changes in the 1/f slope at the individual level.

In an attempt to mitigate inter-individual variability, we employed a paired cTBS paradigm to harness the brain's metaplasticity and drive plasticity in a specific direction. No evidence of a significant change in 1/f slope was observed after paired blocks of stimulation, suggesting that paired cTBS did not induce a more robust change in the 1/f slope as has been seen in MEP literature[13,22].

While not statistically significant, a tendency for the 1/f slope to flatten in the 3-30 Hz range was observed. The ability of cTBS to modulate neural activity at the lower end of the frequency spectrum has been previously reported in EEG literature, such as Rocchi et al.'s[27] finding that cTBS decreased the power of delta (1-4 Hz) brainwaves. However, the exact neurophysiological mechanisms underpinning this flattening of the 1/f slope are unclear. One possible explanation is offered by Gao et al.[5], who hypothesised that neural noise reflects E:I balance. According to computational/animal modelling and human pharmacological studies, increasing the ratio of

inhibition causes a steepening of the 1/f slope[4,5]. Our finding is therefore incongruent with an increase in inhibition in the majority of participants. Instead, 1/f slope flattening may be explained by past research that showed cTBS to M1 decreased the level of SICI[13]. In our study, this reduction in inhibition may have been stronger than any reduction in excitation, causing a higher ratio of excitation and therefore a flattening of the 1/f slope. However, this explanation remains speculative. EEG is non-invasive and it is therefore difficult to postulate the exact mechanisms that contributed to the perceived changes in the 1/f slope. Although E:I balance is one potential mechanism, other mechanisms may also alter 1/f slope characteristics such as changes in voltage-gated ion channels[39].

1/f Slope Is Unreliable in the 20-40 Hz Range

In contrast to the 3-30 Hz range, the 1/f slope in the 20-40 Hz range presented as a highly unreliable metric for capturing neural noise at scalp level. The variability of neural noise in this range is evidenced by: the low reliability of the 1/f slope at baseline across sessions; the steepening of the 1/f slope in the control sham condition; and the poor reproducibility of cTBS effects across real sessions. This unreliability may be explained by facial or scalp muscle activity, where actions such as clenching the jaw produce contaminating artefacts in the higher frequency ranges. In a study using a paralysing neuromuscular blockade, Whitham et al.[40] found that muscle contamination of the frequency spectrum began at 20 Hz in a regular resting state EEG, such that by 40 Hz there was five times more power (μV) than in EEG taken in the paralysed state. Although we used ICA to minimise this source of contamination, smaller muscle artefacts may pass undetected through cleaning procedures[41].

Another potential source of unreliability in the 20-40 Hz range is that the 1/f slope may be sensitive to changes in arousal. For example, Lendner et al.[2] found that the 1/f slope in the 30-45 Hz range became steeper when transitioning from wakefulness to rapid eye movement sleep in humans. It is thus possible in this study that participants became drowsy over the course of the session, resulting

in a steeper 1/f slope in the 20-40 Hz range at later timepoints in the session as compared to baseline.

Limitations and Future Directions

There are some limitations to this study. First, we did not report on the change in broadband offset when analysing the change in 1/f slope exponent (see Equation 1). As such, we are unable to deduce whether the flattening we observed was caused by an increase in power at the higher end of the 1/f slope or a decrease in power at the lower end of the 1/f slope (or both). Furthermore, we assumed that removing the knee to model a straight line was the best fit. Future work should factor in the offset and knee variables to precisely detail the effect of cTBS on the 1/f slope.

Additional future directions from our research include the use of other rTMS paradigms such as intermittent theta burst stimulation, which is thought to increase cortical excitability, to compare its effects on the 1/f slope. Larger frequency limits such as 30-70 Hz could also be tested, as Gao et al. stipulated that their modelling of the 1/f slope best correlated with E:I balance in this frequency range. Finally, as aforementioned, future work should adopt a personalised approach to explore whether cTBS reliably induces changes in the 1/f slope on the level of the individual.

To conclude, the results of the current study yielded no evidence that cTBS alters neural noise at group level in the 3-30 Hz range. However, considerable inter-individual variability was noted in directionality and magnitude of the 1/f slope of neural noise following cTBS, which was moderately reproducible within individuals in the 3-30 Hz range and may thus represent a neurophysiological outcome of stimulation. Contrastingly, the 1/f slope in the 20-40 Hz range presented as an unreliable metric for measuring changes in neural noise at scalp level. Further work is required to investigate the effects of cTBS on the individual level to more precisely define the impact of non-

invasive stimulation on the neurophysiological mechanisms underpinning the neural noise signal. Overall, our results add further weight to arguments in favour of personalising rTMS protocols.

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References

1. Haller, M., Donoghue, T., Peterson, E., Varma, P., Sebastian, P., Gao, R., Noto, T., Knight, R. T., Shestyuk, A., & Voytek, B. (2018). Parameterizing neural power spectra. *bioRxiv* DOI: https://doi.org/10.1101/299859.
2. Lendner, J. D., Helfrich, R. F., Mander, B. A., Romundstad, L., Lin, J. J., Walker, M. P., Larsson, P. G., & Knight, R. T. (2020). An electrophysiological marker of arousal level in humans. *eLife* **9**, e55092.
3. Miskovic, V., MacDonald, K. J., Rhodes, L. J., & Cote, K. A. (2019). Changes in EEG multiscale entropy and power-law frequency scaling during the human sleep cycle. *Human Brain Mapping* **40**(2), 538–551.
4. Colombo, M. A., Napolitani, M., Boly, M., Gosseries, O., Casarotto, S., Rosanova, M., Brichant, J. F., Boveroux, P., Rex, S., Laureys, S., Massimini, M., Chieregato, A., & Sarasso, S. (2019). The spectral exponent of the resting EEG indexes the presence of consciousness during unresponsiveness induced by propofol, xenon, and ketamine. *NeuroImage* **189**, 631–644.
5. Gao, R., Peterson, E. J., & Voytek, B. (2017). Inferring synaptic excitation/inhibition balance from field potentials. *NeuroImage* **158,** 70-78.
6. Gao, R., & Penzes, P. (2015). Common mechanisms of excitatory and inhibitory imbalance in schizophrenia and autism spectrum disorders. *Curr Mol Med* **15**(2), 146-167.
7. Peterson, E. J., Rosen, B. Q., Campbell, A. M., Belger, A., & Voytek, B. (2018). 1/f neural noise is a better predictor of schizophrenia than neural oscillations. *bioRxiv* DOI: https://doi.org/10.1101/113449.
8. Voytek, B., Kramer, M. A., Case, J., Lepage, K. Q., Tempesta, Z. R., Knight, R. T., & Gazzaley, A. (2015). Age-Related Changes in 1/f Neural Electrophysiological Noise. *J Neurosci* **35**(38), 13257-13265.
9. Lim, S., & Goldman, M. S. (2013). Balanced cortical microcircuitry for maintaining information in working memory. *Nature neuroscience* **16**(9), 1306–1314.

10. Hallett, M. (2007). Transcranial magnetic stimulation: a primer. *Neuron* **55**(2), 187-199.

11. Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P., & Rothwell, J. C. (2005). Theta burst stimulation of the human motor cortex. *Neuron* **45**(2), 201-206.

12. Kujirai, T., Caramia, M.D., Rothwell, J.C., Day, B.L., Thompson, P.D., Ferbert, A., Wroe, S., Asselman, P., and Marsden, C.D. (1993). Corti- cocortical inhibition in human motor cortex. *J Physiol **471***, 501–519.

13. Goldsworthy, M. R., Pitcher, J. B., & Ridding, M. C. (2013). Neuroplastic modulation of inhibitory motor cortical networks by spaced theta burst stimulation protocols. *Brain stimulation* **6**(3), 340–345.

14. Stagg, C. J. (2014). Magnetic Resonance Spectroscopy as a tool to study the role of GABA in motor-cortical plasticity. *NeuroImage* **86**, 19-27.

15. Stagg, C. J., Wylezinska, M., Matthews, P. M., Johansen-Berg, H., Jezzard, P., Rothwell, J. C., & Bestmann, S. (2009). Neurochemical effects of theta burst stimulation as assessed by magnetic resonance spectroscopy. *J Neurophysiol* **101**(6), 2872-2877.

16. Vallence, A. M., Goldsworthy, M. R., Hodyl, N. A., Semmler, J. G., Pitcher, J. B., & Ridding, M. C. (2015). Inter- and intra-subject variability of motor cortex plasticity following continuous theta-burst stimulation. *Neuroscience* **304,** 266–278.

17. López-Alonso, V., Cheeran, B., Río-Rodríguez, D., & Fernández-Del-Olmo, M. (2014). Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain stimulation* **7**(3), 372–380.

18. Hamada, M., Murase, N., Hasan, A., Balaratnam, M., & Rothwell, J. C. (2013). The role of interneuron networks in driving motor cortex plasticity. *Cerebral Cortex* **23**(7), 1593-1605.

19. Chung, S. W., Sullivan, C. M., Rogasch, N. C., Hoy, K. E., Bailey, N. W., Cash, R., & Fitzgerald, P. B. (2019). The effects of individualised intermittent theta burst stimulation in the prefrontal cortex: A TMS-EEG study. *Human brain mapping* **40**(2), 608–627.

20. Fung, P. K., & Robinson, P. A. (2014). Neural field theory of synaptic metaplasticity with applications to theta burst stimulation. *Journal of theoretical biology* **340**, 164–176.
21. Gentner, R., Wankerl, K., Reinsberger, C., Zeller, D., & Classen, J. (2008). Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: evidence of rapid polarity-reversing metaplasticity. *Cerebral Cortex* **18**(9), 2046–2053.
22. Goldsworthy, M. R., Pitcher, J. B., & Ridding, M. C. (2012). The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. *The European journal of neuroscience* **35**(1), 125–134.
23. Gamboa, O. L., Antal, A., Laczo, B., Moliadze, V., Nitsche, M. A., & Paulus, W. (2011). Impact of repetitive theta burst stimulation on motor cortex excitability. *Brain stimulation* **4**(3), 145–151.
24. Noh, N. A., Fuggetta, G., Manganotti, P., & Fiaschi, A. (2012). Long lasting modulation of cortical oscillations after continuous theta burst transcranial magnetic stimulation. *PloS one* **7**(4), e35080.
25. Vernet, M., Bashir, S., Yoo, W. K., Perez, J. M., Najib, U., and Pascual-Leone, A. (2013). Insights on the neural basis of motor plasticity induced by theta burst stimulation from TMS-EEG. *Eur. J. Neurosci.* **37**, 598–606.
26. McAllister, S. M., Rothwell, J. C., and Ridding, M. C. (2011). Cortical oscillatory activity and the induction of plasticity in the human motor cortex. *Eur. J. Neurosci.* **33**, 1916–1924.
27. Rocchi, L., Ibáñez, J., Benussi, A., Hannah, R., Rawji, V., Casula, E., & Rothwell, J. (2018). Variability and Predictors of Response to Continuous Theta Burst Stimulation: A TMS-EEG Study. *Frontiers in neuroscience* **12**, 400.
28. Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2011). Screening questionnaire before TMS: an update. *Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology* **122**(8), 1686.

29. Sale, M.V., Ridding, M.C., and Nordstrom, M.A. (2008). Cortisol inhibits neuroplasticity induction in human motor cortex. *J Neurosci*. **28**, 8285-8293.

30. Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of neuroscience methods* **134**(1), 9–21.

31. Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J. M. (2011). FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Computational intelligence and neuroscience* **2011**, 156869.

32. Hyvärinen, A., & Oja, E. (2000). Independent component analysis: algorithms and applications. *Neural networks: the official journal of the International Neural Network Society* **13**(4-5), 411–430.

33. Rogasch, N. C., Sullivan, C., Thomson, R. H., Rose, N. S., Bailey, N. W., Fitzgerald, P. B., et al. (2017). Analysing concurrent transcranial magnetic stimulation and electroencephalographic data: a review and introduction to the open-source TESA software. *NeuroImage* **147**, 934–951.

34. Van Rossum, G., & Drake, F. L. (2009). *Python 3 Reference Manual*. Scotts Valley, CA: CreateSpace.

35. Koo, T. K., & Li, M. Y. (2016). A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of chiropractic medicine* **15**(2), 155–163.

36. Pernet, C. R., Latinus, M., Nichols, T. E., & Rousselet, G. A. (2015). Cluster-based computational methods for mass univariate analyses of event-related brain potentials/fields: A simulation study. *Journal of neuroscience methods* **250**, 85–93.

37. Hordacre, B., Goldsworthy, M. R., Vallence, A. M., Darvishi, S., Moezzi, B., Hamada, M., Rothwell, J. C., & Ridding, M. C. (2017). Variability in neural excitability and plasticity induction in the human cortex: A brain stimulation study. *Brain stimulation* **10**(3), 588–595.

38. Ridding, M. C., & Ziemann, U. (2010). Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *The Journal of physiology* **588**(13), 2291–2304.

39. O'Donnell, C., & van Rossum, M. C. (2014). Systematic analysis of the contributions of stochastic voltage gated channels to neuronal noise. *Frontiers in computational neuroscience* **8**, 105.

40. Whitham, E. M., Pope, K. J., Fitzgibbon, S. P., Lewis, T., Clark, C. R., Loveless, S., Broberg, M., Wallace, A., DeLosAngeles, D., Lillie, P., Hardy, A., Fronsko, R., Pulbrook, A., & Willoughby, J. O. (2007). Scalp electrical recording during paralysis: quantitative evidence that EEG frequencies above 20 Hz are contaminated by EMG. *Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology* **118**(8), 1877–1888.

41. Muthukumaraswamy S. D. (2013). High-frequency brain activity and muscle artifacts in MEG/EEG: a review and recommendations. *Frontiers in human neuroscience* **7**, 138.