# Modulation of intra- and inter-hemispheric connectivity between primary and premotor cortex during speech perception

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## Abstract

Primary motor (M1) areas for speech production activate during speech perception. It has been suggested that such activation may be dependent upon modulatory inputs from premotor cortex (PMv). If and how PMv differentially modulates M1 activity during perception of speech that is easy or challenging to understand, however, is unclear. This study aimed to test the link between PMv and M1 during challenging speech perception in two experiments. The first experiment investigated intra-hemispheric connectivity between left hemisphere PMv and left M1 lip area during comprehension of speech under clear and distorted listening conditions. Continuous theta burst stimulation (cTBS) was applied to left PMv in eighteen participants (aged 18-35). Post-cTBS, participants performed a sentence verification task on distorted (imprecisely articulated), and clear speech, whilst also undergoing stimulation of the lip representation in the left M1 to elicit motor evoked potentials (MEPs). In a second, separate experiment, we investigated the role of inter-hemispheric connectivity between right hemisphere PMv and left hemisphere M1 lip area. Dual-coil transcranial magnetic stimulation was applied to right PMv and left M1 lip in eighteen participants (aged 18-35). Results indicated that disruption of PMv during speech perception affects comprehension of distorted speech specifically. Furthermore, our data suggest that listening to distorted speech modulates the balance of intra- and inter-hemispheric interactions, with a larger sensorimotor network implicated during comprehension of distorted speech than when speech perception is optimal. The present results further understanding of PMv-M1 interactions during auditory-motor integration.

## 1. Introduction

After decades of research, the neurobiological network subserving speech perception remains unclear. Without this knowledge, we are limited in our ability to understand how humans perceive and use language (Skipper, Devlin, & Lametti, 2017). The role of motor regions in the speech perception network, in particular, is still hotly debated. Involvement of motor areas in speech perception is based on the observation that speech perception activates speech production brain regions (Silbert, Honey, Simony, Poeppel, & Hasson, 2014; Skipper, Nusbaum, & Small, 2005; Wilson, Saygin, Sereno, & Iacoboni, 2004). However, discussion continues as to whether observed motor activity during situations requiring auditory-motor integration really is essential to speech processing (Meister, Wilson, Deblieck, & Wu, 2007), complementary (Möttönen & Watkins, 2009), or epiphenomenal (Hickok, Houde, & Rong, 2011). This has led to the division of 'fractionated' and 'integrated' views of speech perception (Schomers & Pulvermüller, 2016). In the fractionated view, the temporal speech perception network is key to recognising speech, but does not significantly depend on fronto-parietal speech production circuits. The 'integrated' view, however, postulates strong reciprocal links between temporal and fronto-parietal areas, yielding multimodal distributed neuronal circuits capable of reciprocal influence that are causally involved in language understanding, and provide the neuronal basis for speech perception and production.

The evidence for the latter 'integrated' network view has been proposed based largely on Transcranial Magnetic Stimulation (TMS) studies. TMS research has demonstrated that primary motor (M1) areas for speech production activate during speech perception. This has been established using single-pulse TMS to the left hemisphere to generate Motor Evoked Potentials (MEPs) in speech articulators such as the lips or tongue, which serve to index the excitability of the underlying motor pathway. Such studies have identified that activity in the corticobulbar motor pathway from left hemisphere M1 lip and tongue regions to the respective speech muscles is facilitated when perceiving speech relative to non-speech sounds (Fadiga et al., 2002; Murakami, Kell, Restle, Ugawa, & Ziemann, 2015; Murakami, Restle, & Ziemann, 2012; Watkins, Strafella, & Paus, 2003). This speech-specific increase in motor pathway excitability is further modulated by listening difficulty, whereby speech-internal distortions, such as unfamiliar manner of speaking, as well as speech-external distortions, such as background noise, have both been found to affect the excitability of the left hemisphere motor pathway for speech production (Murakami, Restle, & Ziemann, 2011; Nuttall, Kennedy-Higgins, Hogan, Devlin, & Adank, 2016). TMS studies have also been used to disrupt activation in M1 lip area, which was found to impair perception of speech sounds produced by the lips, suggesting a potentially causal role for lip M1 under ambiguous listening conditions (Möttönen & Watkins, 2009). Both types of effects of TMS to articulatory speech regions have been found to be muscle-specific, with no corresponding effects on speech perception or MEPs after TMS to M1 hand area (Möttönen & Watkins, 2009; Nuttall et al., 2016).

Although such findings are striking, articulatory M1 is not known to receive direct inputs from auditory areas, raising the question of how is auditory information able to influence activity in M1, when M1 is not connected to auditory temporal cortex. One candidate possibility is that effects observed at M1 during speech perception are mediated by ventral premotor cortex (PMv), which is thought to receive auditory inputs and is linked to temporal auditory association areas via the superior longitudinal and arcuate fasciculi. Using fMRI multivariate analysis in conjunction with probabilistic fibre tracking based on diffusion tensor imaging data, Saur and colleagues (2010) identified that the posterior Superior Temporal Gyrus (pSTG) exhibits extensive direct interactions with PMv nodes, mediated via the dorsal arcuate fasciculus/superior longitudinal fasciculus system (Frey, Campbell, Pike, & Petrides, 2008; Saur et al., 2008). This temporo–premotor interaction via the dorsal pathway is suggested to be important for a rapid, automated conversion of acoustic representations into motor representations (Vigneau et al., 2006).

It has also been suggested that right PMv mediates mirror facilitation effects observed in left M1 hand area, where it is thought that learned associations between multi-modal inputs at PMv contribute to facilitation of the corticospinal motor pathway to the hand (Catmur, Mars, Rushworth, & Heyes, 2011). In humans, the motor system governing the fingers of the hand has been found to be specifically facilitated by mirror facilitation. Mirror facilitation refers to the idea that an MEP from a finger muscle will be greater in size when a subject observes a movement performed involving that muscle, relative to a movement involving a separate muscle. Indeed, Catmur et al (2011) observed that inter-hemispheric PMv-M1 connections modulate the M1 corticospinal response to observed actions, and suggest that MEP mirror facilitation may be governed by PMv. This finding also raises the question of the role of inter-hemispheric connectivity in action observation. It stands to reason, therefore, that articulatory M1 facilitation measured during perception of speech may be underpinned by a similar PMv mechanism, if Catmur et al.'s observation can be generalised to the corticobulbar motor system. However, the intra- and inter-hemispheric significance of activity in articulatory motor networks during speech perception is not clear, as it has not been explored.

Consistent with the possibility of a (intra- or inter-hemispheric) mediating connection between PMv and M1 during speech perception, a body of neuroimaging evidence indicates that frontal brain areas involved in the planning and execution of speech gestures, i.e., the posterior part of the left inferior frontal gyrus and the PMv are activated during passive speech perception (Pulvermüller et al., 2006; Skipper et al., 2005; Wilson & Iacoboni, 2006; Wilson et al., 2004).

Disruptive TMS to left hemisphere PMv has indeed indicated a mediating role for PMv in understanding speech, particularly during phonemic segmentation (Sato, Tremblay, & Gracco, 2009) and syllable discrimination in background noise (Meister et al., 2007). This has led to the hypothesis that PMv, during language learning, may mediate the comparison of sensory representations of speech against stored articulatory productions held in repertoire, and similarly, these comparisons may further assist listening in difficult environments by helping to disambiguate auditory information (Sato et al., 2009) in line with M1 observations (D'Ausilio, Bufalari, Salmas, & Fadiga, 2012).

However, if, and how, PMv differentially modulates M1 activity during perception of speech that is easy or challenging to understand is unknown. Correlational evidence from PET and MEPs (Watkins & Paus, 2004) suggests that increased excitability of the left articulatory M1 during speech perception is significantly related to an increase in blood flow to left hemisphere frontal brain area BA 44 (Watkins & Paus, 2004). This led the authors to propose that BA 44 (pars opercularis of Broca's area) may directly, or indirectly via PMv, 'prime' the motor system during speech perception, even when no speech output is required.

Taken together, the role of PMv in speech perception remains unclear, particularly the intra- and inter-hemispheric association between PMv and articulatory M1. During effortful listening, accumulating evidence from TMS, fMRI, and PET studies has demonstrated that PMv is active relative to control conditions (Meister et al., 2007; Sato et al., 2009; Watkins & Paus, 2004; Wilson et al., 2004). Relatedly, evidence from hand MEP studies has indicated a mediating role of PMv on M1 during hand action observation, indicating that PMv may govern activity in M1 during hand perception. To further understand the role of PMv in speech perception, two outstanding issues need to be resolved. Firstly, how does disrupting activity in PMv affect speech perception behaviourally, and what effect does this disruption have on articulatory M1? Secondly, what is the significance of inter-hemispheric PMv-M1 connectivity during speech perception?

The present study aimed to build on and extend observations from Watkins and Paus (2004), by examining connectivity between left articulatory M1 and left and right PMv in two experiments. The first experiment investigated intra-hemispheric connectivity between left hemisphere PMv and M1 lip area during comprehension of speech under clear and distorted listening conditions. To this end, continuous theta burst stimulation (cTBS) was applied to PMv to directly manipulate brain activity in that region. Post-cTBS, participants performed a sentence verification task on distorted (imprecisely articulated), and clear speech, whilst also undergoing single-pulse stimulation of left M1 lip area to elicit MEPs. In a second experiment, we investigated the role of inter-hemispheric connectivity between right hemisphere PMv and left hemisphere M1 lip area using an inter-hemispheric inhibition TMS protocol to moderate lip MEPs, whilst subjects listened to clear and distorted speech, and a no-speech control. Taken together, these experiments allowed us to assess how manipulating the influence of ipsilateral and contralateral PMv impacted left hemisphere M1 lip when listening to speech.

## 2. Method

### 2.1. Subjects

In Experiment 1, twenty-two subjects took part (eight males; average age: 22 years 8 months (± SD 3 months); age range: 18–28 years). Four subjects could not tolerate cTBS to PMv and withdrew from participation. Twenty-one subjects took part in Experiment 2 (seven males; average age: 22 years 6 months (± SD 3.8 months); age range: 18–30 years), two of whom had also participated in Experiment 1. Three subjects could not tolerate the dual-pulse protocol to right PMv and left M1 lip, and withdrew from participation.  All subjects in Experiments 1 and 2 were right-handed, monolingual, native speakers of British English, with normal language function and hearing thresholds. Handedness was established via self-report. Pure-tone audiometric hearing thresholds were established using a diagnostic audiometer (AD229b, Interacoustic A/S, Denmark) in accordance with The British Society of Audiology Recommended Procedure (The British Society of Audiology, 2011), across 0.25, 0.5, 1, 2, 4, and 8 kHz bilaterally. All subjects had clinically normal thresholds (≤20 dB HL). Subjects presented no TMS contraindications as assessed by the University College London TMS safety screening form. All subjects had a minimum high school-level education, with the majority currently studying at University level. Experiments were undertaken with the understanding and written consent of each subject, according to Research Ethics Board of University College London.

### 2.2. Speech stimuli

For Experiment 1, 160 unique sentences were recorded from the Speed and Capacity of Language Processing (SCOLP) stimuli set (Baddeley, Emslie, and Nimmo-Smith, 1992). The SCOLP sentences are not matched for psycholinguistic variables. However, SCOLP sentences have been found to be a sensitive and reliable measure of the speed of language comprehension, as errors tend to be low across patient and control groups (Adank, Evans, Stuart-Smith, & Scott, 2009; Baddeley, Emslie, & Nimmo-Smith, 1992; Bayre, Geffen, & McFarland, 1997). These comprised two sets of clear sentences, and two sets of distorted sentences based on motor distortion (40 sentences for each set), to ensure unique sentences were tested at baseline and post-cTBS. The average duration of clear sentences was 2008 ms (SD 351 ms), and for distorted sentences was 2585 ms (SD 516 ms). These durations reflect the natural articulation of both types of speech, with the distorted sentences being more difficult and taking longer to articulate. Stimuli were presented in blocks of clear and distorted sentences, with one block of each stimulus type. The order of stimulus block type was counter-balanced across subjects. The SCOLP sentences are designed to be used for semantic verification; are all obviously true or are false, with false sentences being based on a mismatch of subject and predicate from true sentences, i.e., *'Melons are people'*. For each subject, and for each stimulus type and time point (baseline or post-cTBS), a stimulus list containing forty stimuli was randomly permuted, and stimuli were presented according to this order in each condition.

In Experiment 2, twenty vowel-consonant vowel (VCV) syllables containing an equal distribution of lip- (/apa/, /aba/) tongue-articulated (/ata/, /ada/) syllables were recorded. Two sets of the same twenty syllables were created: a clear set based on natural articulation, and a set based on motor distortion. All stimuli were naturally produced to be of approximately the same duration (mean 2864 ms) but were not synthetically manipulated to be precisely the same length. Stimuli varied by a standard deviation of 573 ms. For each subject, and for each condition, a stimulus list containing five occurrences of /apa/, /aba/, /ata/ and /ada/ stimuli was randomly permuted, and stimuli were presented according to this order (20 stimuli in total per condition).

Distorted stimuli were always based on a motor distortion, where the speaker produced the stimuli whilst speaking with a tongue depressor. The tongue depressor was a flat wooden spatula with rounded ends, and was five inches long and one inch wide. A tongue depressor was specifically chosen so as to introduce a motor-based distortion into the speech signal, to relate the speech perception challenge to a speech production difficulty (for further information about these stimuli, and how they are perceived, please see Nuttall et al., 2016). This enabled us to contrast clear speech against distorted speech produced by the same speaker, in contrast to imposing synthetic manipulations upon the spectral characteristics of the original clear speech. Clear speech comprised naturally articulated, normal speech.

Stimuli were produced by a female British English speaker aged 27 years old for Experiment 1, and by a male British English speaker aged 23 for Experiment 2. Stimuli were recorded in a sound-attenuated room and audio digitized at 44.1 kHz with 16 bits. All stimuli were amplitude root-mean-square normalized offline using Praat (Boersma and Weenink, 2016), and then presented using Matlab (The Mathworks, Inc., Natick, MA) through ultra-shielded insert earphones (ER-3A; Etymotic Research, Inc., IL), at a comfortable listening level of around 65 dB SPL.

## 2.3. Design

Two separate experiments were undertaken to assess how PMv-M1 interactions are modulated when listening to clear and distorted speech, as shown in Figure 1. In Experiment 1, continuous theta burst stimulation (cTBS) TMS was applied to modulate cortico-cortical interactions between left PMv and left M1 lip. This allowed us to test the contribution of left PMv to lip MEPs evoked during speech perception, as well as perception of the associated speech. Subjects were instructed to semantically verify the sentences at baseline and post-cTBS as quickly as possible without compromising accuracy. Subjects were asked to respond using the index finger of the left hand, and to press the left arrow key '<' if a sentence was true, and the right arrow key '>' if a sentence was false. The left hand was used instead of the dominant right hand in order to avoid any motor preparation and execution effects affecting global motor activity in left hemisphere M1. The order of experimental conditions in Experiment 1 was counter-balanced. The following two experimental conditions were tested:

1) Distorted: Listening to motor-distorted speech produced using a tongue depressor.
2) Clear: Listening to naturally articulated clear speech.

In Experiment 2, an inter-hemispheric TMS protocol was used to modulate inter-hemispheric connectivity between right PMv, and left M1 lip (Chen, 2004; Ni et al., 2009). This allowed us to examine if right hemisphere PMv exerts an influence over left M1 lip during speech perception. Subjects were instructed to listen passively to the speech stimuli. The order of experimental conditions in Experiment 2 was randomised. The following three experimental conditions were tested in the inter-hemispheric double-pulse protocol and were all expressed relative to the single-pulse control condition, which was measured using single-pulse stimulation to left M1 lip, without any auditory stimulation:

1) Distorted: Listening to motor-distorted speech produced using a tongue depressor.
2) Clear: Listening to naturally articulated clear speech.
3) No-speech Control: No auditory stimulation.

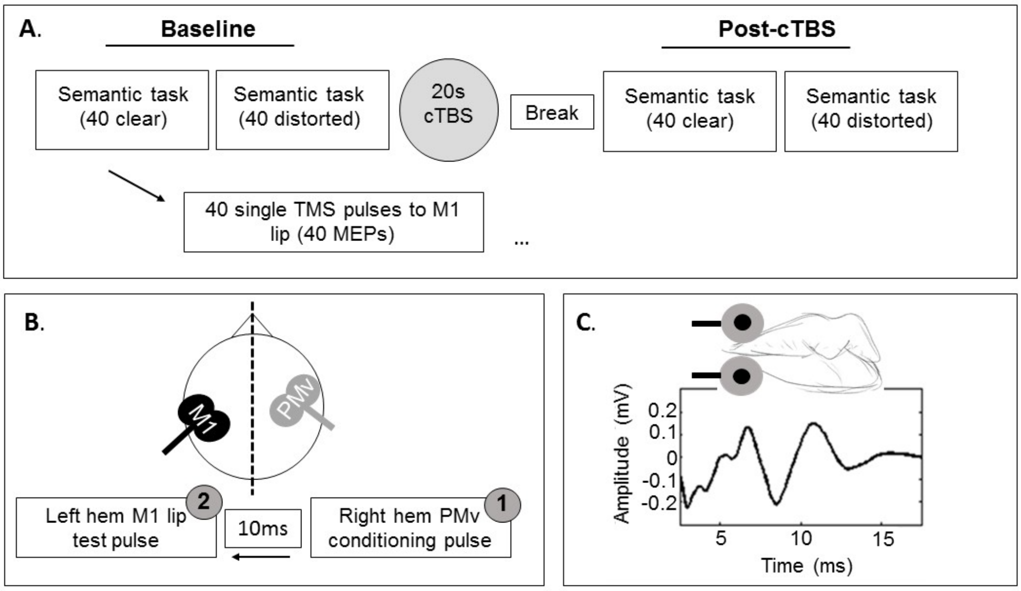

Figure 1. A. Schematic of the cTBS design in Experiment 1. Subjects semantically verified 40 clear sentences and 40 distorted sentences at baseline. At the same time, subjects received single-pulse TMS to M1 lip area. Each sentence was accompanied by one TMS pulse, generating one lip MEP per sentence (example MEP given in C.). Subjects received 20 seconds of cTBS. After a 5 minute break, subjects then performed the semantic verification task again with MEP measurement, as described at baseline. Note that baseline measurements were performed before cTBS, as shown in A., or at the end of the experiment. B. Schematic of inter-hemispheric TMS design in Experiment 2. One conditioning pulse was applied at 120% aMT to right PMv (1). A test pulse was then applied after 10 ms at 0.5mV threshold intensity to left M1 lip area (2), generating an MEP. This procedure was performed during perception of clear speech, distorted speech, and without auditory stimulation (no-speech control).

## 2.4. Transcranial magnetic stimulation

### 2.4.1 MRI acquisition and co-registration

T1-weighted structural magnetic resonance imaging (MRI) scans were acquired using a Siemens Avanto 1.5T MRI scanner and a 32 channel head coil (Siemens Healthcare, GmbH, Germany) at the Birkbeck-UCL Centre for Neuroimaging (BUCNI). A structural image for each participant was obtained using an MP-RAGE sequence [repetition time (TR) = 2730ms; echo time (TE) = 3.57ms;

voxel size = 1x1x1mm; slices = 176]. Once obtained, the structural scans were later used in the main TMS session in conjunction with Brainsight frameless stereotaxy (Rogue Research, Montreal, Canada). For each participant, we performed co-registration between the participant's head and MRI using four anatomical landmarks (tip of the nose, bridge of the nose, and intertragal notch on the left and right ears), which were first identified and marked on the participant's MRI. Accuracy of co-registration was assessed visually using an infrared tracking system (Polaris, Northern Digital, Waterloo, Canada). Upon successful co-registration, infrared tracking was used throughout the experiments in order to maintain coil position during the stimulation.

### 2.4.2. Motor thresholds

In both Experiments 1 and 2, monophasic single TMS pulses were generated by a Magstim $200^2$ unit and delivered to left M1 by a 70mm diameter figure-of-eight coil (Magstim, Dyfed, UK) set to simultaneous discharge mode (inter-pulse spacing of 1 ms). The coil was placed tangential to the skull at a 45 degree angle such that the induced current flowed from posterior to anterior under the junction of the two wings of the figure-of-eight coil. The lip area of M1 was found by using the functional 'hot spot' localization method, whereby application of TMS elicits an MEP from the contralateral muscle. Here, the coil position on the precentral gyrus is adjusted in millimetre movements to ascertain the location on the scalp at which the most robust MEPs are elicited. This location was then marked on a cap and the motor threshold (MT) determined. Before finding the lip area, we first located the hand area by asking subjects to perform a pinching action where the index finger was held against the thumb to activate first dorsal interosseous. Following this, the lip area 'hot spot' was identified by moving the coil ventrally and slightly anterior until an MEP was observed in the contralateral lip muscle. In Experiment 1, the active MT was identified, which constitutes the intensity at which TMS pulses elicited 5 out of 10 MEPs with an amplitude of at least 0.2 mV during 20% of maximum voluntary muscle contraction (Möttönen, Rogers, & Watkins, 2014). The intensity of the stimulator was then set to 120% of aMT for the single-pulse stimulations applied during the experiment. The mean stimulator intensity (120% aMT ± SD) used to elicit lip MEPs in Experiment 1 was 51.4% (±4).

In Experiment, 2 a dual-pulse inter-hemispheric inhibition protocol was utilised, which comprised a conditioning pulse to right PMv followed by a test pulse to left M1 lip (Chen, 2004; Ni et al., 2009). First, aMT was established as detailed above in right M1 lip area, though a 50mm diameter figure-of-eight coil (Magstim, Dyfed, UK) was used in thresholding, which was the same coil used for delivering TMS to the right hemisphere in the dual-pulse protocol (please see section

2.4.3.2 for further details). However, we were unable to record robust lip MEPs from right M1 lip in three subjects, who did not continue on with the study. The intensity of the TMS pulse to right PMv (conditioning stimulus) was subsequently set to 120% of right M1 lip aMT (mean 61.5% ±5.7). For left M1 lip, we found the hot spot using the method detailed above using a 70mm diameter figure-of-eight coil (Magstim, Dyfed, UK), and then set the intensity such that the TMS pulse elicited a lip MEP of on average 0.5 mV in 5 out of 10 MEPs (test stimulus), without any conditioning pulse stimulation, in line with inter-hemispheric inhibition protocols based on M1 hand (Di Lazzaro et al., 1999; Mochizuki, Huang, & Rothwell, 2004). The mean stimulation intensity for the test pulse was 67.6% (±5.6) of maximum stimulator output. All test pulses were applied using the 70mm coil that had been used for left M1 lip thresholding.

### 2.4.3. TMS protocols

#### 2.4.3.1. Experiment 1

After establishing TMS test intensity, half of the subjects then received two blocks of single-pulse TMS to the lip area of M1 in the left hemisphere to measure baseline MEPs during perception of blocks of clear and distorted speech. This was followed immediately by 20s cTBS to PMv, and then two more test blocks of single-pulse TMS to measure MEPs during perception of clear and distorted blocks of speech, post-cTBS. The other half of the subjects received cTBS first, and then two test blocks of single-pulse TMS during perception of clear and distorted speech to record post-cTBS MEPs. This was followed by a break, and then repeated in order to record baseline MEPs during perception of clear and distorted speech. The baseline MEP measurements were always performed at least 30 minutes after administering cTBS to ensure that baseline performance had returned, as 20s of cTBS is thought to affect the brain for around 20 minutes (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). The order of baseline testing was counter-balanced to control for order effects. This design mitigates against post-TMS results being confounded by practice effects. Baseline order showed no significant interactions with any of the experimental variables (all $p > 0.1$), which suggests that it is highly unlikely that cTBS contaminated the baseline data for subjects who performed baseline measurements after cTBS.

During the single-pulse TMS test blocks, subjects were presented with the speech stimuli (see Methods section 2) and were asked to semantically verify the sentences. During the presentation of each speech stimulus, Matlab was used to externally trigger the TMS system at a jittered time point towards the middle or end of the sentence to avoid intersensory facilitation effects, i.e., where reaction times to the auditory stimuli is primed due to the sensation of a TMS pulse occurring at a predictable time. All speech stimuli were accompanied by a TMS pulse; therefore, all trials were presented with TMS. The timing of the single-pulse TMS delivery was not manipulated to coincide specifically with a particular phoneme; therefore, MEPs did not represent specific time-locked phoneme-based MEPs. TMS test blocks lasted for approximately 3-4 minutes, allowing for the application of 40 TMS pulses per block. Single-pulse TMS was always performed using a Magstim $200^2$ unit and delivered by a 70mm diameter figure-of-eight coil (Magstim, Dyfed, UK).

For cTBS to PMv, a $\text{Rapid}^2$ stimulator and 70mm diameter figure-of-eight coil (Magstim, Dyfed, UK) was always used. The intensity of cTBS was fixed for all subjects at 40%, as it is not feasible to record robust lip MEPs using a biphasic pulse, as delivered by the $\text{Rapid}^2$, and nor is it possible to extrapolate motor thresholds obtained using a monophasic stimulator (Magstim $200^2$ unit), to a biphasic stimulator. The stimulation site for cTBS to left PMv was based on the average MNI space co-ordinate from Meister et al., 2007: -53, -4 and -49, which fell within the superior portion of the PMv. This was marked in each subject’s anatomical scan using Brainsight software (Rogue Research Ltd, Montreal, Canada). Across subjects, the co-ordinate fell within premotor cortex, but not always within superior PMv. For the stimulation, we used 20s of cTBS in one offline train of 300 pulses. cTBS is a patterned form of repetitive TMS. The standard theta burst pattern consists of three pulses given in a 50 Hz burst and repeated every 200 ms (5 Hz). We allowed for a 5 minute interval immediately after stimulation to allow for stimulation effects to stabilise, in line with published literature (Huang et al., 2005), after which the single-pulse protocol was administered.

#### 2.4.3.2 Experiment 2

In Experiment 2 a dual-pulse inter-hemispheric inhibition protocol was utilised involving a conditioning pulse to right PMv and a test pulse to left M1 lip. Test pulses in the dual-pulse TMS conditions, and the single-pulse control TMS condition were always performed using a Magstim $200^2$ unit and delivered by a 70mm diameter figure-of-eight coil for left hemisphere stimulation. A 50mm dimeter figure-of-eight coil was always used for the conditioning pulses to the right hemisphere in the dual-pulse conditions (Magstim, Dyfed, UK). It was necessary to use the 50mm coil on the right side of the head as two 70mm coils will not fit when both are positioned at a $45^o$ angle tangential to the skull, which we did not wish to compromise as different coil orientations target different populations of neurons (D’Ostilio et al., 2016; Hannah & Rothwell, 2017). The stimulation site for the conditioning pulse to right PMv was based on the MNI space co-ordinate from Catmur et al., (2011):

57, 12, and 23. This was marked in each subject's anatomical scan using Brainsight software (Rogue Research Ltd, Montreal, Canada). We used a 10ms inter-pulse-interval after the conditioning pulse before administering the subsequent test pulse to left M1 lip area, consistent with inter-hemispheric inhibition protocols (Di Lazzaro et al., 1999; Mochizuki et al., 2004). This dual-pulse protocol was always administered by two experimenters who held one coil each, as it is not feasible for one experimenter to hold both coils at the same time.

### 2.4.4 Electromyography

In both Experiments 1 and 2, electromyographic (EMG) activity was recorded from the lip muscle, orbicularis oris, using surface electrodes (Ag/AgCl; 10-mm diameter) in a non-Faraday caged, double-walled sound-attenuating booth. Electrodes were attached to the orbicularis oris on the right side of the mouth in a bipolar belly-belly montage, with an electrode placed at the right temple serving as a common ground. To stabilize background EMG activity, subjects were trained for approximately five minutes to produce a constant level of contraction (approximately 20% of maximum voluntary contraction) of the lip muscles by pursing, which was verified via visual feedback of the ongoing EMG signal (Möttönen et al., 2014; Watkins et al., 2003). Contraction of the lip muscle also facilitates a lower motor threshold relative to when the muscle is at rest, enabling the use of lower levels of stimulation during the experiment. The raw EMG signal was amplified by a factor of 1000, band-pass filtered between 100–2000 Hz, and sampled at 5000 Hz online using a 1902 amplifier (Cambridge Electronic Design, Cambridge), and analog-to-digital converted using a Micro1401-3 unit (Cambridge Electronic Design, Cambridge). Continuous data were acquired and recorded using Spike2 software (version 8, Cambridge Electronic Design, Cambridge).

## 2.5. Data analysis

In Experiment 1, the proportion of correct responses and associated relative reaction times for the distorted and clear speech pre- and post-cTBS were calculated for each individual. Reaction times were expressed relative to the end of each stimulus, which shows how long after the end of the stimulus a response was made. This is in contrast to expressing the response from the onset of the stimulus, which does not take into account differences in stimulus length. The end of each stimulus was visually identified from the waveform and spectrogram by a trained phonetician using Praat software, who located the final voicing cycle of a vowel, release of a consonant, or cessation of frication, for example, in the spectrogram. In Experiments 1 and 2, for the MEP data, individual EMG sweeps starting 40 ms before the TMS pulse and ending 1000 ms post-stimulation were exported offline from the recording software into Matlab. Individual MEPs were identified in each trial and rectified. The integrated area under the curve (AUC) of the rectified EMG signal of each individual mean MEP was then calculated. In Experiment 2, dual-coil MEP ratios were calculated for by dividing each dual-coil condition MEP (clear, distorted, and no-speech control) by the MEPs in the single-pulse no-speech control condition, to express the influence of the dual-coil protocol on MEPs relative to the single-pulse baseline measure. MEP AUCs were then converted into standard scores within subjects, to control for inter-subject variability. The standardized AUCs of MEPs were used in the statistical analyses. The average height of the pre-TMS baseline EMG activity was also computed, and paired t-tests confirmed that there were no significant differences between baseline activity levels between any conditions in Experiment 1 nor 2, indicating that baseline activity did not influence MEPs across conditions.

Statistical analyses were carried out using SPSS (version 22.0, IBM). In Experiment 1, two-way repeated measures analysis of variance (RMANOVA) were conducted on reaction time, accuracy, and standardised MEP AUC as the dependent variables, with stimulus type (clear, distorted), and time (baseline, post-cTBS), as within-subjects factors. In Experiment 2, a one-way RMANOVA was conducted on standardised MEP AUC ratio, with stimulus type (clear, distorted, no-speech control) as the within-subject factor. Planned comparisons were subsequently computed where appropriate (alpha level = .05).

## 3. Results

### 3.1. Experiment 1

### 3.1.1. Accuracy

Experiment 1 tested how cTBS to left PMv affected behaviour and MEP responses from left M1 lip during perception of clear and distorted speech. The mean difference in accuracy as a function of time and stimulus type is shown in Figure 2. On average, there was a difference in the accuracy of responses to clear and distorted speech at baseline and after cTBS. A two-way repeated measures ANOVA demonstrated a significant main effect of stimulus type on accuracy (F(1,17) = 117.68, p < 0.001, partial eta = .87), confirming that subjects were less accurate in identifying distorted stimuli (73.3%, SE 2.8%)  relative to clear (95.1%, SE 1.15%). The main effect of time was not significant (F(1,17) = 2.9, p = .10), nor was the time x stimulus type interaction (F(1,17) = 2.1, p =

.16), indicating that cTBS did not modulate the accuracy of subjects' responses to clear and distorted stimuli.

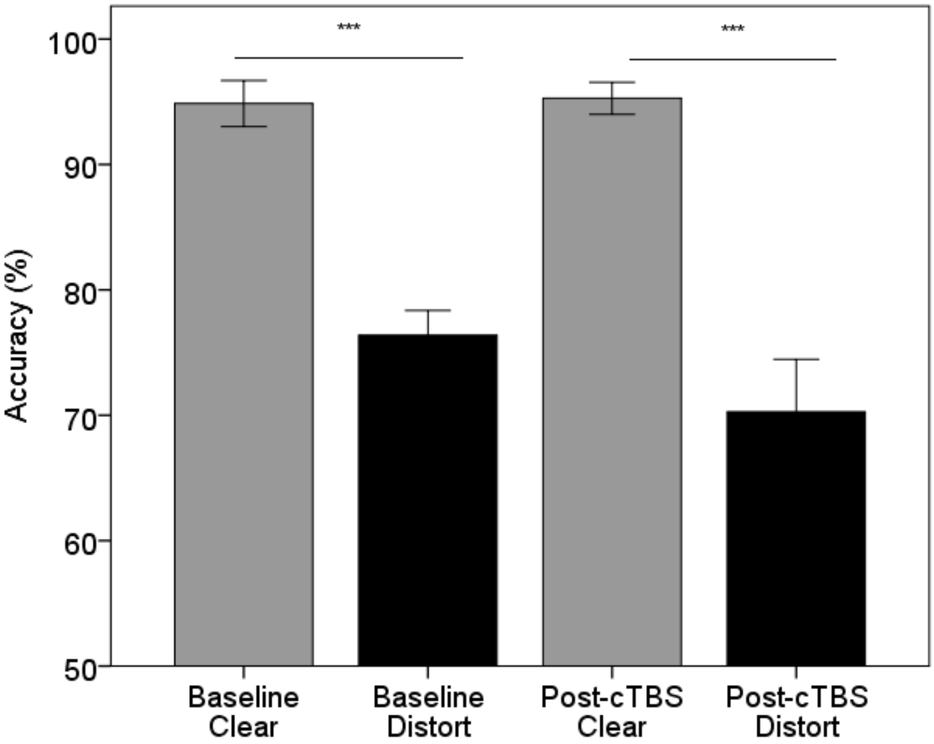

Figure 2. Average percent correct performance for clear (grey) and distorted (black) stimuli at baseline and after cTBS to left PMv. Error bars represent +/- 1 SE.

3.1.2. Reaction time

The mean difference in reaction time as a function of time and stimulus type is shown in Figure 3. On average, there was little difference between reaction times to clear and distorted speech at baseline. The difference between reaction times to clear and distorted speech increased after cTBS. A two-way repeated measures ANOVA found that the interaction between stimulus type and time was significant (F(1,17) = 5.35; p = 0.033), suggesting that cTBS affected reaction times differently depending on the type of speech stimulus perceived. Paired t-tests (two-tailed) confirmed a significant difference between reaction times post-cTBS to clear and distorted speech (t(17) = 2.13, p = 0.048 [clear = 433.37 ms, SE 37.45 ms; distorted = 350.43 ms, SE 23.16 ms]), which was not present at baseline (t(17) = -0.22, p=0.83 [clear = 405.82 ms, SE 48.57 ms; distort = 413.94, 37.52]). No main effects were significant: time = F(1,17) = 0.37, p = 0.55; stimulus type = F(1,17) =

1.29, p = 0.27. There was a trend towards a significant reduction in reaction times post-cTBS relative to baseline for distorted speech (p = 0.08 [baseline: 413.94 ms, 37.52 SE ms, post-cTBS: 350.43 ms, SE 23.16 ms]), which was not evident in reaction times to clear speech post-cTBS (p = 0.4). These data indicate, therefore, that the significant interaction is driven by cTBS to PMv reducing response time to distorted speech specifically, without changing response time to clear stimuli.

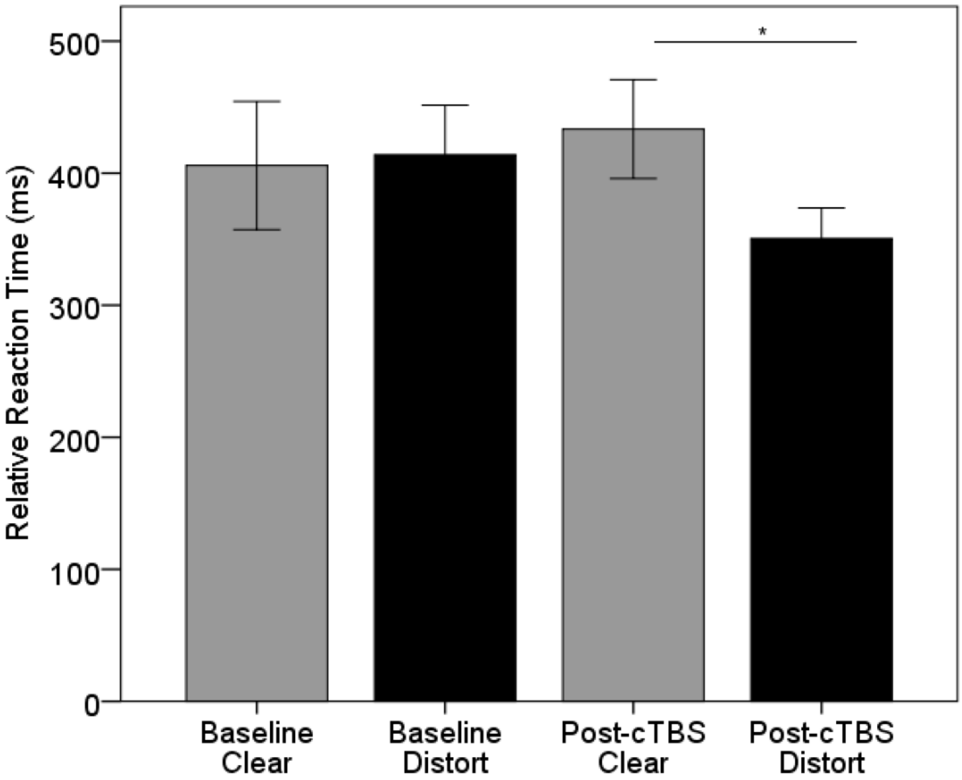

Figure 3. Average relative reaction time to clear (grey) and distorted (black) stimuli at baseline and after cTBS to left PMv. Error bars represent +/-1 SE.

### 3.1.3. Effect of cTBS on Lip MEPs

The mean difference between MEPs elicited during perception of clear and distorted speech at baseline and post-cTBS is shown in Figure 4. Two-way RMANOVA indicated that MEPs were not modulated by stimulus type (F(1,17) = 0.27, p = 0.61) or by time (F(1,17) = 0.30, p = 0.44). The interaction was also not significant (F(1,17) = .94, p = .17). These data confirmed that cTBS to PMv did not influence MEPs during perception of clear or distorted stimuli.

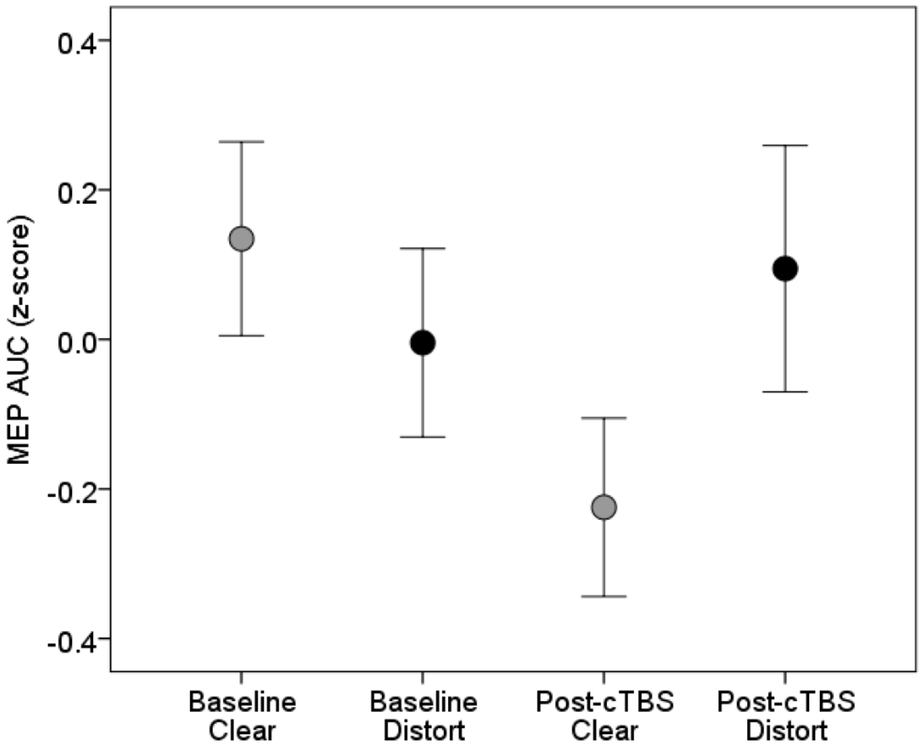

Figure 4. Average MEP area during perception of clear (grey) and distorted (black) speech at baseline and after cTBS to left PMv. Error bars represent +/-1 SE.

Notably, there was considerable variability in the effect of cTBS on motor excitability. Individual subject’s responses to cTBS for each condition are shown for in Figure 5. In some subjects, cTBS caused MEP facilitation, whereas in other subjects, cTBS caused MEP inhibition. The direction of the effect was also not consistent across speech conditions.

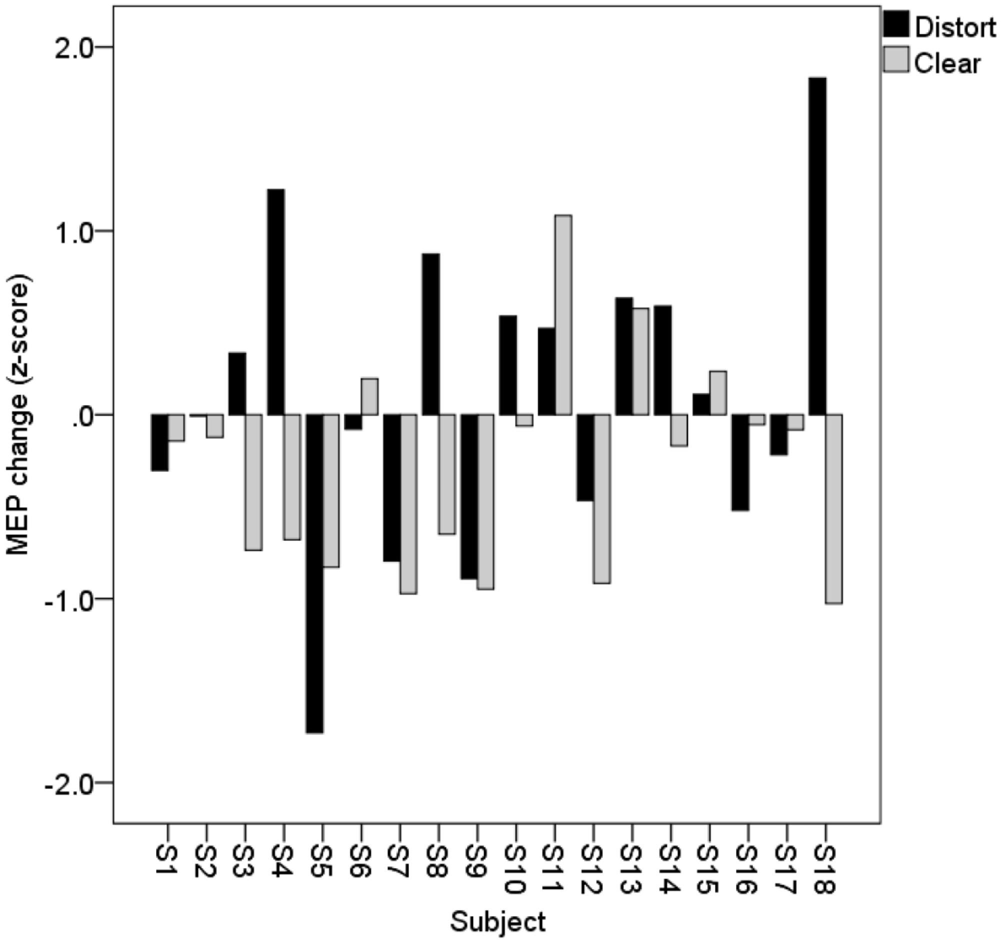

Figure 5. Bars express individual participant's change scores (Δ) in MEP area from baseline, to post-cTBS, in both Distorted (black) and Clear (grey) conditions.

### 3.2. Experiment 2

#### 3.2.1. Motor Evoked Potentials

Experiment 2 tested how a dual-pulse inter-hemispheric inhibition protocol to right PMv affected MEP responses from left M1 lip during perception of clear and distorted speech, as well as during the no-speech control condition. Mean MEP data are shown in Figure 6, where it can be observed that distorted MEP data are further away from the mean than either clear or control MEP data. A one-way RMANOVA indicated that there was a significant effect of stimulus type on the extent of the inter-hemispheric MEP inhibition resulting from disruption of right PMv (F(1,14) = 3.5, p = 0.044, partial eta squared 0.2). Planned comparisons confirmed a significant difference between MEPs during perception of clear and distorted speech elicited after dual-pulse TMS (p = 0.035). The difference between control and distorted MEPs showed a trend towards a significant difference (p = 0.08). There was no significant difference between control MEPs and MEPs elicited during perception of clear speech (p = 0.3). These data indicate that when listening to distorted speech, inhibiting right PMv inhibits MEPs from left M1 lip area. These findings suggest that right PMv may play a role during perception of distorted speech specifically

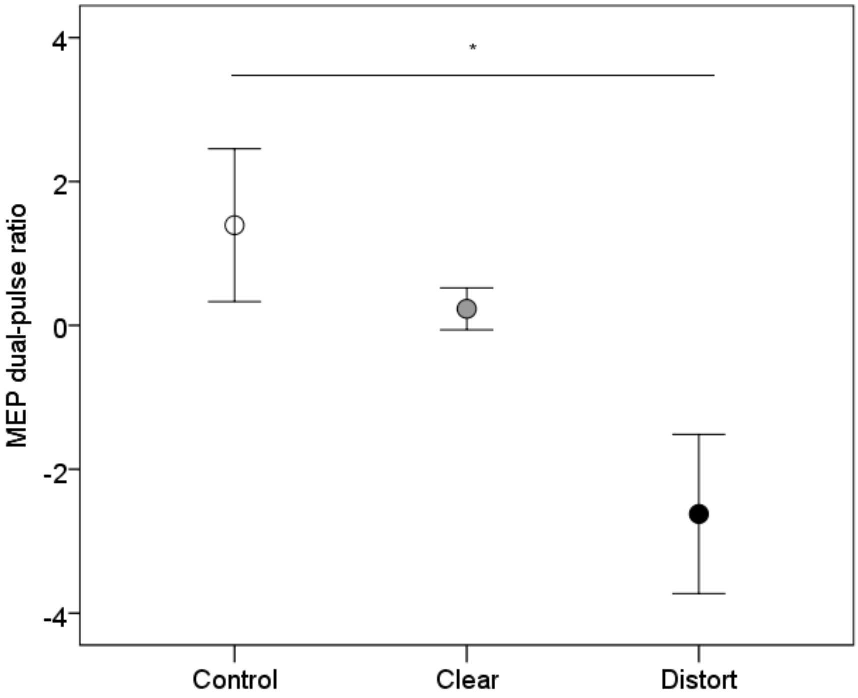

Figure 6. MEP ratios following dual-pulse inter-hemispheric TMS to right PMv and left M1 lip area, during perception of clear (grey) and distorted (black) speech, and no-speech control (no auditory stimulation, open circle) conditions. MEP ratios were calculated by dividing each dual-coil condition MEP (clear, distorted, and no-speech control) by the MEPs in the single-pulse no-speech control condition, to express the influence of the dual-coil protocol on MEPs relative to the single-pulse baseline measure.

## 4. Discussion

The present study aimed to investigate the intra- and inter-hemispheric influence of PMv on left M1 during speech perception. Experiment 1 showed that disruption to PMv causally affected reaction time to speech under distorted listening conditions specifically, but did not affect responses to clear speech. Effects were specific to reaction times and not accuracy. This is in keeping with previous reports of behavioural changes post-TMS, which predominantly manifest in a change in response time (Devlin, Matthews, & Rushworth, 2003; Krieger-Redwood, Gaskell, Lindsay, & Jefferies, 2013; Pobric, Jefferies, & Ralph, 2007; Whitney, Kirk, O'Sullivan, Lambon Ralph, & Jefferies, 2011). Surprisingly, MEPs were not modulated by distorted sentences, despite this form of distortion modulating MEPs to pre-lexical stimuli (Nuttall, Kennedy-Higgins, Devlin, & Adank, 2017; Nuttall et al., 2016). MEPs were not affected by cTBS when considered at group level. In Experiment 2, we found an inter-hemispheric influence of PMv on left M1 lip MEPs. This finding was specific to the distorted listening condition only, in line with the behavioural findings from Experiment 1, with no modulation of control MEPs, or MEPs elicited during perception of clear speech, by right PMv. However, we used syllables instead of sentences in Experiment 2, meaning that different patterns of connectivity were potentially evaluated in both experiments. As such, data should be interpreted in light of this methodological difference.

Our observation in Experiment 1 that PMv influences behavioural responses to distorted but not clear speech resonates with previous findings regarding the role of PMv in speech perception. Specifically, PMv may be causally involved in accurate identification and discrimination of speech that is difficult to understand (Meister et al., 2007; Sato et al., 2009). Here, we demonstrate that PMv also plays a role in higher-level speech comprehension. The task employed in Experiment 1 involved the semantic verification of sentences, relative to lower-level phonetic identification or discrimination tasks that have previously been used in some PMv studies (D'Ausilio et al., 2012; Krieger-Redwood et al., 2013; Meister et al., 2007; Sato et al., 2009). This is in line with previous findings that point to a role of motor areas in speech comprehension (Fadiga et al., 2002; Murakami et al., 2015; Schomers, Kirilina, Weigand, Bajbouj, & Pulvermüller, 2015; Watkins et al., 2003).

Importantly, however, previous studies that used TMS to disrupt PMv found that behavioural performance became worse and not better after the stimulation, as our data might suggest. One reason for this difference may be due to the effect of the TMS paradigm used, as the effect of cTBS appears to vary considerably across people (Hannah, Rocchi, Tremblay, & Rothwell, 2016; Hordacre et al., 2016; Volz, Hamada, Rothwell, & Grefkes, 2014). In our subject sample, five participants showed increased reaction times, whereas thirteen showed decreased reaction times, leading to a reduction in reaction time on average. These different effects may reflect the highly variable response profile associated with cTBS. Future studies should seek to replicate involvement of PMv in speech comprehension paradigms using alternative TMS protocols, to further clarify the role of PMv in speech perception. Furthermore, it would also be informative to pre-screen subjects to investigate the nature of their response profile; i.e., whether they show an inhibitory response to cTBS, or a facilitatory response to cTBS. This would allow for response profile to be included in the design and analysis, and the influence of cTBS to be accurately measured. It cannot necessarily be assumed that the same form of stimulation will affect all subjects equally,

Surprisingly, however, we did not observe facilitation of lip MEPs to the complex sentential stimuli used. One reason for this may be that the complexity of the task, and/or stimulus type, did not draw on resources from articulatory M1 in the same way that distorted syllables modulate M1 activity. Indeed, MEP studies observing facilitation to speech stimuli are largely based on passive protocols, or low-level pre-lexical speech stimuli (Fadiga et al., 2002; Möttönen & Watkins, 2009; Roy, Craighero, Fabbri-Destro, & Fadiga, 2008; Watkins et al., 2003). Therefore, it may be the case that M1 is not recruited to the same extent when this type of perceptual ambiguity can be resolved using other mechanisms and resources, or resolution is not solely dependent upon phonetic analysis by the subject. Indeed, presence of extensive semantic top-down information, as in the semantic verification task, may render phonetic analysis unnecessary. However, it should be noted that Dial & Martin's (2017) data suggest that pre-lexical access may also occur in spite of such semantic information being available. Nonetheless, the lack of MEP changes by cTBS of left PMv indicates no modulation of intra-hemispheric PMv-M1 connectivity on lip motor excitability, under the conditions used in this experiment.

We also did not observe any effect of cTBS on group-level lip MEPs. This is line with previous findings regarding cTBS effects on M1 lip, where MEPs were found to reveal no measurable change in motor excitability following 40 seconds of cTBS (Rogers, Mottonen, Boyles, & Watkins, 2014). However, in accordance with our results, Rogers and colleagues also observed an influence of cTBS on behaviour, despite finding no effect of cTBS on MEPs, the lack of which they attribute to inter-individual variability. Indeed, we also found highly variable MEP responses to cTBS, to both types of speech stimuli. This is in line with recent observations of highly variable MEP response profiles following cTBS (Goldsworthy et al., 2016; Hannah et al., 2016; Hordacre et al., 2016; Vallence et al., 2015; Vernet et al., 2014). Whilst progress has been made in understanding the causes of MEP variability (for review see Ridding & Ziemann, 2010), a large component of this variability remains unexplained, and may contribute to non-significant group results after cTBS. Identifying additional factors contributing to response variability is important for improving understanding of the physiology underpinning MEP responses to cTBS.

In Experiment 2, we found that disruption of right PMv interacted with the type of speech stimulus being perceived during lip MEP recording. Specifically, during perception of speech that was difficult to understand, we observed that disrupting right PMv inhibited left M1 lip MEPs. This may indicate that inter-hemispheric connectivity between right PMv and M1 lip is therefore modulated for distorted listening conditions, such that right PMv may be recruited when speech perception is challenging.

Context-dependent modulation of PMv-M1 interactions has been observed intra-cortically in hand action observation and execution literature. During hand action observation, PMv facilitates grip-specific representations in M1, but only while grip formation is observed (de Beukelaar, Alaerts, Swinnen, & Wenderoth, 2016). These findings suggest that PMv and M1 interact temporarily to facilitate grip-specific representations in M1, but no sustained input from PMv to M1 seems to be required to maintain action representations that are anticipated based on contextual information or once the grip is formed. These findings are also in line with the intra-hemispheric relationship between PMv and M1 during hand action execution, where it is known that PMv-M1 interactions are selectively modulated during specific types of grasp (Davare, Lemon, & Olivier, 2008). Considerably less, however, is known about modulation of inter-hemispheric PMv-M1 interactions during action observation.

Taken together, data from Experiments 1 and 2 highlight several findings with regards to the intra- and inter-hemispheric influence of PMv during speech perception. With respect to intra-hemispheric effects, we observed a reduction in reaction time to distorted speech specifically in Experiment 1. There was no corresponding effect on clear speech. TMS timing was jittered substantially for both types of stimuli, which should mitigate against a general inter-sensory explanation for this TMS effect, though we cannot rule it out completely. The influence of cTBS to PMv on lip MEPs appeared to be bidirectional; i.e., for some subjects, cTBS suppressed MEPs, whereas for other subjects, cTBS facilitated MEPs.  Due to this difference in response profile, the net effect of the cTBS influence is obscured when considered at group-level. With regards to the inter-hemispheric influence of right PMv on left M1, we observed a specific pattern of results that indicated an involvement of right PMv in the sensorimotor network only under distorted listening conditions. This differed from the MEP results from Experiment 1, where there was no difference between clear and distorted speech, and suggests the PMv-M1 intra-hemispheric interactions may be relevant for both clear and distorted speech perception, but PMv-M1 inter-hemispheric interactions are influential only during perception of distorted speech. In other words, the entire system for speech perception works harder when listening becomes more difficult. As we did not measure behavioural responses in Experiment 2, we cannot attest to the necessity of right PMv in perception of distorted speech. It must be noted, however, that our interpretation of PMv is based on two protocols that use different speech stimuli. For a full understanding of the role of PMv in speech perception, future work should seek to use different types of speech stimuli within the same protocol, in order to determine how speech stimulus type affects involvement of PMv.

Data presented within this paper feed into speech perception models that are characterised by reverberant, bilateral speech-motor circuits, which adapt dynamically based on context. As emphasised by Skipper and colleagues (Skipper et al., 2017), it is not the case that the neurobiological network for speech perception should be conceived of as fixed, as one might interpret from dual stream models (Hickok, 2012). It is indeed unambiguous that the brain is adaptable and highly plastic. Likewise, we are in agreement with Skipper and colleagues that it is equally possible that multiple permutations of different speech perception and speech production brain networks exist. The specifics of which particular network is brought online will be most likely moderated by context demands. For example, the engagement of a particular speech perception network is highly dependent on listening difficulty. This is evidenced by how clear and distorted speech differentially modulate intra-cortical and inter-cortical PMv-M1 interactions, which may reflect the influence of PMv operating within two different types of neurobiological networks, dependent upon the auditory stimulus.

The functional role of PMv and articulatory M1 regions in speech perception and language comprehension is likely to help disambiguate auditory information that is hard to understand. Under this interpretation, motor regions provide a supportive, but perhaps not essential role, relative to how they have been measured in the present study. The size of the effects reported here may also corroborate the suggestion that motor regions play a supportive, but non-essential role. However, given that the involvement of motor regions is likely to adapt dynamically in response to situational demands, the role of motor regions should not be considered fixed, and may increase in influence depending on the listening context. Neurobiological interactions, of course, do not map neatly onto behavioural relevance, which we believe poses a different question (for extensive treatment see Schomers & Pulvermüller, 2016).

In summary, the present study explored whether PMv exerts an influence intra-cortically, and inter-cortically, during perception of speech that is easy or challenging to understand. Data indicated that left PMv may exert online influence over perception of distorted speech. We also found evidence to suggest that right PMv influences left M1 lip only when listening conditions are challenging. These data support bilateral models of speech perception, where sensorimotor interaction is adaptive depending upon context and stimulus (Schomers & Pulvermüller, 2016; Skipper et al., 2017).

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# Highlights

- Disruption to left PMv modulates perception of distorted speech
- Disruption to right PMv inhibits left M1 lip area during perception of distorted speech
- Intra- and inter-hemispheric PMv-M1 interactions are modulated by listening context

# Modulation of intra- and inter-hemispheric connectivity between primary and premotor cortex during speech perception

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# Abstract

Primary motor (M1) areas for speech production activate during speech perception. It has been suggested that such activation may be dependent upon modulatory inputs from premotor cortex (PMv). If and how PMv differentially modulates M1 activity during perception of speech that is easy or challenging to understand, however, is unclear. This study aimed to test the link between PMv and M1 during challenging speech perception in two experiments. The first experiment investigated intra-hemispheric connectivity between left hemisphere PMv and left M1 lip area during comprehension of speech under clear and distorted listening conditions. Continuous theta burst stimulation (cTBS) was applied to left PMv in eighteen participants (aged 18-35). Post-cTBS, participants performed a sentence verification task on distorted (imprecisely articulated), and clear speech, whilst also undergoing stimulation of the lip representation in the left M1 to elicit motor evoked potentials (MEPs). In a second, separate experiment, we investigated the role of inter-hemispheric connectivity between right hemisphere PMv and left hemisphere M1 lip area. Dual-coil transcranial magnetic stimulation was applied to right PMv and left M1 lip in eighteen participants (aged 18-35). Results indicated that disruption of PMv during speech perception affects comprehension of distorted speech specifically. Furthermore, our data suggest that listening to distorted speech modulates the balance of intra- and inter-hemispheric interactions, with a larger sensorimotor network implicated during comprehension of distorted speech than when speech perception is optimal. The present results further understanding of PMv-M1 interactions during auditory-motor integration.

# 1. Introduction

After decades of research, the neurobiological network subserving speech perception remains unclear. Without this knowledge, we are limited in our ability to understand how humans perceive and use language (Skipper, Devlin, & Lametti, 2017). The role of motor regions in the speech perception network, in particular, is still hotly debated. Involvement of motor areas in speech perception is based on the observation that speech perception activates speech production brain regions (Silbert, Honey, Simony, Poeppel, & Hasson, 2014; Skipper, Nusbaum, & Small, 2005; Wilson, Saygin, Sereno, & Iacoboni, 2004). However, discussion continues as to whether observed motor activity during situations requiring auditory-motor integration really is essential to speech processing (Meister, Wilson, Deblieck, & Wu, 2007), complementary (Möttönen & Watkins, 2009), or epiphenomenal (Hickok, Houde, & Rong, 2011). This has led to the division of 'fractionated' and 'integrated' views of speech perception (Schomers & Pulvermüller, 2016). In the fractionated view, the temporal speech perception network is key to recognising speech, but does not significantly depend on fronto-parietal speech production circuits. The 'integrated' view, however, postulates strong reciprocal links between temporal and fronto-parietal areas, yielding multimodal distributed neuronal circuits capable of reciprocal influence that are causally involved in language understanding, and provide the neuronal basis for speech perception and production.

The evidence for the latter 'integrated' network view has been proposed based largely on Transcranial Magnetic Stimulation (TMS) studies. TMS research has demonstrated that primary motor (M1) areas for speech production activate during speech perception. This has been established using single-pulse TMS to the left hemisphere to generate Motor Evoked Potentials (MEPs) in speech articulators such as the lips or tongue, which serve to index the excitability of the underlying motor pathway. Such studies have identified that activity in the corticobulbar motor pathway from left hemisphere M1 lip and tongue regions to the respective speech muscles is facilitated when perceiving speech relative to non-speech sounds (Fadiga et al., 2002; Murakami, Kell, Restle, Ugawa, & Ziemann, 2015; Murakami, Restle, & Ziemann, 2012; Watkins, Strafella, & Paus, 2003). This speech-specific increase in motor pathway excitability is further modulated by listening difficulty, whereby speech-internal distortions, such as unfamiliar manner of speaking, as well as speech-external distortions, such as background noise, have both been found to affect the excitability of the left hemisphere motor pathway for speech production (Murakami, Restle, & Ziemann, 2011; Nuttall, Kennedy-Higgins, Hogan, Devlin, & Adank, 2016). TMS studies have also been used to disrupt activation in M1 lip area, which was found to impair perception of speech sounds produced by the lips, suggesting a potentially causal role for lip M1 under ambiguous listening conditions (Möttönen & Watkins, 2009). Both types of effects of TMS to articulatory speech regions have been found to be muscle-specific, with no corresponding effects on speech perception or MEPs after TMS to M1 hand area (Möttönen & Watkins, 2009; Nuttall et al., 2016).

Although such findings are striking, articulatory M1 is not known to receive direct inputs from auditory areas, raising the question of how is auditory information able to influence activity in M1, when M1 is not connected to auditory temporal cortex. One candidate possibility is that effects observed at M1 during speech perception are mediated by ventral premotor cortex (PMv), which is thought to receive auditory inputs and is linked to temporal auditory association areas via the superior longitudinal and arcuate fasciculi. Using fMRI multivariate analysis in conjunction with probabilistic fibre tracking based on diffusion tensor imaging data, Saur and colleagues (2010) identified that the posterior Superior Temporal Gyrus (pSTG) exhibits extensive direct interactions with PMv nodes, mediated via the dorsal arcuate fasciculus/superior longitudinal fasciculus system (Frey, Campbell, Pike, & Petrides, 2008; Saur et al., 2008). This temporo–premotor interaction via the dorsal pathway is suggested to be important for a rapid, automated conversion of acoustic representations into motor representations (Vigneau et al., 2006).

It has also been suggested that right PMv mediates mirror facilitation effects observed in left M1 hand area, where it is thought that learned associations between multi-modal inputs at PMv contribute to facilitation of the corticospinal motor pathway to the hand (Catmur, Mars, Rushworth, & Heyes, 2011). In humans, the motor system governing the fingers of the hand has been found to be specifically facilitated by mirror facilitation. Mirror facilitation refers to the idea that an MEP from a finger muscle will be greater in size when a subject observes a movement performed involving that muscle, relative to a movement involving a separate muscle. Indeed, Catmur et al (2011) observed that inter-hemispheric PMv-M1 connections modulate the M1 corticospinal response to observed actions, and suggest that MEP mirror facilitation may be governed by PMv. This finding also raises the question of the role of inter-hemispheric connectivity in action observation. It stands to reason, therefore, that articulatory M1 facilitation measured during perception of speech may be underpinned by a similar PMv mechanism, if Catmur et al.'s observation can be generalised to the corticobulbar motor system. However, the intra- and inter-hemispheric significance of activity in articulatory motor networks during speech perception is not clear, as it has not been explored.

Consistent with the possibility of a (intra- or inter-hemispheric) mediating connection between PMv and M1 during speech perception, a body of neuroimaging evidence indicates that frontal brain areas involved in the planning and execution of speech gestures, i.e., the posterior part of the left inferior frontal gyrus and the PMv are activated during passive speech perception (Pulvermüller et al., 2006; Skipper et al., 2005; Wilson & Iacoboni, 2006; Wilson et al., 2004).

Disruptive TMS to left hemisphere PMv has indeed indicated a mediating role for PMv in understanding speech, particularly during phonemic segmentation (Sato, Tremblay, & Gracco, 2009) and syllable discrimination in background noise (Meister et al., 2007). This has led to the hypothesis that PMv, during language learning, may mediate the comparison of sensory representations of speech against stored articulatory productions held in repertoire, and similarly, these comparisons may further assist listening in difficult environments by helping to disambiguate auditory information (Sato et al., 2009) in line with M1 observations (D'Ausilio, Bufalari, Salmas, & Fadiga, 2012).

However, if, and how, PMv differentially modulates M1 activity during perception of speech that is easy or challenging to understand is unknown. Correlational evidence from PET and MEPs (Watkins & Paus, 2004) suggests that increased excitability of the left articulatory M1 during speech perception is significantly related to an increase in blood flow to left hemisphere frontal brain area BA 44 (Watkins & Paus, 2004). This led the authors to propose that BA 44 (pars opercularis of Broca's area) may directly, or indirectly via PMv, 'prime' the motor system during speech perception, even when no speech output is required.

Taken together, the role of PMv in speech perception remains unclear, particularly the intra- and inter-hemispheric association between PMv and articulatory M1. During effortful listening, accumulating evidence from TMS, fMRI, and PET studies has demonstrated that PMv is active relative to control conditions (Meister et al., 2007; Sato et al., 2009; Watkins & Paus, 2004; Wilson et al., 2004). Relatedly, evidence from hand MEP studies has indicated a mediating role of PMv on M1 during hand action observation, indicating that PMv may govern activity in M1 during hand perception. To further understand the role of PMv in speech perception, two outstanding issues need to be resolved. Firstly, how does disrupting activity in PMv affect speech perception behaviourally, and what effect does this disruption have on articulatory M1? Secondly, what is the significance of inter-hemispheric PMv-M1 connectivity during speech perception?

The present study aimed to build on and extend observations from Watkins and Paus (2004), by examining connectivity between left articulatory M1 and left and right PMv in two experiments. The first experiment investigated intra-hemispheric connectivity between left hemisphere PMv and M1 lip area during comprehension of speech under clear and distorted listening conditions. To this end, continuous theta burst stimulation (cTBS) was applied to PMv to directly manipulate brain activity in that region. Post-cTBS, participants performed a sentence verification task on distorted (imprecisely articulated), and clear speech, whilst also undergoing single-pulse stimulation of left M1 lip area to elicit MEPs. In a second experiment, we investigated the role of inter-hemispheric connectivity between right hemisphere PMv and left hemisphere M1 lip area using an inter-hemispheric inhibition TMS protocol to moderate lip MEPs, whilst subjects listened to clear and distorted speech, and a no-speech control. Taken together, these experiments allowed us to assess how manipulating the influence of ipsilateral and contralateral PMv impacted left hemisphere M1 lip when listening to speech.

## 2. Method

### 2.1. Subjects

In Experiment 1, twenty-two subjects took part (eight males; average age: 22 years 8 months (± SD 3 months); age range: 18–28 years). Four subjects could not tolerate cTBS to PMv and withdrew from participation. Twenty-one subjects took part in Experiment 2 (seven males; average age: 22 years 6 months (± SD 3.8 months); age range: 18–30 years), two of whom had also participated in Experiment 1. Three subjects could not tolerate the dual-pulse protocol to right PMv and left M1 lip, and withdrew from participation. All subjects in Experiments 1 and 2 were right-handed, monolingual, native speakers of British English, with normal language function and hearing thresholds. Handedness was established via self-report. Pure-tone audiometric hearing thresholds were established using a diagnostic audiometer (AD229b, Interacoustic A/S, Denmark) in accordance with The British Society of Audiology Recommended Procedure (The British Society of Audiology, 2011), across 0.25, 0.5, 1, 2, 4, and 8 kHz bilaterally. All subjects had clinically normal thresholds (≤20 dB HL). Subjects presented no TMS contraindications as assessed by the University College London TMS safety screening form. All subjects had a minimum high school-level education, with the majority currently studying at University level. Experiments were undertaken with the understanding and written consent of each subject, according to Research Ethics Board of University College London.

### 2.2. Speech stimuli

For Experiment 1, 160 unique sentences were recorded from the Speed and Capacity of Language Processing (SCOLP) stimuli set (Baddeley, Emslie, and Nimmo-Smith, 1992). The SCOLP sentences are not matched for psycholinguistic variables. However, SCOLP sentences have been found to be a sensitive and reliable measure of the speed of language comprehension, as errors tend to be low across patient and control groups (Adank, Evans, Stuart-Smith, & Scott, 2009; Baddeley, Emslie, & Nimmo-Smith, 1992; Bayre, Geffen, & McFarland, 1997). These comprised two sets of clear sentences, and two sets of distorted sentences based on motor distortion (40 sentences for each set), to ensure unique sentences were tested at baseline and post-cTBS. The average duration of clear sentences was 2008 ms (SD 351 ms), and for distorted sentences was 2585 ms (SD 516 ms). These durations reflect the natural articulation of both types of speech, with the distorted sentences being more difficult and taking longer to articulate. Stimuli were presented in blocks of clear and distorted sentences, with one block of each stimulus type. The order of stimulus block type was counter-balanced across subjects. The SCOLP sentences are designed to be used for semantic verification; are all obviously true or are false, with false sentences being based on a mismatch of subject and predicate from true sentences, i.e., *'Melons are people'*. For each subject, and for each stimulus type and time point (baseline or post-cTBS), a stimulus list containing forty stimuli was randomly permuted, and stimuli were presented according to this order in each condition.

In Experiment 2, twenty vowel-consonant vowel (VCV) syllables containing an equal distribution of lip- (/apa/, /aba/) tongue-articulated (/ata/, /ada/) syllables were recorded. Two sets of the same twenty syllables were created: a clear set based on natural articulation, and a set based on motor distortion. All stimuli were naturally produced to be of approximately the same duration (mean 2864 ms) but were not synthetically manipulated to be precisely the same length. Stimuli varied by a standard deviation of 573 ms. For each subject, and for each condition, a stimulus list containing five occurrences of /apa/, /aba/, /ata/ and /ada/ stimuli was randomly permuted, and stimuli were presented according to this order (20 stimuli in total per condition).

Distorted stimuli were always based on a motor distortion, where the speaker produced the stimuli whilst speaking with a tongue depressor. The tongue depressor was a flat wooden spatula with rounded ends, and was five inches long and one inch wide. A tongue depressor was specifically chosen so as to introduce a motor-based distortion into the speech signal, to relate the speech perception challenge to a speech production difficulty (for further information about these stimuli, and how they are perceived, please see Nuttall et al., 2016). This enabled us to contrast clear speech against distorted speech produced by the same speaker, in contrast to imposing synthetic manipulations upon the spectral characteristics of the original clear speech. Clear speech comprised naturally articulated, normal speech.

Stimuli were produced by a female British English speaker aged 27 years old for Experiment 1, and by a male British English speaker aged 23 for Experiment 2. Stimuli were recorded in a sound-attenuated room and audio digitized at 44.1 kHz with 16 bits. All stimuli were amplitude root-mean-square normalized offline using Praat (Boersma and Weenink, 2016), and then presented using Matlab (The Mathworks, Inc., Natick, MA) through ultra-shielded insert earphones (ER-3A; Etymotic Research, Inc., IL), at a comfortable listening level of around 65 dB SPL.

### 2.3. Design

Two separate experiments were undertaken to assess how PMv-M1 interactions are modulated when listening to clear and distorted speech, as shown in Figure 1. In Experiment 1, continuous theta burst stimulation (cTBS) TMS was applied to modulate cortico-cortical interactions between left PMv and left M1 lip. This allowed us to test the contribution of left PMv to lip MEPs evoked during speech perception, as well as perception of the associated speech. Subjects were instructed to semantically verify the sentences at baseline and post-cTBS as quickly as possible without compromising accuracy. Subjects were asked to respond using the index finger of the left hand, and to press the left arrow key ‘<’ if a sentence was true, and the right arrow key ’>’ if a sentence was false. The left hand was used instead of the dominant right hand in order to avoid any motor preparation and execution effects affecting global motor activity in left hemisphere M1. The order of experimental conditions in Experiment 1 was counter-balanced. The following two experimental conditions were tested:

1) Distorted: Listening to motor-distorted speech produced using a tongue depressor.
2) Clear: Listening to naturally articulated clear speech.

In Experiment 2, an inter-hemispheric TMS protocol was used to modulate inter-hemispheric connectivity between right PMv, and left M1 lip (Chen, 2004; Ni et al., 2009). This allowed us to examine if right hemisphere PMv exerts an influence over left M1 lip during speech perception. Subjects were instructed to listen passively to the speech stimuli. The order of experimental conditions in Experiment 2 was randomised. The following three experimental conditions were tested in the inter-hemispheric double-pulse protocol and were all expressed relative to the single-pulse control condition, which was measured using single-pulse stimulation to left M1 lip, without any auditory stimulation:

1) Distorted: Listening to motor-distorted speech produced using a tongue depressor.
2) Clear: Listening to naturally articulated clear speech.
3) No-speech Control: No auditory stimulation.

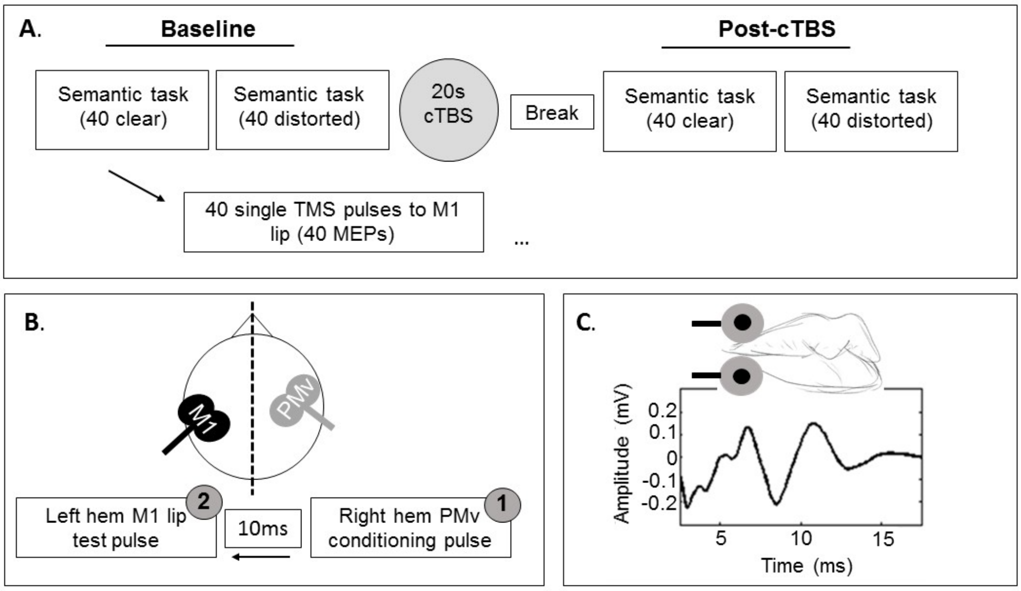

Figure 1. A. Schematic of the cTBS design in Experiment 1. Subjects semantically verified 40 clear sentences and 40 distorted sentences at baseline. At the same time, subjects received single-pulse TMS to M1 lip area. Each sentence was accompanied by one TMS pulse, generating one lip MEP per sentence (example MEP given in C.). Subjects received 20 seconds of cTBS. After a 5 minute break, subjects then performed the semantic verification task again with MEP measurement, as described at baseline. Note that baseline measurements were performed before cTBS, as shown in A., or at the end of the experiment. B. Schematic of inter-hemispheric TMS design in Experiment 2. One conditioning pulse was applied at 120% aMT to right PMv (1). A test pulse was then applied after 10 ms at 0.5mV threshold intensity to left M1 lip area (2), generating an MEP. This procedure was performed during perception of clear speech, distorted speech, and without auditory stimulation (no-speech control).

## 2.4. Transcranial magnetic stimulation

### 2.4.1 MRI acquisition and co-registration

T1-weighted structural magnetic resonance imaging (MRI) scans were acquired using a Siemens Avanto 1.5T MRI scanner and a 32 channel head coil (Siemens Healthcare, GmbH, Germany) at the Birkbeck-UCL Centre for Neuroimaging (BUCNI). A structural image for each participant was obtained using an MP-RAGE sequence [repetition time (TR) = 2730ms; echo time (TE) = 3.57ms;

voxel size = 1x1x1mm; slices = 176]. Once obtained, the structural scans were later used in the main TMS session in conjunction with Brainsight frameless stereotaxy (Rogue Research, Montreal, Canada). For each participant, we performed co-registration between the participant's head and MRI using four anatomical landmarks (tip of the nose, bridge of the nose, and intertragal notch on the left and right ears), which were first identified and marked on the participant's MRI. Accuracy of co-registration was assessed visually using an infrared tracking system (Polaris, Northern Digital, Waterloo, Canada). Upon successful co-registration, infrared tracking was used throughout the experiments in order to maintain coil position during the stimulation.

### 2.4.2. Motor thresholds

In both Experiments 1 and 2, monophasic single TMS pulses were generated by a Magstim $200^2$ unit and delivered to left M1 by a 70mm diameter figure-of-eight coil (Magstim, Dyfed, UK) set to simultaneous discharge mode (inter-pulse spacing of 1 ms). The coil was placed tangential to the skull at a 45 degree angle such that the induced current flowed from posterior to anterior under the junction of the two wings of the figure-of-eight coil. The lip area of M1 was found by using the functional 'hot spot' localization method, whereby application of TMS elicits an MEP from the contralateral muscle. Here, the coil position on the precentral gyrus is adjusted in millimetre movements to ascertain the location on the scalp at which the most robust MEPs are elicited. This location was then marked on a cap and the motor threshold (MT) determined. Before finding the lip area, we first located the hand area by asking subjects to perform a pinching action where the index finger was held against the thumb to activate first dorsal interosseous. Following this, the lip area 'hot spot' was identified by moving the coil ventrally and slightly anterior until an MEP was observed in the contralateral lip muscle. In Experiment 1, the active MT was identified, which constitutes the intensity at which TMS pulses elicited 5 out of 10 MEPs with an amplitude of at least 0.2 mV during 20% of maximum voluntary muscle contraction (Möttönen, Rogers, & Watkins, 2014). The intensity of the stimulator was then set to 120% of aMT for the single-pulse stimulations applied during the experiment. The mean stimulator intensity (120% aMT ± SD) used to elicit lip MEPs in Experiment 1 was 51.4% (±4).

In Experiment, 2 a dual-pulse inter-hemispheric inhibition protocol was utilised, which comprised a conditioning pulse to right PMv followed by a test pulse to left M1 lip (Chen, 2004; Ni et al., 2009). First, aMT was established as detailed above in right M1 lip area, though a 50mm diameter figure-of-eight coil (Magstim, Dyfed, UK) was used in thresholding, which was the same coil used for delivering TMS to the right hemisphere in the dual-pulse protocol (please see section

2.4.3.2 for further details). However, we were unable to record robust lip MEPs from right M1 lip in three subjects, who did not continue on with the study. The intensity of the TMS pulse to right PMv (conditioning stimulus) was subsequently set to 120% of right M1 lip aMT (mean 61.5% ±5.7). For left M1 lip, we found the hot spot using the method detailed above using a 70mm diameter figure-of-eight coil (Magstim, Dyfed, UK), and then set the intensity such that the TMS pulse elicited a lip MEP of on average 0.5 mV in 5 out of 10 MEPs (test stimulus), without any conditioning pulse stimulation, in line with inter-hemispheric inhibition protocols based on M1 hand (Di Lazzaro et al., 1999; Mochizuki, Huang, & Rothwell, 2004). The mean stimulation intensity for the test pulse was 67.6% (±5.6) of maximum stimulator output. All test pulses were applied using the 70mm coil that had been used for left M1 lip thresholding.

### 2.4.3. TMS protocols

#### 2.4.3.1. Experiment 1

After establishing TMS test intensity, half of the subjects then received two blocks of single-pulse TMS to the lip area of M1 in the left hemisphere to measure baseline MEPs during perception of blocks of clear and distorted speech. This was followed immediately by 20s cTBS to PMv, and then two more test blocks of single-pulse TMS to measure MEPs during perception of clear and distorted blocks of speech, post-cTBS. The other half of the subjects received cTBS first, and then two test blocks of single-pulse TMS during perception of clear and distorted speech to record post-cTBS MEPs. This was followed by a break, and then repeated in order to record baseline MEPs during perception of clear and distorted speech. The baseline MEP measurements were always performed at least 30 minutes after administering cTBS to ensure that baseline performance had returned, as 20s of cTBS is thought to affect the brain for around 20 minutes (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). The order of baseline testing was counter-balanced to control for order effects. This design mitigates against post-TMS results being confounded by practice effects. Baseline order showed no significant interactions with any of the experimental variables (all $p > 0.1$), which suggests that it is highly unlikely that cTBS contaminated the baseline data for subjects who performed baseline measurements after cTBS.

During the single-pulse TMS test blocks, subjects were presented with the speech stimuli (see Methods section 2) and were asked to semantically verify the sentences. During the presentation of each speech stimulus, Matlab was used to externally trigger the TMS system at a jittered time point towards the middle or end of the sentence to avoid intersensory facilitation effects, i.e., where reaction times to the auditory stimuli is primed due to the sensation of a TMS pulse occurring at a predictable time. All speech stimuli were accompanied by a TMS pulse; therefore, all trials were presented with TMS. The timing of the single-pulse TMS delivery was not manipulated to coincide specifically with a particular phoneme; therefore, MEPs did not represent specific time-locked phoneme-based MEPs. TMS test blocks lasted for approximately 3-4 minutes, allowing for the application of 40 TMS pulses per block. Single-pulse TMS was always performed using a Magstim $200^2$ unit and delivered by a 70mm diameter figure-of-eight coil (Magstim, Dyfed, UK).

For cTBS to PMv, a $Rapid^2$ stimulator and 70mm diameter figure-of-eight coil (Magstim, Dyfed, UK) was always used. The intensity of cTBS was fixed for all subjects at 40%, as it is not feasible to record robust lip MEPs using a biphasic pulse, as delivered by the $Rapid^2$, and nor is it possible to extrapolate motor thresholds obtained using a monophasic stimulator (Magstim $200^2$ unit), to a biphasic stimulator. The stimulation site for cTBS to left PMv was based on the average MNI space co-ordinate from Meister et al., 2007: -53, -4 and -49, which fell within the superior portion of the PMv. This was marked in each subject's anatomical scan using Brainsight software (Rogue Research Ltd, Montreal, Canada). Across subjects, the co-ordinate fell within premotor cortex, but not always within superior PMv. For the stimulation, we used 20s of cTBS in one offline train of 300 pulses. cTBS is a patterned form of repetitive TMS. The standard theta burst pattern consists of three pulses given in a 50 Hz burst and repeated every 200 ms (5 Hz). We allowed for a 5 minute interval immediately after stimulation to allow for stimulation effects to stabilise, in line with published literature (Huang et al., 2005), after which the single-pulse protocol was administered.

#### 2.4.3.2 Experiment 2

In Experiment 2 a dual-pulse inter-hemispheric inhibition protocol was utilised involving a conditioning pulse to right PMv and a test pulse to left M1 lip.  Test pulses in the dual-pulse TMS conditions, and the single-pulse control TMS condition were always performed using a Magstim $200^2$ unit and delivered by a 70mm diameter figure-of-eight coil for left hemisphere stimulation. A 50mm dimeter figure-of-eight coil was always used for the conditioning pulses to the right hemisphere in the dual-pulse conditions (Magstim, Dyfed, UK). It was necessary to use the 50mm coil on the right side of the head as two 70mm coils will not fit when both are positioned at a $45^o$ angle tangential to the skull, which we did not wish to compromise as different coil orientations target different populations of neurons (D'Ostilio et al., 2016; Hannah & Rothwell, 2017). The stimulation site for the conditioning pulse to right PMv was based on the MNI space co-ordinate from Catmur et al., (2011):

57, 12, and 23. This was marked in each subject's anatomical scan using Brainsight software (Rogue Research Ltd, Montreal, Canada). We used a 10ms inter-pulse-interval after the conditioning pulse before administering the subsequent test pulse to left M1 lip area, consistent with inter-hemispheric inhibition protocols (Di Lazzaro et al., 1999; Mochizuki et al., 2004). This dual-pulse protocol was always administered by two experimenters who held one coil each, as it is not feasible for one experimenter to hold both coils at the same time.

### 2.4.4 Electromyography

In both Experiments 1 and 2, electromyographic (EMG) activity was recorded from the lip muscle, orbicularis oris, using surface electrodes (Ag/AgCl; 10-mm diameter) in a non-Faraday caged, double-walled sound-attenuating booth. Electrodes were attached to the orbicularis oris on the right side of the mouth in a bipolar belly-belly montage, with an electrode placed at the right temple serving as a common ground. To stabilize background EMG activity, subjects were trained for approximately five minutes to produce a constant level of contraction (approximately 20% of maximum voluntary contraction) of the lip muscles by pursing, which was verified via visual feedback of the ongoing EMG signal (Möttönen et al., 2014; Watkins et al., 2003). Contraction of the lip muscle also facilitates a lower motor threshold relative to when the muscle is at rest, enabling the use of lower levels of stimulation during the experiment. The raw EMG signal was amplified by a factor of 1000, band-pass filtered between 100–2000 Hz, and sampled at 5000 Hz online using a 1902 amplifier (Cambridge Electronic Design, Cambridge), and analog-to-digital converted using a Micro1401-3 unit (Cambridge Electronic Design, Cambridge). Continuous data were acquired and recorded using Spike2 software (version 8, Cambridge Electronic Design, Cambridge).

## 2.5. Data analysis

In Experiment 1, the proportion of correct responses and associated relative reaction times for the distorted and clear speech pre- and post-cTBS were calculated for each individual. Reaction times were expressed relative to the end of each stimulus, which shows how long after the end of the stimulus a response was made. This is in contrast to expressing the response from the onset of the stimulus, which does not take into account differences in stimulus length. The end of each stimulus was visually identified from the waveform and spectrogram by a trained phonetician using Praat software, who located the final voicing cycle of a vowel, release of a consonant, or cessation of frication, for example, in the spectrogram. In Experiments 1 and 2, for the MEP data, individual EMG sweeps starting 40 ms before the TMS pulse and ending 1000 ms post-stimulation were exported offline from the recording software into Matlab. Individual MEPs were identified in each trial and rectified. The integrated area under the curve (AUC) of the rectified EMG signal of each individual mean MEP was then calculated. In Experiment 2, dual-coil MEP ratios were calculated for by dividing each dual-coil condition MEP (clear, distorted, and no-speech control) by the MEPs in the single-pulse no-speech control condition, to express the influence of the dual-coil protocol on MEPs relative to the single-pulse baseline measure. MEP AUCs were then converted into standard scores within subjects, to control for inter-subject variability. The standardized AUCs of MEPs were used in the statistical analyses. The average height of the pre-TMS baseline EMG activity was also computed, and paired t-tests confirmed that there were no significant differences between baseline activity levels between any conditions in Experiment 1 nor 2, indicating that baseline activity did not influence MEPs across conditions.

Statistical analyses were carried out using SPSS (version 22.0, IBM). In Experiment 1, two-way repeated measures analysis of variance (RMANOVA) were conducted on reaction time, accuracy, and standardised MEP AUC as the dependent variables, with stimulus type (clear, distorted), and time (baseline, post-cTBS), as within-subjects factors. In Experiment 2, a one-way RMANOVA was conducted on standardised MEP AUC ratio, with stimulus type (clear, distorted, no-speech control) as the within-subject factor. Planned comparisons were subsequently computed where appropriate (alpha level = .05).

## 3. Results

### 3.1. Experiment 1

#### 3.1.1. Accuracy

Experiment 1 tested how cTBS to left PMv affected behaviour and MEP responses from left M1 lip during perception of clear and distorted speech. The mean difference in accuracy as a function of time and stimulus type is shown in Figure 2. On average, there was a difference in the accuracy of responses to clear and distorted speech at baseline and after cTBS. A two-way repeated measures ANOVA demonstrated a significant main effect of stimulus type on accuracy (F(1,17) = 117.68, p < 0.001, partial eta = .87), confirming that subjects were less accurate in identifying distorted stimuli (73.3%, SE 2.8%)  relative to clear (95.1%, SE 1.15%). The main effect of time was not significant (F(1,17) = 2.9, p = .10), nor was the time x stimulus type interaction (F(1,17) = 2.1, p =

.16), indicating that cTBS did not modulate the accuracy of subjects' responses to clear and distorted stimuli.

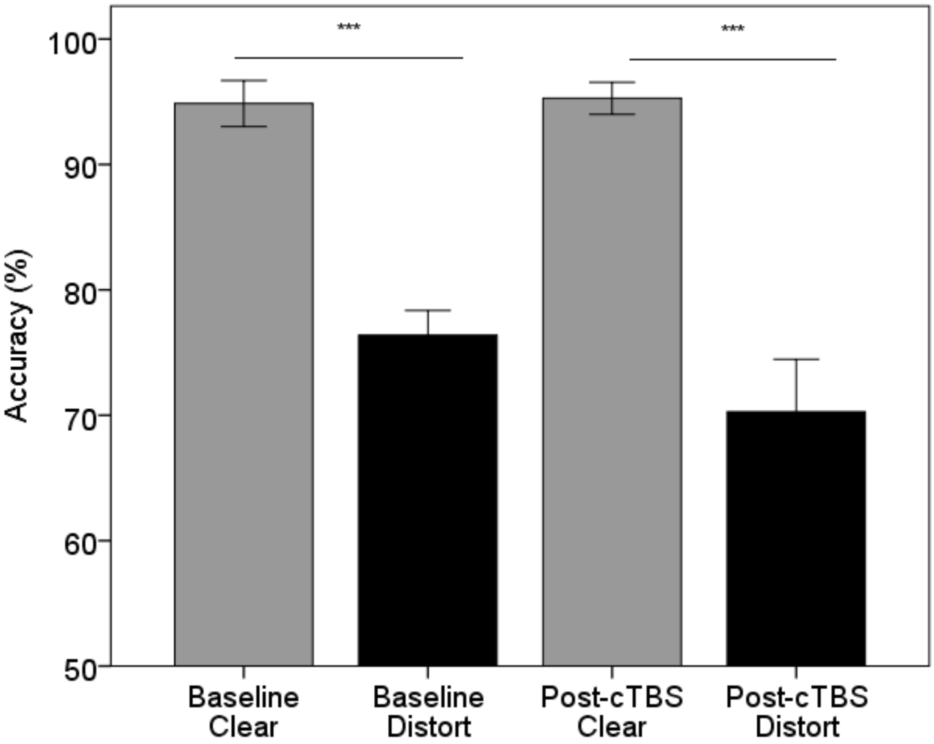

Figure 2. Average percent correct performance for clear (grey) and distorted (black) stimuli at baseline and after cTBS to left PMv. Error bars represent +/- 1 SE.

### 3.1.2. Reaction time

The mean difference in reaction time as a function of time and stimulus type is shown in Figure 3. On average, there was little difference between reaction times to clear and distorted speech at baseline. The difference between reaction times to clear and distorted speech increased after cTBS. A two-way repeated measures ANOVA found that the interaction between stimulus type and time was significant (F(1,17) = 5.35; p = 0.033), suggesting that cTBS affected reaction times differently depending on the type of speech stimulus perceived. Paired t-tests (two-tailed) confirmed a significant difference between reaction times post-cTBS to clear and distorted speech (t(17) = 2.13, p = 0.048 [clear = 433.37 ms, SE 37.45 ms; distorted = 350.43 ms, SE 23.16 ms]), which was not present at baseline (t(17) = -0.22, p=0.83 [clear = 405.82 ms, SE 48.57 ms; distort = 413.94, 37.52]). No main effects were significant: time = F(1,17) = 0.37, p = 0.55; stimulus type = F(1,17) =

1.29, p = 0.27. There was a trend towards a significant reduction in reaction times post-cTBS relative to baseline for distorted speech (p = 0.08 [baseline: 413.94 ms, 37.52 SE ms, post-cTBS: 350.43 ms, SE 23.16 ms]), which was not evident in reaction times to clear speech post-cTBS (p = 0.4). These data indicate, therefore, that the significant interaction is driven by cTBS to PMv reducing response time to distorted speech specifically, without changing response time to clear stimuli.

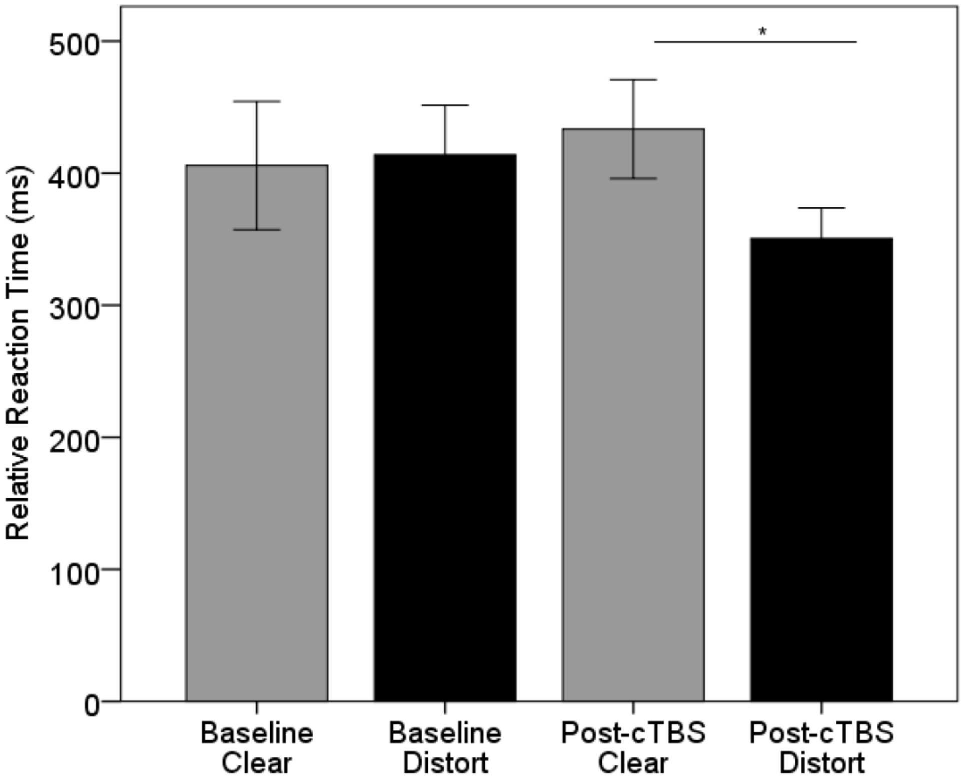

Figure 3. Average relative reaction time to clear (grey) and distorted (black) stimuli at baseline and after cTBS to left PMv. Error bars represent +/-1 SE.

### 3.1.3. Effect of cTBS on Lip MEPs

The mean difference between MEPs elicited during perception of clear and distorted speech at baseline and post-cTBS is shown in Figure 4. Two-way RMANOVA indicated that MEPs were not modulated by stimulus type (F(1,17) = 0.27, p = 0.61) or by time (F(1,17) = 0.30, p = 0.44). The interaction was also not significant (F(1,17) = .94, p = .17). These data confirmed that cTBS to PMv did not influence MEPs during perception of clear or distorted stimuli.

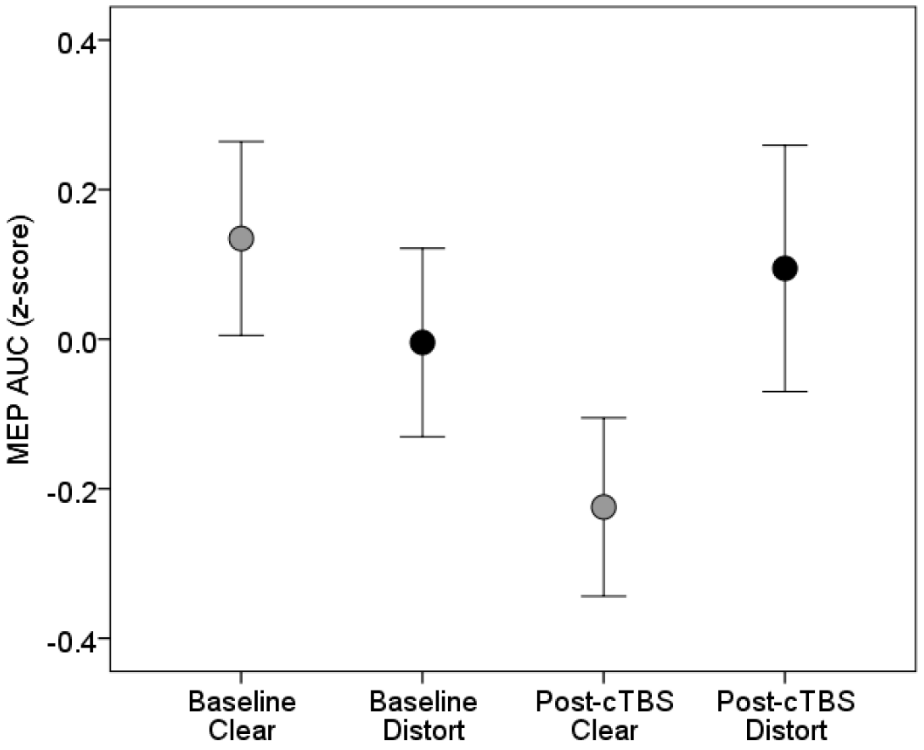

Figure 4. Average MEP area during perception of clear (grey) and distorted (black) speech at baseline and after cTBS to left PMv. Error bars represent +/-1 SE.

Notably, there was considerable variability in the effect of cTBS on motor excitability. Individual subject's responses to cTBS for each condition are shown for in Figure 5. In some subjects, cTBS caused MEP facilitation, whereas in other subjects, cTBS caused MEP inhibition. The direction of the effect was also not consistent across speech conditions.

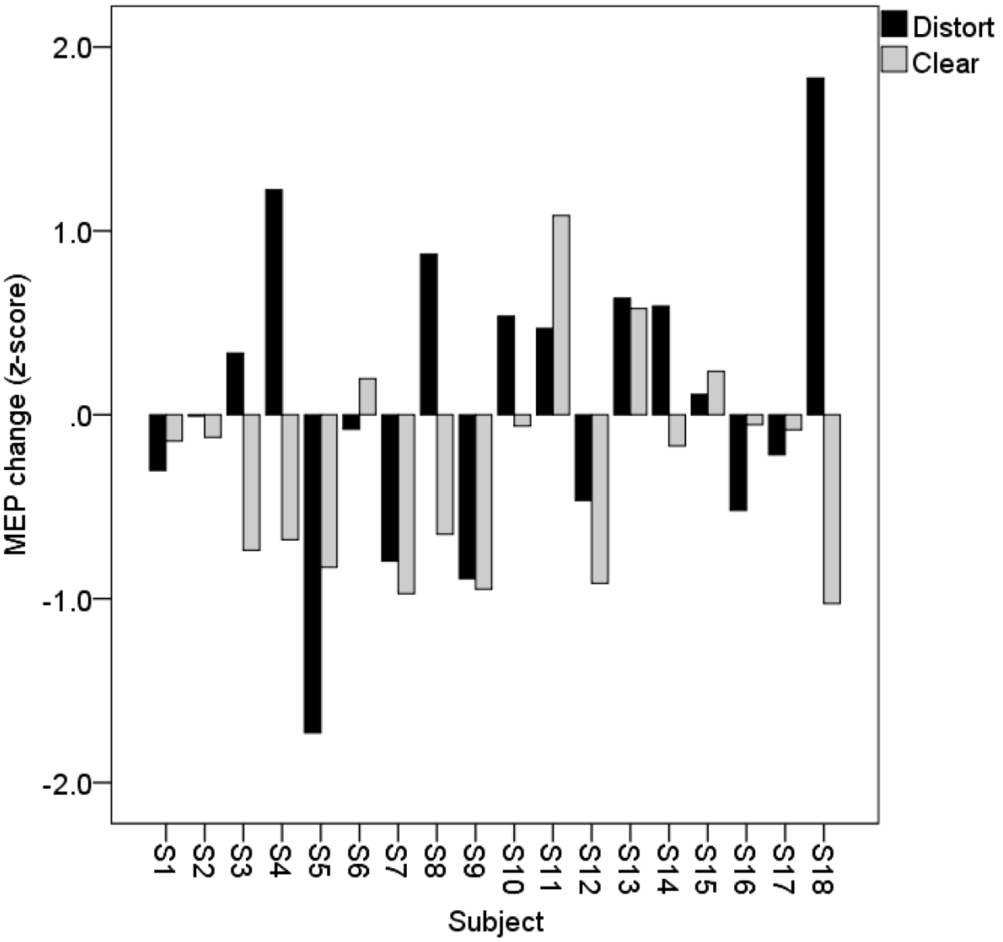

Figure 5. Bars express individual participant’s change scores (Δ) in MEP area from baseline, to post-cTBS, in both Distorted (black) and Clear (grey) conditions.

### 3.2. Experiment 2

#### 3.2.1. Motor Evoked Potentials

Experiment 2 tested how a dual-pulse inter-hemispheric inhibition protocol to right PMv affected MEP responses from left M1 lip during perception of clear and distorted speech, as well as during the no-speech control condition. Mean MEP data are shown in Figure 6, where it can be observed that distorted MEP data are further away from the mean than either clear or control MEP data. A one-way RMANOVA indicated that there was a significant effect of stimulus type on the extent of the inter-hemispheric MEP inhibition resulting from disruption of right PMv (F(1,14) = 3.5, p = 0.044, partial eta squared 0.2). Planned comparisons confirmed a significant difference between MEPs during perception of clear and distorted speech elicited after dual-pulse TMS (p = 0.035). The difference between control and distorted MEPs showed a trend towards a significant difference (p = 0.08). There was no significant difference between control MEPs and MEPs elicited during perception of clear speech (p = 0.3). These data indicate that when listening to distorted speech, inhibiting right PMv inhibits MEPs from left M1 lip area. These findings suggest that right PMv may play a role during perception of distorted speech specifically

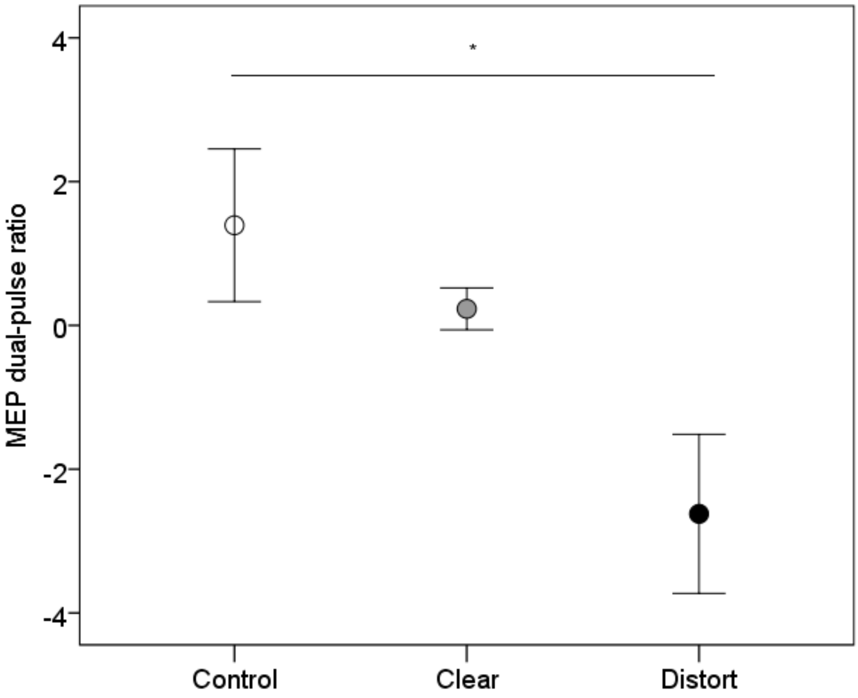

Figure 6. MEP ratios following dual-pulse inter-hemispheric TMS to right PMv and left M1 lip area, during perception of clear (grey) and distorted (black) speech, and no-speech control (no auditory stimulation, open circle) conditions. MEP ratios were calculated by dividing each dual-coil condition MEP (clear, distorted, and no-speech control) by the MEPs in the single-pulse no-speech control condition, to express the influence of the dual-coil protocol on MEPs relative to the single-pulse baseline measure.

## 4. Discussion

The present study aimed to investigate the intra- and inter-hemispheric influence of PMv on left M1 during speech perception. Experiment 1 showed that disruption to PMv causally affected reaction time to speech under distorted listening conditions specifically, but did not affect responses to clear speech. Effects were specific to reaction times and not accuracy. This is in keeping with previous reports of behavioural changes post-TMS, which predominantly manifest in a change in response time (Devlin, Matthews, & Rushworth, 2003; Krieger-Redwood, Gaskell, Lindsay, & Jefferies, 2013; Pobric, Jefferies, & Ralph, 2007; Whitney, Kirk, O'Sullivan, Lambon Ralph, & Jefferies, 2011). Surprisingly, MEPs were not modulated by distorted sentences, despite this form of distortion modulating MEPs to pre-lexical stimuli (Nuttall, Kennedy-Higgins, Devlin, & Adank, 2017; Nuttall et al., 2016). MEPs were not affected by cTBS when considered at group level. In Experiment 2, we found an inter-hemispheric influence of PMv on left M1 lip MEPs. This finding was specific to the distorted listening condition only, in line with the behavioural findings from Experiment 1, with no modulation of control MEPs, or MEPs elicited during perception of clear speech, by right PMv. However, we used syllables instead of sentences in Experiment 2, meaning that different patterns of connectivity were potentially evaluated in both experiments. As such, data should be interpreted in light of this methodological difference.

Our observation in Experiment 1 that PMv influences behavioural responses to distorted but not clear speech resonates with previous findings regarding the role of PMv in speech perception. Specifically, PMv may be causally involved in accurate identification and discrimination of speech that is difficult to understand (Meister et al., 2007; Sato et al., 2009). Here, we demonstrate that PMv also plays a role in higher-level speech comprehension. The task employed in Experiment 1 involved the semantic verification of sentences, relative to lower-level phonetic identification or discrimination tasks that have previously been used in some PMv studies (D'Ausilio et al., 2012; Krieger-Redwood et al., 2013; Meister et al., 2007; Sato et al., 2009). This is in line with previous findings that point to a role of motor areas in speech comprehension (Fadiga et al., 2002; Murakami et al., 2015; Schomers, Kirilina, Weigand, Bajbouj, & Pulvermüller, 2015; Watkins et al., 2003).

Importantly, however, previous studies that used TMS to disrupt PMv found that behavioural performance became worse and not better after the stimulation, as our data might suggest. One reason for this difference may be due to the effect of the TMS paradigm used, as the effect of cTBS appears to vary considerably across people (Hannah, Rocchi, Tremblay, & Rothwell, 2016; Hordacre et al., 2016; Volz, Hamada, Rothwell, & Grefkes, 2014). In our subject sample, five participants showed increased reaction times, whereas thirteen showed decreased reaction times, leading to a reduction in reaction time on average. These different effects may reflect the highly variable response profile associated with cTBS. Future studies should seek to replicate involvement of PMv in speech comprehension paradigms using alternative TMS protocols, to further clarify the role of PMv in speech perception. Furthermore, it would also be informative to pre-screen subjects to investigate the nature of their response profile; i.e., whether they show an inhibitory response to cTBS, or a facilitatory response to cTBS. This would allow for response profile to be included in the design and analysis, and the influence of cTBS to be accurately measured. It cannot necessarily be assumed that the same form of stimulation will affect all subjects equally,

Surprisingly, however, we did not observe facilitation of lip MEPs to the complex sentential stimuli used. One reason for this may be that the complexity of the task, and/or stimulus type, did not draw on resources from articulatory M1 in the same way that distorted syllables modulate M1 activity. Indeed, MEP studies observing facilitation to speech stimuli are largely based on passive protocols, or low-level pre-lexical speech stimuli (Fadiga et al., 2002; Möttönen & Watkins, 2009; Roy, Craighero, Fabbri-Destro, & Fadiga, 2008; Watkins et al., 2003). Therefore, it may be the case that M1 is not recruited to the same extent when this type of perceptual ambiguity can be resolved using other mechanisms and resources, or resolution is not solely dependent upon phonetic analysis by the subject. Indeed, presence of extensive semantic top-down information, as in the semantic verification task, may render phonetic analysis unnecessary. However, it should be noted that Dial & Martin’s (2017) data suggest that pre-lexical access may also occur in spite of such semantic information being available. Nonetheless, the lack of MEP changes by cTBS of left PMv indicates no modulation of intra-hemispheric PMv-M1 connectivity on lip motor excitability, under the conditions used in this experiment.

We also did not observe any effect of cTBS on group-level lip MEPs. This is line with previous findings regarding cTBS effects on M1 lip, where MEPs were found to reveal no measurable change in motor excitability following 40 seconds of cTBS (Rogers, Mottonen, Boyles, & Watkins, 2014). However, in accordance with our results, Rogers and colleagues also observed an influence of cTBS on behaviour, despite finding no effect of cTBS on MEPs, the lack of which they attribute to inter-individual variability. Indeed, we also found highly variable MEP responses to cTBS, to both types of speech stimuli. This is in line with recent observations of highly variable MEP response profiles following cTBS (Goldsworthy et al., 2016; Hannah et al., 2016; Hordacre et al., 2016; Vallence et al., 2015; Vernet et al., 2014). Whilst progress has been made in understanding the causes of MEP variability (for review see Ridding & Ziemann, 2010), a large component of this variability remains unexplained, and may contribute to non-significant group results after cTBS. Identifying additional factors contributing to response variability is important for improving understanding of the physiology underpinning MEP responses to cTBS.

In Experiment 2, we found that disruption of right PMv interacted with the type of speech stimulus being perceived during lip MEP recording. Specifically, during perception of speech that was difficult to understand, we observed that disrupting right PMv inhibited left M1 lip MEPs. This may indicate that inter-hemispheric connectivity between right PMv and M1 lip is therefore modulated for distorted listening conditions, such that right PMv may be recruited when speech perception is challenging.

Context-dependent modulation of PMv-M1 interactions has been observed intra-cortically in hand action observation and execution literature. During hand action observation, PMv facilitates grip-specific representations in M1, but only while grip formation is observed (de Beukelaar, Alaerts, Swinnen, & Wenderoth, 2016). These findings suggest that PMv and M1 interact temporarily to facilitate grip-specific representations in M1, but no sustained input from PMv to M1 seems to be required to maintain action representations that are anticipated based on contextual information or once the grip is formed. These findings are also in line with the intra-hemispheric relationship between PMv and M1 during hand action execution, where it is known that PMv-M1 interactions are selectively modulated during specific types of grasp (Davare, Lemon, & Olivier, 2008). Considerably less, however, is known about modulation of inter-hemispheric PMv-M1 interactions during action observation.

Taken together, data from Experiments 1 and 2 highlight several findings with regards to the intra- and inter-hemispheric influence of PMv during speech perception. With respect to intra-hemispheric effects, we observed a reduction in reaction time to distorted speech specifically in Experiment 1. There was no corresponding effect on clear speech. TMS timing was jittered substantially for both types of stimuli, which should mitigate against a general inter-sensory explanation for this TMS effect, though we cannot rule it out completely. The influence of cTBS to PMv on lip MEPs appeared to be bidirectional; i.e., for some subjects, cTBS suppressed MEPs, whereas for other subjects, cTBS facilitated MEPs.  Due to this difference in response profile, the net effect of the cTBS influence is obscured when considered at group-level. With regards to the inter-hemispheric influence of right PMv on left M1, we observed a specific pattern of results that indicated an involvement of right PMv in the sensorimotor network only under distorted listening conditions. This differed from the MEP results from Experiment 1, where there was no difference between clear and distorted speech, and suggests the PMv-M1 intra-hemispheric interactions may be relevant for both clear and distorted speech perception, but PMv-M1 inter-hemispheric interactions are influential only during perception of distorted speech. In other words, the entire system for speech perception works harder when listening becomes more difficult. As we did not measure behavioural responses in Experiment 2, we cannot attest to the necessity of right PMv in perception of distorted speech. It must be noted, however, that our interpretation of PMv is based on two protocols that use different speech stimuli. For a full understanding of the role of PMv in speech perception, future work should seek to use different types of speech stimuli within the same protocol, in order to determine how speech stimulus type affects involvement of PMv.

Data presented within this paper feed into speech perception models that are characterised by reverberant, bilateral speech-motor circuits, which adapt dynamically based on context. As emphasised by Skipper and colleagues (Skipper et al., 2017), it is not the case that the neurobiological network for speech perception should be conceived of as fixed, as one might interpret from dual stream models (Hickok, 2012). It is indeed unambiguous that the brain is adaptable and highly plastic. Likewise, we are in agreement with Skipper and colleagues that it is equally possible that multiple permutations of different speech perception and speech production brain networks exist. The specifics of which particular network is brought online will be most likely moderated by context demands. For example, the engagement of a particular speech perception network is highly dependent on listening difficulty. This is evidenced by how clear and distorted speech differentially modulate intra-cortical and inter-cortical PMv-M1 interactions, which may reflect the influence of PMv operating within two different types of neurobiological networks, dependent upon the auditory stimulus.

The functional role of PMv and articulatory M1 regions in speech perception and language comprehension is likely to help disambiguate auditory information that is hard to understand. Under this interpretation, motor regions provide a supportive, but perhaps not essential role, relative to how they have been measured in the present study. The size of the effects reported here may also corroborate the suggestion that motor regions play a supportive, but non-essential role. However, given that the involvement of motor regions is likely to adapt dynamically in response to situational demands, the role of motor regions should not be considered fixed, and may increase in influence depending on the listening context. Neurobiological interactions, of course, do not map neatly onto behavioural relevance, which we believe poses a different question (for extensive treatment see Schomers & Pulvermüller, 2016).

In summary, the present study explored whether PMv exerts an influence intra-cortically, and inter-cortically, during perception of speech that is easy or challenging to understand. Data indicated that left PMv may exert online influence over perception of distorted speech. We also found evidence to suggest that right PMv influences left M1 lip only when listening conditions are challenging. These data support bilateral models of speech perception, where sensorimotor interaction is adaptive depending upon context and stimulus (Schomers & Pulvermüller, 2016; Skipper et al., 2017).

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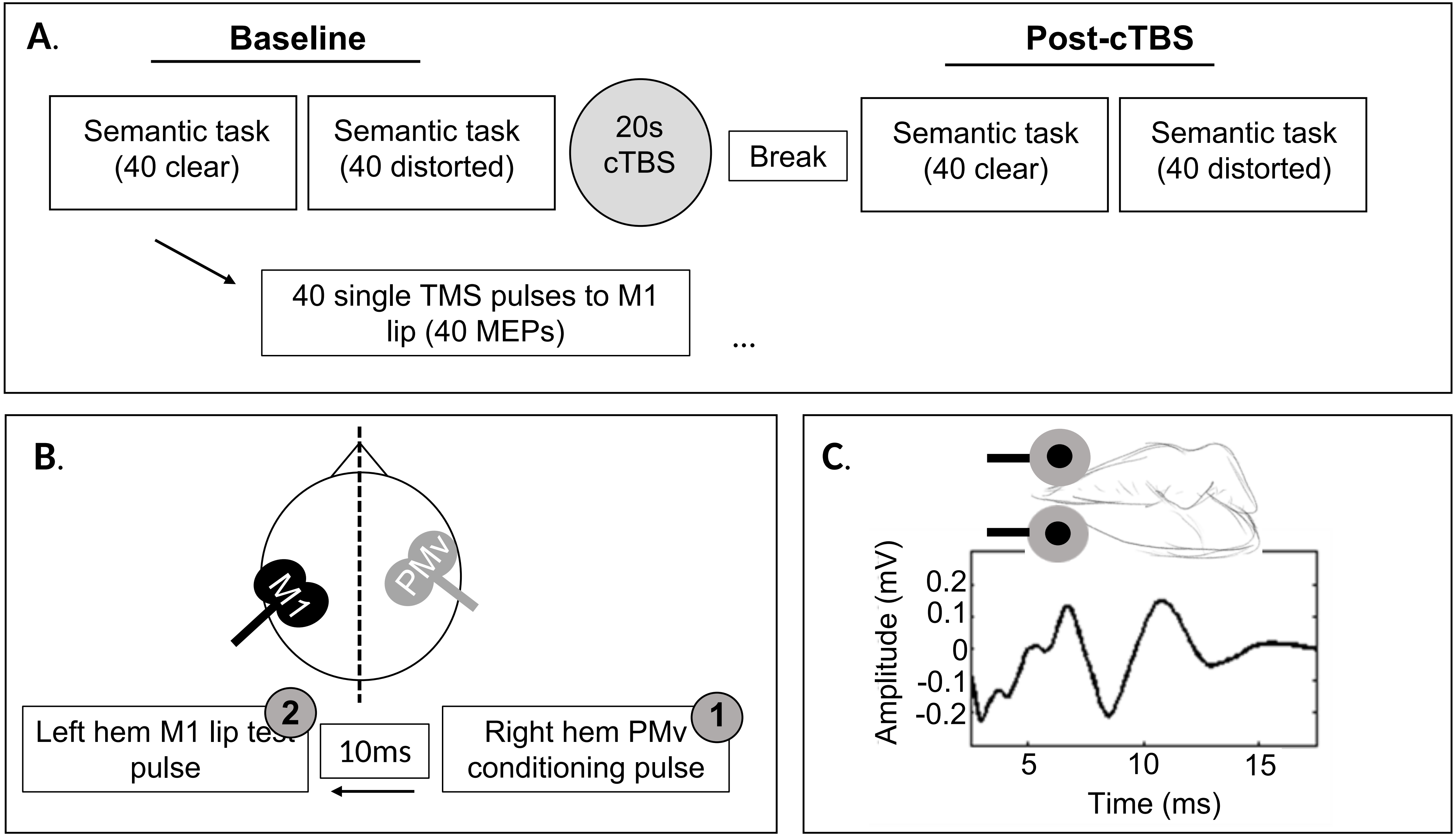
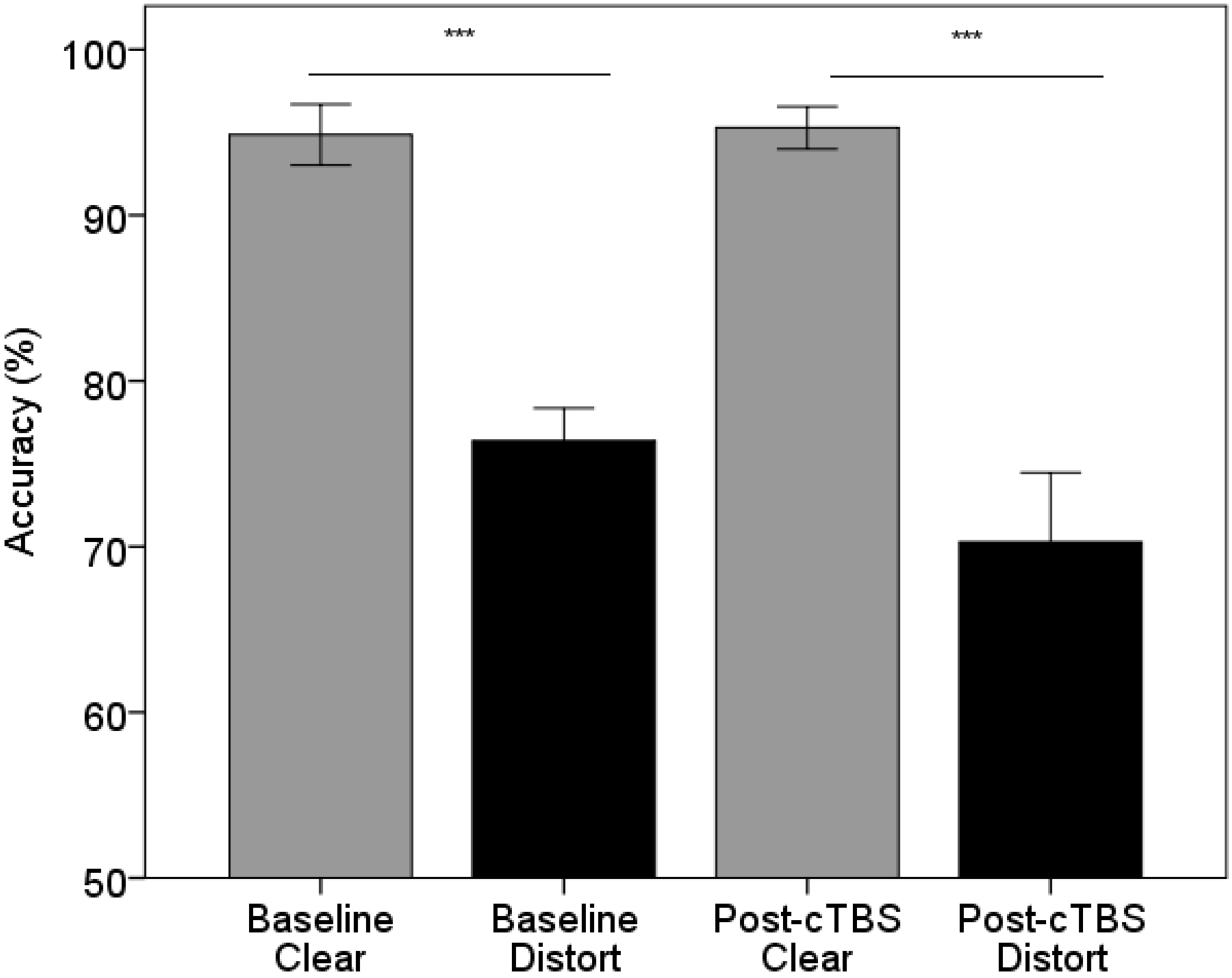

A.
Baseline
Post-cTBS
Semantic task (40 clear)
Semantic task (40 distorted)
20s cTBS
Break
Semantic task (40 clear)
Semantic task (40 distorted)
40 single TMS pulses to M1 lip (40 MEPs)
...
B.
M1
PMv
Left hem M1 lip test pulse
2
10ms
Right hem PMv conditioning pulse
1
C.
Amplitude (mV)
0.2
0.1
0
-0.1
-0.2
5
10
15
Time (ms)


***
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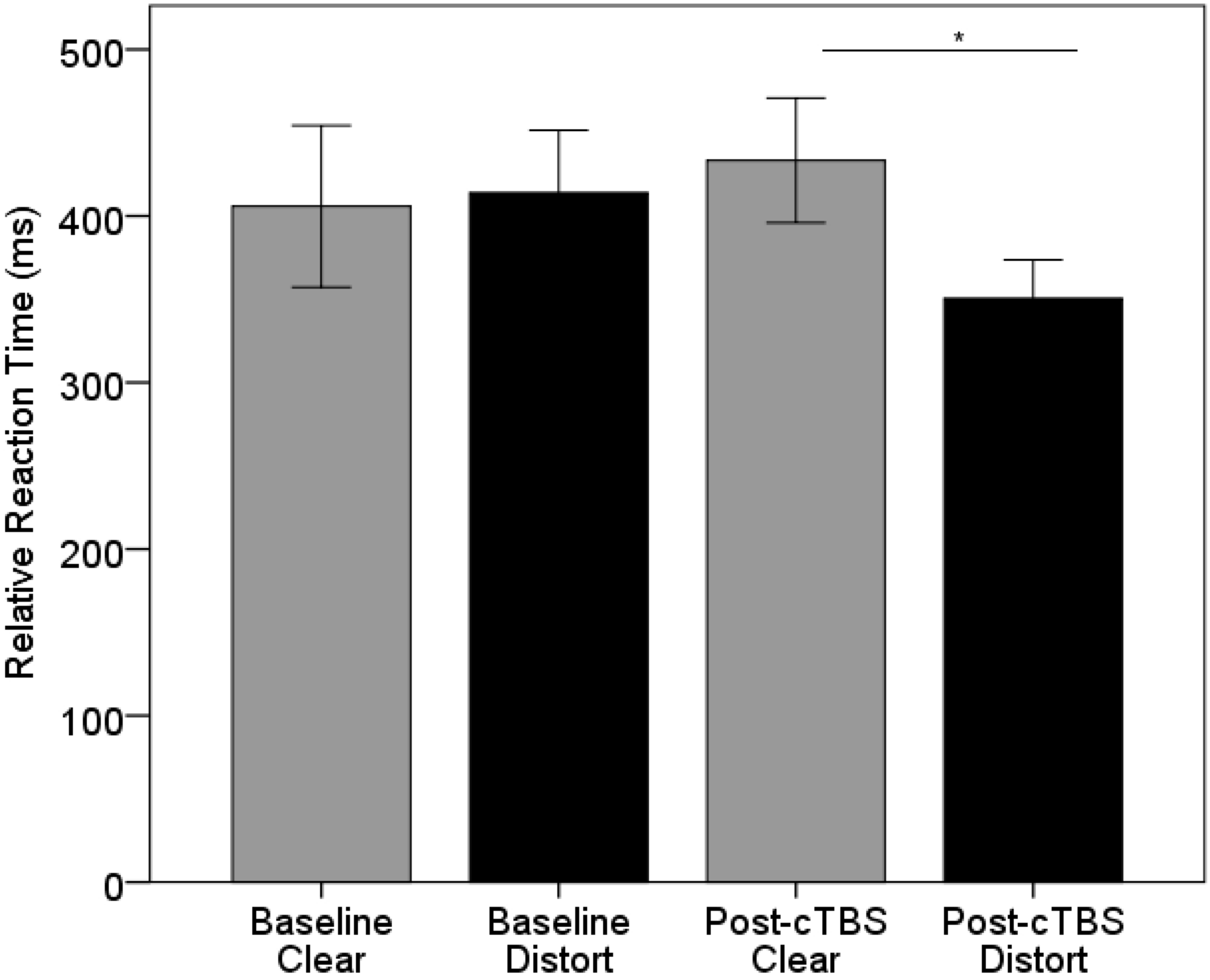
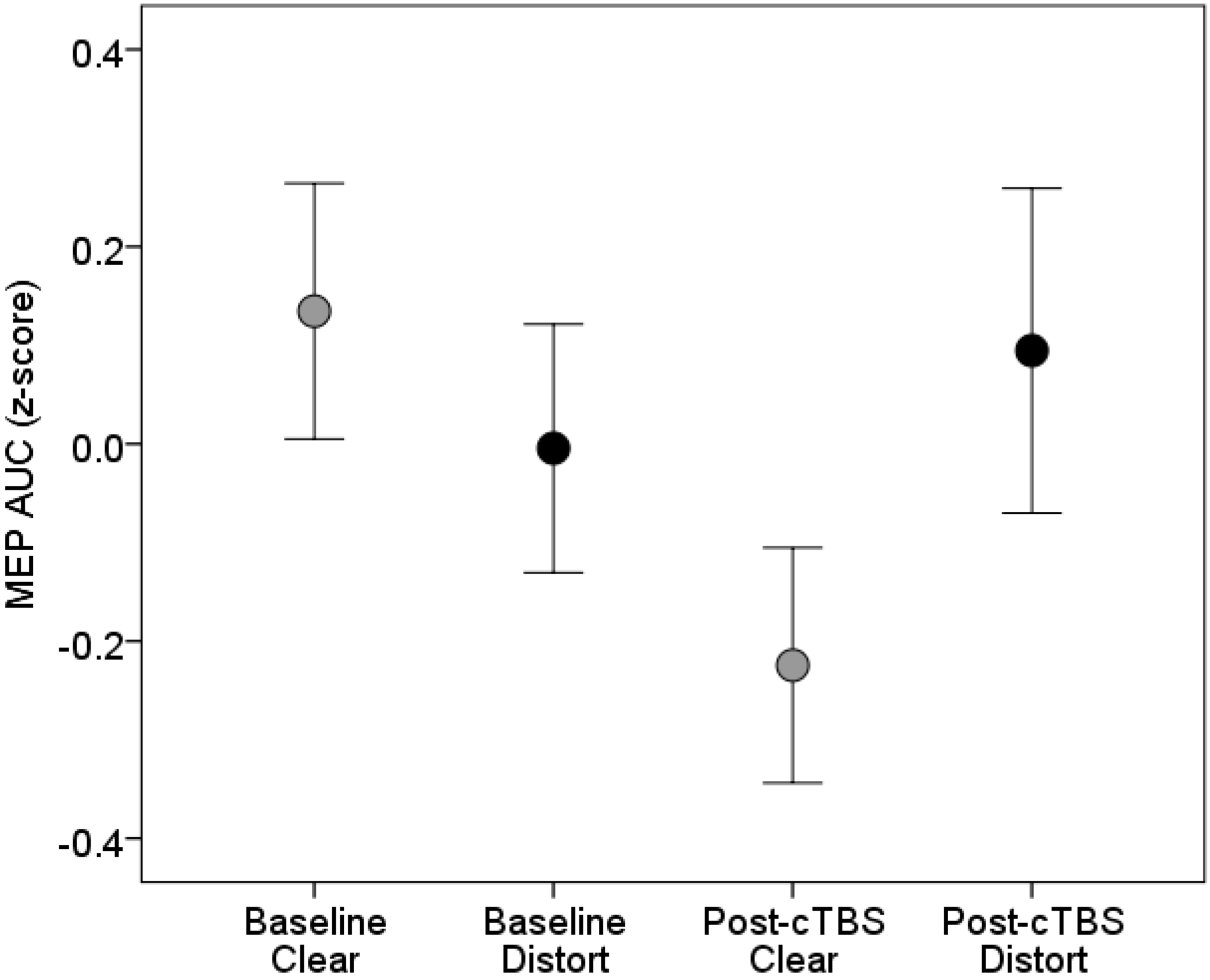
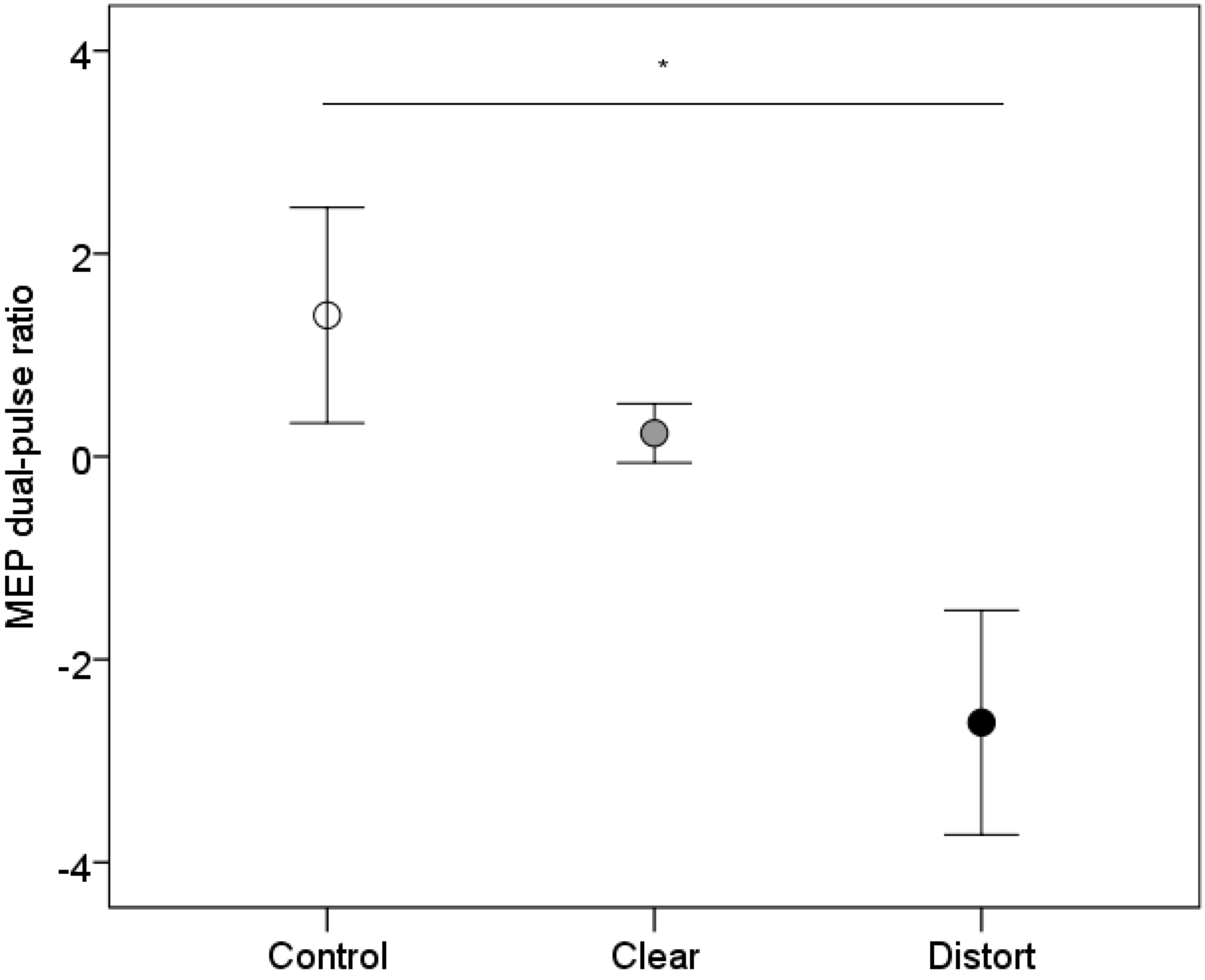
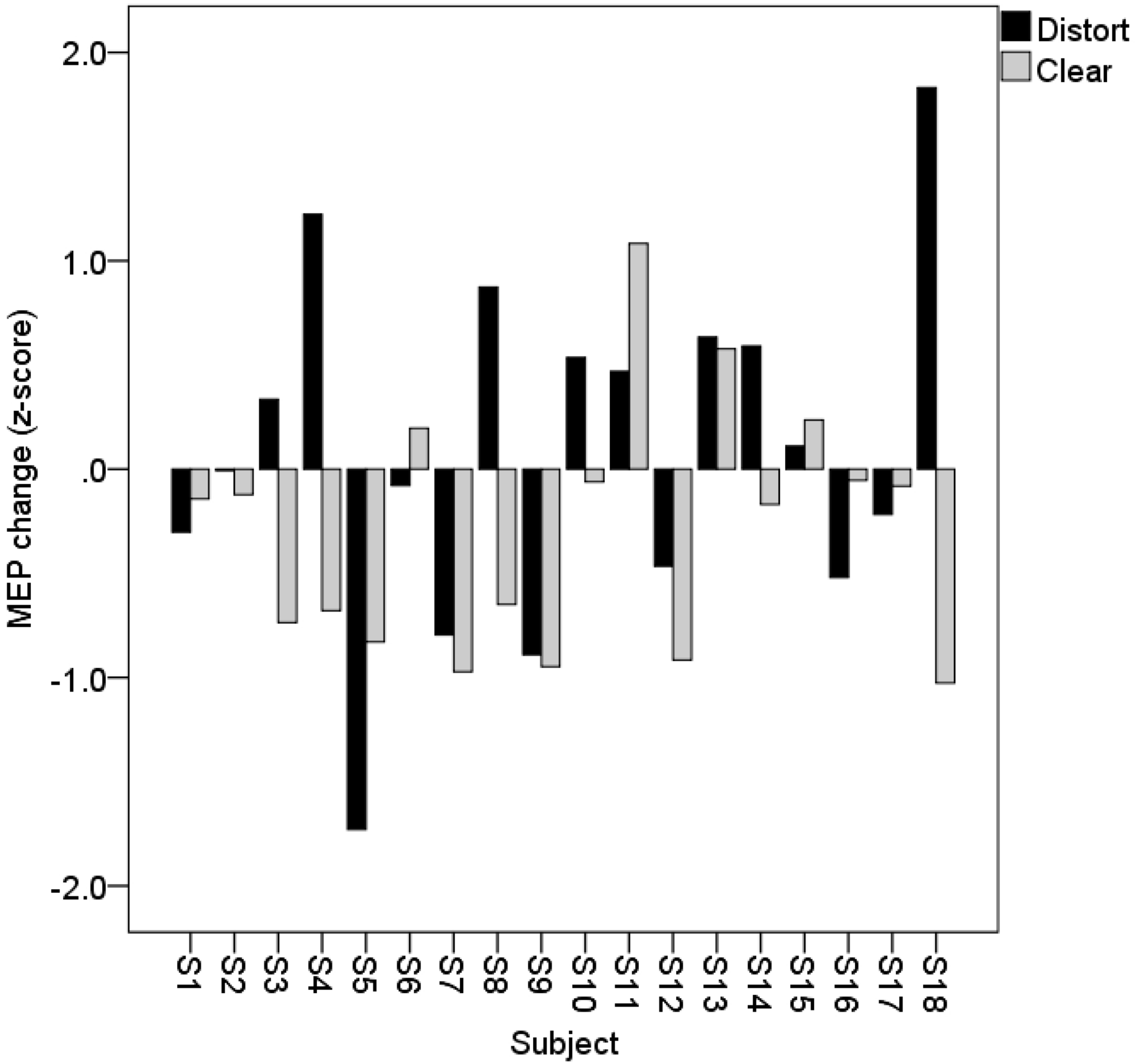
Accuracy (%)
100
90
80
70
60
50
Baseline Clear
Baseline Distort
Post-cTBS Clear
Post-cTBS Distort


500
400
300
200
100
0
Relative Reaction Time (ms)
*
Baseline Clear
Baseline Distort
Post-cTBS Clear
Post-cTBS Distort


MEP AUC (z-score)
0.4
0.2
0.0
-0.2
-0.4
Baseline Clear
Baseline Distort
Post-cTBS Clear
Post-cTBS Distort


4
2
0
-2
-4
MEP dual-pulse ratio
*
Control
Clear
Distort


Distort
Clear
2.0
1.0
.0
-1.0
-2.0
MEP change (z-score)
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12
S13
S14
S15
S16
S17
S18
Subject

# Statement of Significance

Data presented within this paper suggest that perceiving speech in challenging listening environments modulates the balance of intra- and inter-hemispheric motor interactions in the neurobiological network for speech perception. Specifically, a more extensive sensorimotor network is implicated during perception of distorted speech than when speech perception is optimal. The present results further understanding of connections between ventral Premotor Cortex and Primary Motor Cortex during auditory-motor integration.