Is there a Relationship between EEG and sTMS Neurophysiological Markers of the Putative Human Mirror Neuron System?

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Abstract

The mirror neuron system (MNS) has been theorised to play a neurobiological role in a number of social cognitive abilities and is commonly indexed putatively in humans via interpersonal motor resonance (IMR) and mu-suppression. Though both indices are thought to measure similar neuronal populations (i.e., ‘mirror neurons’), it has been suggested that these methods are unrelated, and therefore, incompatible. However, prior studies reporting no relationships were typically conducted in small and underpowered samples. Thus, we aimed to investigate this potential association in a large sample of neurotypical adults ($N = 116$; 72 females). Participants underwent TMS, electromyography (EMG), and electroencephalography (EEG) during the observation of videos of actors performing grasping actions in order to index IMR and mu-suppression (in beta, lower alpha, and upper alpha bandwidths). A series of linear regressions revealed no associations between IMR and each of the mu-suppression bandwidths. Supplementary Bayesian analyses provided further evidence in favour of the null ($B_{01} = 8.85 - 8.93$), providing further support for no association between the two indices of MNS activity. Our findings suggest that these two measures may indeed be unrelated indices that perhaps assess different neurophysiological aspects of the MNS. These results have important implications for future studies examining the MNS.

Key words: Interpersonal Motor Resonance; Mu-Suppression; Mirror Neuron System; TMS; EEG

1. Introduction

The mirror neuron system (MNS, hereafter) has been heavily investigated in recent years given its theorised role in a number of important social cognitive processes, including, but not limited to, empathy, imitation, action-understanding, theory of mind (ToM), and facial-emotion processing (Bekkali, Youssef, Donaldson, Albein-Urios, et al., 2020; Jeon & Lee, 2018). This network has also been implicated in a number of psychiatric and neurodevelopmental disorders (such as autism spectrum disorder; (Buccino & Amore, 2008; Gonzalez-Liencre, Shamay-Tsoory, & Brüne, 2013), whereby the MNS is thought to be ‘broken’ or functioning aberrantly, and thus, a potential contributor towards the social cognitive impairments relevant to these conditions. However, despite intuitive theoretical propositions regarding the role of the MNS in social cognition (e.g., simulation mechanisms, the perception-action model, and associative learning theory; for a review, see: Bekkali, Youssef, Donaldson, Albein-Urios, et al., 2020), there have been many apprehensions regarding the strength of evidence for this role, including concerns pertaining to the limitations of different measures used to index the MNS in humans.

While mirror neurons in non-human primates are measured via depth electrodes, the MNS is predominantly studied in humans using ‘indirect’ measures, including functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and transcranial magnetic stimulation (TMS; Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Muthukumaraswamy, Johnson, & McNair, 2004; Rizzolatti & Craighero, 2004; Strafella & Paus, 2000). Each of these methods require action observation protocols to putatively elicit a mirror neuron response. That is, each method involves presenting stimuli of human actions (such as hand-action sequences, finger movements, facial expressions, or biological motion) during neurophysiological measurement, relative to a control condition, whereby a ‘mirroring’ index is calculated via a subtraction method (Iacoboni, 2009; Rizzolatti & Craighero, 2004). Here, researchers are effectively looking for activation during the observation of action that is similar to that seen during action execution (Rizzolatti & Craighero, 2004). Two of the most commonly used markers of putative MNS activity are (1) ‘interpersonal motor resonance’, or IMR, via TMS, and (2) mu-suppression via EEG.

IMR assesses variations in corticospinal excitability (CSE) via TMS, a non-invasive neuromodulation technique whereby electrical currents are induced in the brain via time varying electromagnetic pulses (Pascual-Leone, 1999). The administration of a TMS pulse

over the primary motor cortex (M1) of sufficient strength can elicit a motor evoked potential (MEP), or a targeted muscle response, which is considered an index of CSE (Fadiga et al., 1995; Strafella & Paus, 2000). When this method is applied during the observation of stimuli depicting the same target muscle executing an action, an increase in MEP amplitude (i.e., CSE) is often observed. This increase in CSE during action observation, relative to a baseline condition, is referred to as 'IMR', and is taken to represent putative MNS activity occurring in the adjacent and anatomically connected premotor cortex via inputs to M1 (Fadiga et al., 1995; Gangitano, Mottaghy, & Pascual-Leone, 2001; Uithol, van Rooij, Bekkering, & Haselager, 2011).

A second common method of putatively measuring MNS activation is referred to as 'mu-suppression'. Using EEG, this method assesses resting motor neuron assemblies over central scalp regions (e.g., CZ, C3, C4), where large amplitude oscillations in the mu frequency band (lower alpha [8 – 10 Hz] and upper alpha [10 – 12 Hz] ranges) occur at rest, reflecting synchronized and spontaneous firing of sensorimotor neurons (Muthukumaraswamy et al., 2004; Oberman et al., 2005). However, when motor neurons become active, such as during observation of motor movement, this alpha rhythm excitability becomes suppressed and/or desynchronized, which is thought to reflect MNS activity variation (Muthukumaraswamy et al., 2004; Oberman et al., 2005). Thus, this suppression in the mu frequency band is termed 'mu-suppression' and is a common putative index of MNS activity. Further, it should also be noted that motor and mirror activity contributes to mu-rhythm on the beta-bandwidth (12 – 30 Hz) during action observation, whereby neuronal activation in the ventral premotor cortex (vPMC) has been demonstrated to be associated with mu-suppression (Bimbi et al., 2018). Thus, both alpha (lower and upper) and beta bandwidths are utilized as indices of mu-suppression in the MNS literature.

Though these methods are conceptually similar, in that they are thought to measure the same neural mechanism and neuronal populations (i.e., 'mirror neurons'), it has been suggested that they may be divergent (Lepage, Jean-François, Saint-Amour, & Théoret, 2008). In their seminal paper, Lepage, Saint-Amour, and Théoret (2008) examined the association between MEP amplitude and mu-rhythm modulation and found no relationship. These findings were unexpected, stimulating further investigations into the nature of the proposed relationship between TMS and EEG measures of the MNS. To our knowledge, an additional two studies have since reported similar null findings in their efforts to establish a direct relationship (Lapenta, Ferrari, Boggio, Fadiga, & D'Ausilio, 2018; Prinsen & Alaerts,

2020). However, one common limitation between these and Lepage and colleagues' original 2008 paper was that small samples were used ($N = 16 - 32$) and thus, they are not only subject to sampling error, but are only powered to detect very large effect sizes. By contrast, a larger sample is needed to detect small to moderate effects (of approximately $r = .20$), should they indeed exist. The question of what neurobiological role the MNS plays in motor and social cognitive abilities is unclear and can only be answered through robust and cumulative studies that assess the reliability of its measurement whilst also addressing the limits of insufficiently powered studies. Using G*Power (Faul, Erdfelder, Lang, & Buchner, 2007), we estimated that in order to detect a small to moderate effect size ($f^2 = .07 \sim r = .20$ [.10 - .30]), an approximate sample size required to achieve 80% power would be $N = 115$ (see supplementary materials). Given the strong implications of sample size on research outcomes, translation, and interpretability, particularly in a research area that is both highly speculative and controversial, it is imperative to conduct these methodological studies in large and sufficient samples. Thus, the current study aimed to investigate the association between IMR and mu-suppression in a large sample of healthy adults. It was hypothesised that the action observation conditions would elicit larger MEPs (reflecting IMR) and stronger mu-suppression (in beta, lower alpha, and upper alpha bandwidths), relative to the observation of no action, thus reflecting mirroring. It was also hypothesized that IMR would be positively associated with each mu-suppression bandwidth.

2. Methods

2.1. Participants.

Data examined in the current study were part of a larger study examining the neurobiological basis of empathy consisting of a sample of 150 right-handed adults (18-40 years) with no neuropsychiatric history and no contraindications to TMS (Rossi, Hallett, Rossini, Pascual-Leone, & Group, 2009). Only participants who completed EEG and/or TMS assessments ($N = 127$) were eligible for the current study. Of these participants, 11 were excluded due to (1) equipment and acquisition failures during data collection ($n = 6$; 4.72%), and (2) termination of the session due to participant fatigue ($n = 5$; 3.93%). The final sample thus comprised 116 ($N_{females} = 72$) neurotypical adults (see Table 1). Participants provided written informed consent and ethics approval for this project was provided by the Deakin University Human Research Ethics Committee (DUHREC 2015-038).

Table 1. Demographic Characteristics of the Current Sample ($N = 116$).

Demographic Variable	<i>M</i>	<i>SD</i>	<i>Range</i>
Age	23.79	4.87	18 - 40
Handedness (EHI <i>laterality quotient</i>)	91.70	15.79	15 - 100
Years of formal education	16.00	2.08	13 - 22
FSIQ	105.71	12.92	71 - 156

Note. EHI = edinburgh handedness inventory where a score of 50+ indicates right-handedness, (Oldfield, 1971); FSIQ = full scale IQ and measured by the Wechsler Abbreviated Scale of Intelligence – Second Edition (WASI-II).

2.2. Materials

Putative MNS measures were *IMR* (via TMS and EMG) and *mu-suppression* (via EEG). These were obtained concurrently during the observation of video displays of biological motion.

2.2.1. Biological Motion Stimulus Videos. Full details of the biological motion stimulus videos are provided in Bekkali et al. (2020: <https://doi.org/10.1093/scan/nsaa106>). Briefly, there were 132 trials (presented in 4 blocks of 33 videos) depicting human actors executing simple hand grasping actions (see Figure 1).

2.2.2. *IMR via Transcranial Magnetic Stimulation (TMS) and Electromyography (EMG).*

TMS was administered using a Magstim 200 stimulator (The Magstim Company Limited, Whitland, UK) and a figure-of-eight coil (d70). EMG was recorded using Powerlab 4/35 (AD Instruments, USA) with single BioAmp and via self-adhesive unipolar surface electrodes placed over the first dorsal interosseous (FDI) muscle (belly-tendon montage), the abductor digiti minimi (ADM; a control muscle), and the ulnar styloid process (ground electrode) of the right hand. EMG muscle responses were recorded using LabChart v8 (AD instruments, USA) at a sampling rate of 10 kHz. Additionally, during the observation of the biological motion videos, each relevant trial had four TMS stimulation timepoints (triggered at: 1 - prior to movement; 2- 90ms post movement; 3- 200ms post movement; and 4- at grasp, where the ‘grasp’ timepoint was approximated manually for each video; see Figure 2; Gangitano et al., 2001; Lepage, Tremblay, & Théoret, 2010). Each trial consisted of one of the four possible stimulation time points, and the order of pulse delivery was randomised. These TMS-pulse timepoints were automatically triggered using a light-

sensor device that detected an intermittent white box embedded in the stimulus videos (not visible to participants).

2.2.3. *Mu-Suppression via Electroencephalography (EEG)*. Participants were fitted with an EEG 40-channel EasyCap (EASYCAP GmbH, Herrsching, Germany) by measuring the head circumference, and nasion to inion distance. Sites of interests (F3, FCz, F4) were determined in reference to the 10-20 system and marked using surgical markers. The cap was then removed. Although EEG sites are typically central (C3, C4, CZ), frontal electrodes were used in the current study due to the concurrent administration of TMS, which is obstructed by electrodes placed over central sites and typically results in increased TMS-induced artefacts. Additionally, mu-suppression has previously been demonstrated to also occur over frontal sites during the observation of action (Cole, Barraclough, & Enticott, 2018). Therefore, we measured from frontal regions to reduce TMS related artefacts and to allow for TMS coil-to-scalp contact. Visual electrooculography (VEOG) was implemented, with electrodes placed above and below the right eye in line with the pupil. Electrodes were also placed over the bilateral mastoids, and the ground electrode was placed on the forehead (determined by marking 10% of the nasion to inion distance). Impedance was consistently maintained below 5 k Ω for all electrodes.

2.3. Procedure

Here we only describe the procedure of the relevant data collected for this study (i.e., TMS and EEG).

Following experimental setup for TMS and EEG and repeated safety screening, TMS was first employed to determine the 1 mV resting motor threshold (RMT) over 10 consecutive trials (Hyde et al., 2018). The average RMT of participants was 51.43% of maximum stimulation output ($SD = 10.68$, $range = 28 - 80\%$). Using the predetermined individual RMT, participants then received 20 pulses delivered 5000 ms apart while observing a fixation cross, which served as the pre-stimulus baseline index of CSE. Next, participants passively observed the biological stimulus videos while receiving TMS at the triggered timepoints (see materials) and concurrent EEG recording took place. Following stimulus videos, a second baseline (post-stimulus videos) identical to the pre-baseline condition was obtained (i.e., 20 pulses delivered 5000 ms apart while observing a fixation cross). A post-baseline condition, which was administered approximately 2-3 minutes

following the fourth and final block of the experiment, was included to assess whether CSE changed over the course of the experiment and to control for the potential effects of repetitive stimulation across time.

A.

B.

Figure 1. A = Visual depiction of action execution sequence of male and female actors. B = Stimulation (TMS) timepoints delivered during observation of action. Note - the blocks in images displayed here have been added to de-identify actors and were not present during the task.

2.4. Data Cleaning and Preparation

Both indices of putative MNS activity (*pMNS*, hereafter) were calculated using the below formula:

$$pMNS\ Index = \frac{Active\ condition - Baseline\ condition^{\dagger}}{Baseline\ condition} \times 100$$

[†]Numerator is reversed when calculating mu-suppression, so positive values reflect increased suppression

Where, '*pMNS index*' reflects the type of putative MNS index acquired (either IMR or mu-suppression), 'active condition' reflects neurophysiological activity occurring during the observation of action, and 'baseline condition' reflects activity during the control condition (i.e., a black screen). Here, positive percentage values reflect greater degrees of *pMNS* activity (i.e., increased MEP facilitation reflecting IMR, and stronger alpha and beta desynchronization for mu-suppression values; see Table 1). This formula is commonly used and has been applied in previous MNS studies (e.g., Bekkali, Youssef, Donaldson, He, et al., 2020; Cole et al., 2018).

Table 2. Calculation of percentage change variables reflecting IMR and Mu-suppression

<i>pMNS</i> Index	Neurophysiological Index	
	<i>IMR</i>	<i>Mu-suppression</i>
Data Index	MEP FDI values per trial	Mu power for each frequency band (beta, lower alpha, upper alpha)
Active Condition	Median raw MEP (FDI) value per trial during the observation of the biological motion stimulus videos	Average mu values occurring during the action observation of the biological motion stimulus videos
Baseline Condition	Median of the 20 MEP (FDI) values acquired during the pre-baseline condition	Average mu power values occurring during the baseline condition (i.e., during the observation of a blank screen)

This table reflects the type of data computed and used in the above formula in the calculation of each *pMNS* activity index. *pMNS* = putative mirror neuron system; MEP = motor evoked potential; FDI = first dorsal interosseous.

2.4.1. TMS and EMG: Interpersonal Motor Resonance. All EMG data were pre-processed and extracted in LabChart Reader v8 (AD Instruments, USA). Given that activation of the ADM data was generally small and unreliable, resting motor threshold was

determined according to the FDI muscle activation. Raw peak-to-peak MEP amplitudes during experimental and baseline conditions were extracted and converted into an IMR percentage change variable using the $pMNS$ index formula above. As it has been previously shown that MEPs suffer from transient inflation, where amplitudes are initially large but decline over time (Schmidt et al., 2009), we used the median value as our measure of central tendency. Further, preliminary analysis revealed that our post-stimulus baseline not only yielded greater MEPs ($M = 1.47$, $SE = .06$) than our pre-stimulus baseline ($M = 1.17$, $SE = .06$, $p < .001$), but was also not significantly different from the MEPs observed during the task ($M = 1.53$, $SE = .06$, $p = .080$), suggesting possible neurophysiological contamination of the post-stimulus baseline, as it occurred following the observation of the experimental task. Considering this, we only included the pre-stimulus baseline in the calculation of IMR, given that this baseline was observed before the stimulus set, and thus was deemed more reflective of a true baseline condition.

2.4.2. EEG: Mu-Suppression. EEG analysis was conducted offline using Curry 7 neuroimaging suite software (Compumedics Ltd, Australia). Epochs were created for the action observation stimulus videos ('active condition') and for the baseline periods (blank screen observation). Though the stimulus videos were 4000 ms in duration, we extracted a 1000 ms epoch (between 1450 ms and 2450 ms) in order to specifically capture the grasping action sequence of each trial whilst also eliminating the TMS pulses that were delivered prior to the grasping action (i.e., prior to movement onset [T1] and 90 ms post movement [T2]; see TMS section above). This resulted in 66 retained trials for the active condition per person. For the baseline period, we extracted a 1000 ms epoch (500 ms to 1500 ms). All data were re-referenced to the average of the mastoids (M1, M2). EEG (F3, FCz, and F4) and EOG channels were bandpass filtered (1-30 Hz, notch filter at 50 Hz), and baseline corrected. Ocular artefacts were detected by scanning EOG electrodes for voltages exceeding $\pm 200 \mu V$. Non-ocular artefacts, such as the TMS pulses administered at 200 ms post movement onset (T3) and before grasp (T4), were detected by searching for voltages $\pm 150 \mu V$. All artefacts exceeding these thresholds (for ocular and non-ocular artefacts) were corrected using covariance analysis (Curry Neuroimaging Suite 7, Compumedics Ltd, Australia).

To calculate mu-suppression, a Fast Fourier Transform (FFT) was applied to each epoch (active and baseline) and averaged for each condition and electrode (F3, FCz,

and F4). We determined mu power for beta (12 – 30 Hz), lower alpha (8 – 10 Hz) and upper alpha (10 – 12 Hz) frequency ranges for each epoch and electrode. A measure of the percentage change from baseline in power for each frequency band and electrode was then calculated using the *p*MNS formula described above (see Table 1). Here ‘baseline mu power’ reflects the mu power values occurring during the baseline condition, ‘active’ reflects the mu values occurring during the action observation trials. We then computed an overall mu-suppression variable by calculating the mean of each electrode site (i.e., the average of F3, F4, and FCz) per frequency band (beta, lower alpha, and upper alpha). These three variables were used as our indices of mu-suppression in our final analysis.

Figure 2. Visual depiction of TMS-EEG protocol set-up. A = biological motion stimulus videos, where participants were seated approximately 600mm away from a 24" LED computer screen. B = TMS/EMG set up C = concurrent EEG set up and montage

2.5. Data Analysis and Statistical Methods

In order to estimate the relationship between the two indices of putative MNS activity, a series of linear regressions were performed in Mplus v8 (Muthén & Muthén, 2012). We ran a total of 3 independent bivariate regressions estimating the association between IMR and: (1) beta desynchronization; (2) lower alpha desynchronization; and (3) upper alpha desynchronization, and controlled for the effects of sex, age, and FSIQ throughout.

Each of our regression analyses utilised robust maximum likelihood (MLR) estimation method which is robust to heteroskedasticity and non-normality (Li, 2016). Of the available sample ($N = 116$), 77% of cases had complete data with missing data ranging from 9 – 15% ($n_{Mu-Suppression} = 10$, $n_{IMR} = 17$, respectively). To account for missingness we used a full information-maximum likelihood (FIML) approach, which is considered a best practice approach to dealing with missingness (Schafer & Graham, 2002).

Following preliminary frequentist estimation of regression relationships, secondary analyses were also conducted where we used a Bayesian statistical estimator in Mplus v8 (Muthén & Muthén, 2012), which allowed us to formally examine the probability of a null association given the data. Bayesian analyses are robust to the distribution of model parameters and accounts for missing data (Van de Schoot, Yerkes, Mouw, & Sonneveld, 2013). We used an uninformed Bayesian approach for analysis presented in the manuscript, which relied on default uninformed priors (Muthén & Muthén, 2012). However, as presented in Supplementary Table I, we conducted sensitivity analyses to explore the influence of varied prior specifications on results. The effect of varying the priors for the model was negligible. Thus, the results of the regression analyses using Bayesian estimation methods reflect default priors. Finally, for each regression analysis, we calculated the respective Bayes factor (BF_{01}), an index reflecting the degree of evidence in support of the null hypothesis over the alternate hypothesis, using the below formula (Faulkenberry, 2018; Wagenmakers, 2007):

$$BF_{01} = \frac{Pr_{BIC}(D | H_0)}{Pr_{BIC}(D | H_1)} = \exp\left(\frac{\Delta BIC_{10}}{2}\right)$$

$$\text{where } \Delta BIC_{10} = BIC(H_1) - BIC(H_0)$$

Here, we computed the BF_{01} for the null hypotheses ($\mu = 0$) over an alternate hypothesis ($\mu \neq 0$), given the data (D), by using a Bayesian information criterion (BIC) approximation.

Interpretations and classifications of BF_{01} were based on empirical guidelines demonstrated by Raftery (1995; weak = 1-3; moderate = 3-20, strong = 20-150; very strong = 150+).

3. Results

3.1. *p*MNS Indices.

Results showed evidence for increased MEP percentage change from baseline during action observation, reflecting motor resonance (i.e., IMR), and thus indexing mirroring. Comparably, mu-suppression was only observed in the beta frequency band (demonstrated as a significant change from baseline; see Table 3). On average, there was no evidence of significant mu-suppression in the lower and upper alpha bandwidths in our sample, which demonstrated large variability in scores and high SDs for each portion of the alpha bandwidth (see Table 3).

Table 3. Descriptive Statistics for *p*MNS indices

<i>p</i> MNS Index	<i>M</i>	<i>SD</i>	<i>t</i>	<i>df</i>	<i>p</i>	95% <i>CI</i>
Mu-suppression						
Beta	15.99%	41.71	3.95	105	<.001	[7.96, 24.03]
Lower Alpha	-6.52%	93.01	- 0.72	105	.472	[-24.43, 11.39]
Upper Alpha	1.58%	95.65	0.170	105	.865	[-16.83, 20.00]
IMR	65.43%	112.33	5.32	98	<.001	[43.01, 87.81]

Note. IMR = interpersonal motor resonance All percentage scores express a change from their respective baselines, and inferential statistics tested for whether these changes were significantly different from zero.

3.2. Relationships between IMR and Mu-Suppression

Results of the regression analyses showed no significant associations between IMR and each of the mu-suppression bandwidths, suggesting no relationship between these two putative measures of MNS activity. Subsequent Bayesian regression analyses further revealed no associations, with posterior mean regression β -coefficients consistently found to be approximately zero. Further, corresponding credible intervals indicated a 95% probability that the true effect could be zero, and thus, a plausible outcome. Finally, Bayes factor (BF_{01}) estimations for each regression analysis (IMR vs beta; IMR vs lower alpha; IMR vs upper alpha; see Table 4) revealed moderate evidence in favour of the null, providing further support for no association between the two indices of *p*MNS activity. Results of the regression analyses using both robust maximum likelihood and Bayesian estimation methods are presented in Table 4.

Table 4. Standardized and Unstandardized Regression Coefficients of p MNS using Frequentist and Bayesian Estimation Methods

Mu Power Bandwidths	Frequentist MLR Estimation					Bayesian Estimation					
	<i>B</i>	Confidence Interval	<i>p</i>	β	Confidence Interval	<i>B</i>	Credible Interval	<i>p</i>	β	Credible Interval	<i>B</i> ₀₁
		(95% LL, UL)			(95% LL, UL)		(95% LL, UL)			(95% LL, UL)	
Beta (12 - 30 Hz)											
Unadjusted	.22	(-.09, .55)	.164	.08	(-.05, .22)	.22	(-.20, .91)	.213	.08	(-.10, .29)	8.57
Adjusted	.24	(-.06, .54)	.119	.09	(-.04, .22)	.25	(-.31, .90)	.180	.09	(-.12, .30)	8.61
Lower Alpha (8 – 10 Hz)											
Unadjusted	.09	(-.07, .26)	.245	.08	(-.08, .25)	.11	(-.15, .31)	.113	.09	(-.13, .26)	8.93
Adjusted	.11	(-.15, .31)	.199	.09	(-.07, .25)	.12	(-.15, .43)	.160	.10	(-.13, .32)	8.81
Upper Alpha (10– 12 Hz)											
Unadjusted	-.15	(-.69, .38)	.568	-.13	(.54, .28)	-.14	(-.36, .09)	.113	-.12	(-.32, .08)	5.85
Adjusted	-.15	(-.67, .36)	.557	-.13	(-.53, .26)	-.15	(-.45, .12)	.200	-.13	(-.34, .09)	5.88

Note. Independent variable = Interpersonal Motor Resonance (IMR). Adjusted = covaried for Sex, Age, and Full-Scale IQ. MLR = robust maximum likelihood; Bayesian regression coefficients presented here reflect the use of default priors; LL = lower level CI; UL = upper level CI; B_{01} = Bayes Factor testing null hypothesis over the alternate hypothesis. B_{01} guidelines: weak evidence = 1-3; moderate = 3-20, strong = 20-150; very strong = 150+

4. Discussion

The current study aimed to investigate the relationship between interpersonal motor resonance (IMR) and mu-suppression indices of putative MNS activity. It was hypothesised that the observation of action would elicit larger MEPs (reflecting IMR) and stronger mu power desynchronization (in beta, lower alpha, and upper alpha bandwidths), relative to the observation of no action. It was also hypothesized that IMR would be positively associated with each mu-suppression bandwidth. Results revealed that mirroring was evident for the IMR index of MNS activity, and there was also evidence of mu-suppression within the beta (12 – 30 Hz) bandwidth. However, this was not the case for the alpha bandwidths (lower [8 – 10 Hz] and upper [10 – 12 Hz]), where suppression, on average, was not elicited. Importantly, we detected no association between IMR and mu-suppression (across all bandwidths), and Bayesian inference provided further evidence in favour of a null association, suggesting the two methods may be dissociable.

4.1. Associations Between IMR and Mu-Suppression

Overall, our preliminary results revealed no significant associations between IMR and mu-suppression (in all bandwidths). In fact, the magnitude of these observed coefficients were extremely small and, arguably, negligible. Supplementary analyses conducted to examine the probability of these results, given the data, provided further evidence of no association. Thus, our results provide strong evidence that these two markers of MNS activity are unrelated, suggesting that they may tap into different neurophysiological aspects of the MNS. Though each measure has been previously demonstrated to be sufficient in putatively evoking ‘mirroring’, the lack of an association between the two indices, though theoretically unintuitive, are empirically supported by previous studies which have similarly failed to demonstrate a relationship (Lapenta et al., 2018; Lepage et al., 2008; Prinsen & Alaerts, 2020). The current study builds on that prior research, with the added strength of a sufficiently powered sample in conjunction with supplementary Bayesian analyses, and thus providing further confidence in this null outcome. Plausible explanations for these relatively surprising outcomes are primarily discussed with regards to differences in the measurements of ‘mirroring’, particularly the vastly different temporal properties of TMS and EEG. While the former assesses excitability at a precise moment in time (with millisecond precision), the latter takes an aggregate of brain activity across a far broader time scale (such as hundreds or thousands of milliseconds). Further, although both indices putatively reflect excitability of the motor cortex (M1), these neuronal populations, though overlapping, are not identical

(Mäki & Ilmoniemi, 2010). Rather, mu oscillations are the sum activity of large neuronal populations, while IMR reflects CSE of select fewer specific neurons and interneurons from regions of M1 that project onto, or control, a specific target muscle (i.e., the FDI muscle). Related to this, IMR is therefore also affected by spinal excitability and its contributions to MEP amplitudes that are not necessarily reflected in mu-suppression indices (Lepage et al., 2008; Mäki & Ilmoniemi, 2010).

Though the methodological differences between EEG and TMS methods are important to consider, the indirect nature of their measurement (a limitation they both share), may also contribute to a lack of association. While both are considered relatively robust measures of MNS activity, the changes in neural activation observed during action observation, relative to a baseline condition, likely reflects a combination of targeted neuronal populations (i.e., mirror neurons) and simultaneous neuronal activity that is co-occurring in response to the visual stimuli (Lamm & Majdandzic, 2015). In other words, neuronal activation detected using these neurophysiological methods are indirect (i.e., putative), relative to more precise (but invasive) methods such as single-cell recordings and are therefore unable to differentiate between MNS-specific activation and that of non-MNS related populations (Fuelscher et al., 2019; Lamm & Majdandzic, 2015). Thus, because both IMR and mu-suppression outcomes are similarly affected by neural ‘noise,’ it cannot be determined with absolute certainty that what is being indexed as ‘mirroring’ is in fact truly reflective of pure MNS activity. Given both the methodological differences and limitations inherent in these techniques, it is not unlikely that these measures are unrelated.

4.2. *p*MNS Indices via IMR and Mu-Suppression

Importantly, while the biological motion stimuli created here were successful in inducing significant ‘mirroring’ as measured by TMS via IMR, this was not entirely reflected in our EEG outcomes. Mu-suppression was only evident in the beta bandwidth, and not in lower or upper alpha. Reasons for this are not entirely clear, particularly considering that these measures were collected concurrently during the observation of the same stimulus videos. However, recent methodological considerations highlighted by Hobson and Bishop (2016) may provide some insight. When investigating the validity of mu-suppression as a measure of MNS activity, it was suggested that three important guidelines should be addressed in order to successfully show evidence of mu-suppression: (1) the inclusion of both action observation *and* action execution conditions; (2) EEG activity from multiple electrode sites should be reported, and; (3) potential attentional confounds should be examined and accounted for if necessary. The current study did not include an action execution condition,

as it was not considered necessary to elicit mu-suppression, which is methodologically consistent with previous studies that have successfully demonstrated mu-suppression (e.g., Bekkali, Youssef, Donaldson, He, et al., 2020; Cole et al., 2018; Lapenta et al., 2018; Prinsen & Alaerts, 2020). However, it has been suggested that the inclusion of a movement condition is necessary, as it serves as a positive control condition (Hobson & Bishop, 2016). Secondly, we note that we did not use electrode sites outside the central sensorimotor strip, and thus, it is difficult to ascertain whether mu-suppression was specifically indexing motor mirroring, as opposed to neuronal changes occurring elsewhere. Finally, though we indeed previously examined the perceptual effects of gaze behaviours on our IMR index of MNS activity (see: Bekkali, Youssef, Donaldson, He, et al., 2020), this was not possible for mu-suppression due to limitations associated with the measurement design. Though, given that IMR and mu-suppression data were collected concurrently and during the observation of identical trials, it was considered plausible that any effects evident in our IMR index would be similarly evident in our mu-suppression index, unless these two methods indeed tap into different neurophysiological outcomes. Given that no major perceptual effects were found overall, we were confident that this would be the case for mu-suppression. However, there is some possibility that this was not translatable, and thus, there may have been evidence of selective suppression. Nonetheless, it remains unclear why alpha desynchronization was not evident in the current study.

4.3. Limitations and Directions for Future Research

The results of the current study extend on previous experiments that have suggested TMS (IMR) and EEG (mu-suppression) putative indices draw on different neurophysiological aspects of the MNS, and are thus, incompatible. Though we have discussed the limitations characteristic of these methodologies throughout, there are additional limits specific to the current study that require some mention. The first relates to the lack of neuronavigation used for coil positioning and placement during TMS. It has been previously asserted that the use of a neuronavigational system is a crucial prerequisite for establishing reliable and stable outcomes via TMS, given the high potential for movement during stimulation (Lepage et al., 2008). Given the magnitude of the original project from which this study was derived, neuro-navigation was not utilised due to time constraints. Though we were nonetheless able to induce IMR via TMS and replicate the outcomes of prior studies that employed neuro-navigation (Lepage et al., 2008), this remains a constraint of the current study, as the use neuro-navigation is considered ideal. Further limitations formerly discussed in our previous study (Bekkali, Youssef, Donaldson, He, et al., 2020) are

also constraints of the current study, given the use of identical data. In particular, the lack of ADM data and the unexpected increase in CSE during the post-baseline condition. Though ADM data was collected as part of the larger project, it was not utilised here as it was not of interest and thus, may not allow for direct comparisons with prior studies that have included control muscles. Further, we unexpectedly observed larger CSE during the post-baseline, relative to both the pre-baseline and experimental conditions, suggesting possible neurophysiological carry-over effects from the observation of action observation stimuli. Given this residual effect, we were unable to include the post-baseline condition. Collectively, these limitations should be taken into consideration when interpreting the findings of the current study and a degree of caution should be maintained.

5. Conclusion

In their original study, Lepage et al. (2008) concluded that *‘the strength of a correlation between two measures is limited by the reliability of the instruments used’* (p. 22). Indeed, though stated over a decade ago, this remains to be the case, and these methodological limitations continue to cloud outcomes. It is clear that this area necessitates not only continued investigations, but also more focussed examinations towards establishing more reliable methods and techniques for measuring MNS activity, in order to develop robust and empirically supported procedures that can be broadly and consistently implemented in this area. This would include the development of reliable stimulus sets that effectively and consistently induce a ‘mirroring’ response in both healthy and clinical populations. The development of such a stimulus set would allow for comparability between studies investigating the same neural system, and potentially more consistent outcomes. Though it cannot be stated with absolute certainty that these two measures are completely unrelated, given the limitations of each respective measurement tool and the general constraints of this study, our findings provide strong evidence for their incompatibility and support prior studies that have reported similar null outcomes. Further, the multi-method approach, large sample size ($N = 116$), and supplementary Bayesian analyses provides a certain degree of confidence in these findings. Of equal importance, it should be highlighted that although our results suggest that these two methods draw on very different neurophysiological aspect of the MNS, our study also indicates that the use of both techniques concurrently may be essential to provide more comprehensive and versatile indices of this neural system, and that solely relying on one method may be insufficient. Thus, until more adequate stimuli and evidence-based procedures are developed, it is recommended that future studies examining the *p*MNS use a multi-method approach (such as

combined TMS-EEG, or fMRI-EEG). Crucially, researchers must also consider this lack of association in their selection of methods in order to make informed decisions regarding what methodological approach is most suitable for their research question and must acknowledge this limitation in their findings.

Conflicts of interest. None declared

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Supplementary Material

Figure I. G*Power Output: Calculation of Approximate Sample Size

Table I. Standardized Regression Coefficients of p MNS across Varied Variance Priors using Bayesian Estimation Methods.

Figure I.

[1] -----

F tests - Linear multiple regression: Fixed model, R^2 deviation from zero

Analysis:	A priori: Compute required sample size		
Input:	Effect size f^2	=	0.07
	α err prob	=	0.05
	Power ($1-\beta$ err prob)	=	0.8
	Number of predictors	=	1
Output:	Noncentrality parameter λ	=	8.0500000
	Critical F	=	3.9250756
	Numerator df	=	1
	Denominator df	=	113
	Total sample size	=	115
	Actual power	=	0.8031981

Table I. Standardized Regression Coefficients of p MNS across Varied Variance Priors using Bayesian Estimation Methods.

Priors (μ, σ^2)	Mu Power Bandwidths											
	Beta (12 - 30 Hz)				Lower Alpha (8 - 10 Hz)				Upper Alpha (10 - 12 Hz)			
			Credible Interval				Credible Interval				Credible Interval	
			<i>Lower</i>	<i>Upper</i>			<i>Lower</i>	<i>Upper</i>			<i>Lower</i>	<i>Upper</i>
β	p			β	p			β	p			
(0, 0.1)	0.046	0.287	-0.067	0.202	0.073	0.24	-0.126	0.226	-0.104	0.127	-0.276	0.097
(0, 0.5)	0.071	0.233	-0.085	0.264	0.083	0.213	-0.126	0.249	-0.116	0.113	-0.316	0.081
(0, 1)	0.078	0.227	-0.088	0.281	0.085	0.213	-0.126	0.253	-0.119	0.113	-0.319	0.08
(0, 2)	0.081	0.227	-0.09	0.291	0.086	0.213	-0.126	0.254	-0.12	0.113	-0.321	0.08
(0, 5)	0.084	0.213	-0.102	0.284	0.087	0.213	-0.126	0.255	-0.12	0.113	-0.322	0.08
(0, 20)	0.085	0.213	-0.102	0.287	0.087	0.213	-0.126	0.256	-0.121	0.113	-0.322	0.08
(0, 50)	0.085	0.213	-0.102	0.287	0.087	0.213	-0.126	0.256	-0.121	0.113	-0.322	0.08

Note. Independent variable = Interpersonal Motor Resonance (IMR). Bseed = 20081991, number of chains = 3.