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Does *f*MRI Repetition Suppression Reveal Mirror Neuron Activity in the Human Brain? Insights from Univariate and Multivariate Analysis

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Abstract

Mirror neurons (MN) have been proposed as the neural substrate for a wide range of clinical, social and cognitive phenomena. Over the last decade, a commonly used tool for investigating MN activity in the human brain has been functional magnetic resonance (*f*MRI) repetition suppression (RS) paradigms. However, the available evidence is mixed, largely owing to inconsistent application of the methodological criteria necessary to infer MN properties. This raises concerns about the degree to which one can infer the presence (or absence) of MN activity from earlier accounts that adopted RS paradigms. We aimed to clarify this issue using a well validated *f*MRI RS paradigm and tested for mirror properties by rigorously applying the widely accepted criteria necessary to demonstrate MN activity using traditional univariate techniques and Multivariate Pattern Analysis (MVPA). While univariate whole brain analysis in healthy adults showed uni-modal RS effects within the supplementary motor area, no evidence for cross-modal RS effects consistent with mirror neuron activity was found. MVPA on the other hand revealed a region along the anterior intraparietal sulcus that met the criteria for MN activity. Taken together, these results clarify disparate evidence from earlier RS studies, highlighting that traditional univariate analysis of RS data may not be sensitive for detecting MN activity when rigorously applying the requisite criteria. In light of these findings, we recommend that short of increasing sample sizes substantially, future studies using RS paradigms to investigate MNs across the human brain consider the use of MVPA.

1. Introduction

Mirror neurons (MN) were first described by di Pelligrino and colleagues (1992), who observed cells within the ventral premotor cortex (F5) of the Macaque that discharged during both action execution and observation of the same goal-directed movement. Since their discovery in the Macaque over 25 years ago, a growing body of neuroimaging and neurophysiological studies have revealed similar neural regions in humans that consistently show MN properties (Cook et al., 2014;

Kilner & Lemon, 2013). These include the human homolog for the F5, the inferior frontal gyrus (IFG; Kilner et al., 2009; Press et al., 2012), and more broadly throughout the cortex including the inferior parietal lobe (IPL; Chong et al., 2008) and supplementary motor area (SMA; Mukamel et al., 2010). Functionally, it has been argued that the MN system matches an observed movement with one's corresponding neural representation for that exact same movement, thus facilitating imitation and motor learning (Casile et al., 2013; Oosterhof et al., 2013; Rizzolatti et al., 2014). In recent years, MNs have become an increasingly popular topic in light of their putative explanatory power for a wide range of clinical (e.g., autism spectrum disorder; Rizzolatti & Fabbri-Destro, 2010), social (e.g., facial emotion recognition; Enticott et al., 2008) and cognitive phenomena (e.g., theory of mind and empathy; Gallese, 2001; Gallese & Goldman, 1998).

With respect to neuroimaging studies, the existence of MNs in the human cortex has traditionally been inferred from spatial convergence between regions that show increased blood oxygenation level dependent (BOLD) responses during both action observation and execution (Oosterhof et al., 2013; Molenberghs et al., 2012). However, the majority of neurons activated during execution and observation are not MN in nature, and in some cases, are not even *motor* specific (Dinstein et al., 2008). Since the typical $3mm^3$ voxel contains approximately 10^5 neurons (Barron et al., 2016), and since increased BOLD activity reflects the combined average neural firing of the entire voxel, numerous neuronal sub-populations can be responsible for the BOLD profile of a single voxel. Accordingly, shared BOLD activity of a voxel during both executed and observed movement can be the product of different neural populations firing (Dinstein et al., 2008; Davis & Poldrack, 2013; Malach, 2012). Thus, while convergence of neural activity during action observation and execution may be predictive of voxels containing MNs, convergence alone does not constitute sufficient evidence to this end.

Several studies have aimed to circumvent this limitation using a functional magnetic resonance imaging (*f*MRI) adaptation technique known as repetition suppression (RS). RS occurs when neural activity is suppressed within a population of neurons following repeated activation by the successive presentation of stimuli to which these neurons are sensitive (Barron et al., 2016; Davis & Poldrack, 2013; Desimone, 1996). While the precise mechanisms underlying RS are not fully understood (see Grill-Sepctor et al., 2006), it is generally argued that RS reflects a rapid form of experience-dependent brain plasticity, possibly linked to optimized predictions about the sensory consequences of actions (Solomon & Kohn, 2014; Stefanics et al., 2018). In the case of MN activity, voxels containing neurons with MN properties should behave in the following three ways: They should show shared convergence, that is, they should activate during both observation and execution of the same object-directed movement (Criterion 1). When the same movement is performed (or observed) repeatedly, suppression of neural activity should take place (uni-modal repetition suppression – uni-modal RS, Criterion 2). RS should also occur when a given action is performed and then observed, or vice versa (cross-modal repetition suppression – cross-modal RS, Criterion 3). As per the critique that convergence of neural involvement at a given site during observed and executed movement is a pre-requisite of MN activity, yet insufficient to conclude their presence, the same can be argued for each of these criteria in isolation. That is, the degree to which MN activity can be reliably inferred is predicated on adherence to each of the abovementioned criteria (see Oosterhof et al., 2013 for a detailed discussion).

Early *f*MRI adaptation studies provided mixed evidence for the existence of MNs with one study reporting cross-modal RS in the inferior parietal lobe (IPL, Chong et al., 2008) while other studies failed to find evidence indicative of MN activity (Dinstein et al., 2007; Lingnau et al., 2009). However, to prevent signal changes from non-motor categorization on the basis of inference about potential motor acts, the actions adopted in these studies were neither goal nor object directed. It has since been argued however that arbitrary motor acts are unlikely to active the MN system. That

is, mirror neurons are thought to be sensitive to object and goal directed behaviour rather than biological motion per se (Enticott et al., 2010; Kilner et al., 2009; Umiltà et al., 2001).

Similarly, studies employing object and goal directed actions have provided mixed results both across and within studies. This may partly be due to inconsistencies in addressing the aforementioned criteria for MN activity (see Table 1). For example, in their seminal study, Kilner et al. (2009) reported cross-modal RS in the inferior frontal gyrus (IFG) using a ROI approach. However, uni-modal RS was not reported at a group level (C2), and no single ROI was tested against all three criteria necessary to infer mirror neuron activity (C1 – C3). Finally, the ROI approach prevented inferences about the spatial distribution of MN activity beyond the IFG. In an attempt to replicate these findings at a whole brain level, Press et al. (2012) demonstrated RS effects in the IFG. Although the study showed shared convergence for peak voxel coordinates bilaterally at the border of BA44/BA6, uni-modal RS and cross-modal RS was reported bilaterally for BA47. Thus, it is unclear whether convergence (C1) was observed in either BA47 sites, limiting the confidence with which cross-modal RS (and uni-modal RS) at this site can be attributed to MN activity.

Finally, de la Rosa et al (2016) identified a small cluster in the IFG that reportedly showed evidence of MN activity during an adaptation paradigm. While cross-modal RS was observed, uni-modal RS (C2) was not reported, limiting the degree to which RS characteristics can be considered to be MN specific. Further, de la Rosa and colleagues presented their stimuli in a block-wise fashion, whereby a block of 10 successive execution trials was followed by a block of 20 consecutive observation trials. However, it is difficult to ascertain the degree to which the BOLD response from the observation block reflected cross-modal RS (as hypothesized) based on this design. Instead, uni-modal RS effects could reasonably have occurred during the observation block given that observation trials were presented consecutively.

To summarize, while *f*MRI RS paradigms have been employed in the MN literature over the last decade, the available evidence is mixed. Where significant effects are reported, these effects are typically modest and are only observed following ROI analysis. Most importantly, however, no study to date has reported MN activity in a single region of the brain that has satisfied the minimum criteria necessary for inferring MN activity (see Table 1). These limitations raise concerns about the degree to which one can reliably measure the presence (or absence) of MN activity within the human brain based on current evidence. Thus, there is a clear need for a controlled study that rigorously applies the necessary criteria to infer MN responses. To this end, we adopted a well validated *f*MRI RS paradigm (Kilner et al., 2009; Press et al., 2012) and tested for regions with mirror properties as per the minimum criteria necessary for inferring MN activity (as mentioned above) using the previously adopted univariate techniques. As a further aim, we adopted multivariate pattern analysis (MVPA) to test these criteria. The latter approach was taken in light of recent evidence (see Oosterhof et al., 2013 and Barron et al., 2016 for detailed discussions) suggesting that MVPA may provide a promising alternative to investigating the MN system in light of its sensitivity in decoding subtle differences between stimulus evoked activity patterns.

2. Experimental Procedures

2.1. Participants

Consistent with comparable *f*MRI RS studies (see Table 1), we included 12 healthy adults aged 18-46 years (mean age = 26.08 years, three females) in the present study. Participants were recruited via advertisements on university websites and notice boards. All participants reported being right-handed and/or showed a right-hand preference during motor function as assessed using the Bruininks-Oseretsky Test of Motor Proficiency (BOT-2, Bruininks, 2005), a well validated standardized measure of motor ability. None of the participants reported any MRI contra-indicators (as assessed by an online safety questionnaire) and/or motor difficulties that significantly impacted

their daily functioning (as assessed by the Adult Dyspraxia Checklist, Kirby & Rosenblum, 2008). The study received approval from the Deakin University (Australia) ethics committee. All participants provided written informed consent prior to participation.

2.2. MRI data acquisition

Six functional MRI (*f*MRI) scans were conducted on each participant using a Siemens Skyra 3T MRI system (Erlangen, Germany) with a 32-channel receive only head coil. Blood oxygenation level dependent (BOLD) echo-planar imaging (EPI) data were acquired of the whole brain using 44 x 3 mm contiguous slices. The data were acquired in the axial plane parallel to the AC-PC line using the following parameters: TR = 3000 ms, TE = 30 ms, flip angle = 90°, acquisition matrix 72 x 72, FoV = 216 mm, 97 whole brain EPI volumes, with an acquisition time of 5.02 minutes. For anatomical reference, T1-weighted 3D MPRAGE images were also acquired for each participant using the following parameters: TR = 1900ms, TI = 900 ms, TE = 2.49 ms, flip angle = 9º, voxel size = 0.9 mm^3, acquisition matrix 256 x 256, FoV = 240 mm, 192 contiguous slices with an acquisition time of 4.26 minutes.

2.3. *f*MRI procedure

The experimental paradigm was based on the seminal study design by Kilner et al (2009) and was programmed in Presentation software (Version 18.0, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com). Participants were required to either observe (O) or execute (E) one of two object directed actions performed by the right hand – a wrist turn or a button press (see Figure 1). These actions were chosen to selectively activate distinguishable populations of neurons (see Oosterhof, 2013) while minimizing intention inference due to the semantic context (see Lingnau et al., 2009). During the scan, participants lay supine on the scanner bed with their right hand holding

onto the button box. The wrist of the participant was slightly elevated (using a triangular prismatic cushion) to allow for execution of the wrist turn and to confine movement to the hand/wrist only (thus avoiding movement artefacts). The button box comprised two diagonally adjacent buttons (see Figure 1). Participants rested their index finger on the upper button (default position) unless prompted to execute a button press, which required them to press the lower button (using their index finger) and then and return to the default position as quickly as possible. The participant's hand was positioned so that the movement took place outside their field of view. Stimuli were shown on a projection screen situated behind the participant's head and reflected via a mirror mounted on the head coil.

On each trial, participants were sequentially presented with pairs of stimuli. These pairs consisted of either two observation conditions (OO), two execution conditions (EE), an observation condition followed by an execution condition (OE), or an execution condition followed by an observation condition (EO). During the observation condition, participants watched a video of one of the actions (i.e., either the wrist turn or the button press), which lasted ~750 ms. During the execution condition, participants were required to execute an action according to a word presented on the screen. This imperative cue was presented for ~750 ms and either read 'Turn', indicating the wrist turn, or 'Press', indicating the button press (see Figure 1). Prior to the scan, participants completed a practice session outside the scanner that trained them to execute the relevant action as quickly as possible following the imperative cue. Participants were also familiarized with the videos used during the observation condition.

We included eight different trial types: observe – observe same action (defined by whether it was a wrist turn or button press; OO_{same}), observe – observe different ($OO_{different}$), execute – execute same (EE_{same}), execute – execute different ($EE_{different}$), observe – execute same (OE_{same}),

observe – execute different ($OE_{different}$), execute – observe same (EO_{same}) and execute – observe different ($EO_{different}$). Within each trial, there was a 500 ms gap between the first and second stimulus. Trials were separated by a jittered wait period of 5000-5500 ms, during which participants were presented with a fixation cross. Participants performed six runs lasting 4.80 min each. Each session consisted of 32 trials with each of the eight trial types presented four times. To control for the potential cofounding effect of expectation suppression (see Barron et al., 2016), trials were randomized within each block. For the execution conditions, we ensured that participants accurately performed the button press by monitoring the signal of the response box (resulting from the button press) and the wrist turn by visual inspection from outside the scanner room respectively. The latter method was also used to confirm that participants did not perform mirror movements with their left (i.e., non-dominant) hand during the scan. Participants accurately observed and executed all conditions during the experiment with the exception of two participants, who incorrectly executed an action during the first observation trial. For these two participants, the relevant block was discontinued and repeated at the end of the experiment.

MR data were pre-processed and analysed in SPM12 (www.fil.ion.ucl.ac.uk/spm/software/spm12/), using the default parameters unless otherwise stated. For each individual, EPIs were realigned and a mean image was generated. In-scanner motion was checked for each participant with all participants displaying minimal translation (x_{mean} = 0.08 mm, x_{range} = 0.04 – 0.16 mm, y_{mean} = 0.10 mm, y_{range} = 0.04 – 0.17 mm, z_{mean} = 0.25 mm, z_{range} = 0.11 – 0.45 mm). The structural image was first co-registered to the mean realigned EPI. Next, the co-registered structural image was normalised to a standard SPM12 T1 template (avg305T1.nii) in MNI space. The parameters of this transformation were then applied to the realigned EPIs, which were subsequently smoothed using an 8 mm full width at half maximum (FWHM) Gaussian kernel. This kernel was chosen to ameliorate differences in intersubject localization (for group level analysis)

while reducing noise at the individual participant level (Poldrack et al., 2008; Woo et al., 2014). Using the General Linear Model (GLM), we created voxel wise t-maps for each participant. Eight boxcar regressors, convolved with the canonical hemodynamic response function (HRF) provided within SPM12, identified the onset and duration of the second within-trial stimulus within each trial. A high-pass filter (128 s) was applied to control for low frequency noise (see Fitzgibbon et al., 2016 for comparable pre-processing/model specification parameters involving MR repetition suppression data). Finally, condition specific estimates of BOLD activity, corresponding to the amplitude of the response to the second within-trial stimulus, were computed at each voxel for each participant.

2.4. Data analysis

2.4.1. Univariate analysis

Data were analysed using a random effects design in SPM12. For each participant, contrasts were defined at the first level (using SPM12's implicit baseline). The contrasts of interest computed at the first level were the effect for each experimental condition (OO, EE, EO, OE combinations for both same and different action pairs), the effect for uni-modal RS ($OO_{same} – OO_{different} + EE_{same} – EE_{different}$), the effect for cross-modal RS ($EO_{same} – EO_{different} + OE_{same} – OE_{different}$), the effect for uni-modal repetition facilitation ($OO_{different} – OO_{same} + EE_{different} – EE_{same}$) and the effect for cross-modal repetition facilitation ($EO_{different} – EO_{same} + OE_{different} – OE_{same}$). At the second level, we specified a 2 x 2 x 2 repeated measures ANOVA, allowing for dependencies between levels of each factor. The factors were trial type (uni-modal or cross-modal), first event modality (observe or execute) and congruence (same or different). Based on this design, we performed a series of conjunction (null) analyses to test the criteria for MN activity (see Table 2 for an overview of the contrasts used to test each criterion). To ensure comparability of our analysis with previous work, we controlled for multiple comparisons at the whole brain level using a cluster threshold of $p < .05$ (FDR corrected, Lingnau et al., 2009), after thresholding the images at an uncorrected threshold of $p < .005$ (Press et al., 2012). Reported

p-values for peak voxel response are based on one-tailed *t*-tests (as calculated by SPM). As per Kilner at al. (2009), we further tested our criteria for MN activity at the individual level by analysing the peak voxel response for each participant separately.

Criterion 1 – overlap between action observation and execution We tested for regions that showed an increased BOLD response during both action observation and execution. To this end, we performed a conjunction analysis designed to test for common areas of increased BOLD signal across observation ($EO_{different}$, $OO_{different}$) and execution conditions ($OE_{different}$, $EE_{different}$). To control for possible repetition suppression effects, this analysis only included conditions where the action of the second within-trial stimulus was different to the action of the first within-trial stimulus.

Criterion 2 – uni-modal RS To identify regions that a) were active during both action observation and execution and b) showed uni-modal repetition suppression (uni-modal RS), we performed a conjunction analysis testing for uni-modal RS (using the first-level contrast specified above) in areas that responded (i.e., areas that showed an increased BOLD response) during action observation ($EO_{different}$) and action execution ($OE_{different}$). To ensure that this analysis was based on a fully balanced orthogonal design (as prescribed by Kriegeskorte et al., 2009), we defined action observation as the response to $EO_{different}$ (rather than $EO_{different}$ + $OO_{different}$) and action execution as the response to $OE_{different}$ (rather than $OE_{different}$ + $EE_{different}$). In support of the validity of this approach, analysis showed that the first event prime did not modulate the RS effect (as indicated by a non-significant interaction between first event prime and congruency – see results section for details). As per Press et al. (2012), we further tested for uni-modal repetition facilitation (uni-modal RF) at a group level using the same conjunction analysis as above but replacing the contrast for uni-modal RS with the contrast for uni-modal RF.

Criterion 3 – cross-modal RS Finally, to test for regions that a) were active during both action observation and execution and b) showed cross-modal repetition suppression (cross-modal RS), we performed a conjunction analysis testing for cross-modal RS (using the first-level contrast specified above) in areas that responded during action observation ($OO_{different}$) and action execution ($EE_{different}$). To ensure that this analysis was based on a fully balanced orthogonal design (see above), we defined action observation as the neural response to $OO_{different}$ and action execution as the response to $EE_{different}$. These contrasts were consequently used in the conjunction analysis. Again, we tested for uni-modal repetition facilitation (uni-modal RF) at a group level using the same conjunction analysis as above but replacing the contrast for uni-modal RS with the contrast for uni-modal RF.

2.4.2. Multivariate pattern analysis

In light of recent evidence that multivariate techniques may provide a more sensitive technique for studying the cross-modality of neural representation such as that expected of MN activity (see Oosterhof et al., 2013 and Barron et al., 2016 for detailed discussions), we further adopted multivariate pattern analysis (MVPA) to challenge the aforementioned criteria for MN activity. MVPA aims to quantify the similarity of response patterns evoked across groups of voxels by specific actions (Haxby et al., 2001; Norman et al., 2006). To test for cross-modality, MVPA identifies areas where the same action produces more similar patterns (across action observation and execution) than different actions would be expected to produce (Oosterhof et al., 2013). Thus, to ensure direct comparability of our univariate and multivariate analyses, we adopted MVPA to test for spatially extended groups of voxels that allowed us to decode RS trials (i.e., trials where the action of the first within-trial stimulus was the same as the action of the second within-trial stimulus) from non-RS trials (i.e., trials where the action of the first within-trial stimulus was different to the action of the second within-trial stimulus). As per the criteria for MN activity,

analysis was thus designed to identify regions that showed both uni-modal (C2) and cross-modal (C3) RS in regions that were active during action observation/execution (C1). In support of this approach, predictive coding theory (Friston, 2005) offers a plausible mechanistic framework that can be used to illustrate that RS trials may produce distinct multivoxel patterns compared to non-RS trials. Specifically, while non-RS trials elicit larger BOLD responses to unpredicted events (possibly reflecting signal increase in areas encoding prediction error), both signal attenuation (RS) and signal enhancement (RE) can co-occur on RS trials in the same region (for a more detailed discussion see Baron et al., 2016 and Stefanics et al., 2018). To this end, we reasoned that MVPA would be well placed to decode these differential patterns of co-occurring RS/RE on RS trials from larger responses on non-RS trials.

We performed MVPA in CoSMoMVPA (v. 1.1, Oosterhof et al., 2016) using a linear support vector machine (SVM) classifier as implemented by MATLAB (vR2015b). For each condition (OO, EE, EO, OE combinations for both same and different action pairs) and participant, we estimated the ß-weights (condition vs implicit baseline) from each of the six runs separately and used the corresponding *t*-values as input for the classifier. Estimation was based on the same pre-processing/design specification parameters as in the univariate analysis (note that the number of trials per condition was fully balanced for each run). The classifier was trained to discriminate RS trials (i.e., trials where the action of the first within-trial stimulus was the same as the action of the second within-trial stimulus) from non-RS trials (i.e., trials where the action of the first within-trial stimulus was different to the action of the second within-trial stimulus).

For uni-modal trials, we trained the classifier to discriminate between OO_{same} and $OO_{different}$ (uni-modal observation, OO) and between EE_{same} and $EE_{different}$ (uni-modal execution, EE). For cross-modal trials, we trained the classifier to discriminate between EO_{same} and $EO_{different}$ (cross-modal

observation, EO) and between OE_{same} and $OE_{different}$ (cross-modal execution, OE). Classification accuracies were computed using *n*-fold cross validation (see Oosterhof et al., 2016). In brief, data from a single run was used as the test set with data from all other runs used for the train set. These steps were repeated for each of the six runs. That is, for the *i*-th repetition, data from the *i*-th run was used for testing after training on data from all other runs. This resulted in a prediction for each sample in the data set. Classification accuracies were then computed by dividing the number of correct predictions by the total number of predictions.

Searchlight based MVPA To identify regions with significant decoding results for either uni-modal (C2) or cross-modal (C3) RS, we performed a searchlight analysis with cross-validation as described above. To this end, we defined a spherical neighbourhood with a constant number of 100 voxels around each voxel and used an *n*-fold partitioner for cross-validation. To restrict this searchlight to regions that responded during both action observation and execution (C1), and to ensure comparability between the multivariate and univariate analyses in testing the criteria for MN activity, we used the group-level convergence mask from the univariate analysis. The searchlight analysis was conducted at the individual level for each participant across each of the uni-modal (OO_{same}/$OO_{different}$ and EE_{same}/$EE_{different}$) and cross-modal conditions (EO_{same}/$EO_{different}$ and OE_{same}/$OE_{different}$). The resulting accuracy maps were then averaged across participants to produce separate group-level accuracy maps for each condition (OO, EE, EO, and OE). Finally, we tested for clusters where decoding accuracy was significantly above chance using the threshold-free cluster enhancement (TFCE) approach (Smith & Nichols, 2009). We reasoned that any surviving clusters would define regions that responded during action observation/execution (C1) with significant decoding for either uni-modal RS (C2) or cross-modal RS (C3), thus providing a direct test of the criteria for MN activity.

ROI based MVPA While our searchlight analysis provided a direct test of the criteria for MN activity, we sought to explore the spatial specificity of regions that showed both uni-modal and cross-modal RS but did not survive cluster level correction. We reasoned that this approach would provide maximum sensitivity for identifying regions that held MN specific response patterns, even if effects were subtle. To this end, we averaged the group-level accuracy maps across the uni-modal (OO, EE) and cross-modal (EO, OE) conditions from the searchlight analysis. The resulting conjunction map was then inspected for extended groups of voxels that showed above chance classification. ROIs were then defined separately for each participant on the basis of their accuracy maps. For each participant, we averaged the accuracy maps across the uni-modal and cross-modal conditions (as per the group level analysis) and defined ROIs as spheres (12 mm radius) around the cortical voxel with peak decoding accuracy, located within a sphere of 12 mm radius (257 voxels) centred around the group peak vertex. Importantly, this analysis was deliberately circular (Etzel et al., 2013; Kriegeskorte et al., 2009) to provide maximum sensitivity for demonstrating the spatial specificity of informative regions that met all three criteria for MN activity, should MN activity be present. We reasoned that this approach was justified given that the results of a searchlight analysis are influenced by parameters and choices specific to the searchlight analysis (e.g., the searchlight radius). That is, it is possible that a searchlight analysis may fail to detect informative areas (clusters) at the whole brain level, or within a ROI, despite significant classification across the whole ROI (see Etzel et al., 2013).

For each participant, classification accuracies were calculated separately for each of the uni-modal (OO_{same}/$OO_{different}$ and EE_{same}/$EE_{different}$) and cross-modal (EO_{same}/$EO_{different}$ and OE_{same}/$OE_{different}$) conditions based on all six (fully balanced) runs. As per our searchlight analysis, we estimated the ß-weights for each condition (OO, EE, EO, OE combinations for both same and different action pairs) from each of the six runs separately using the same pre-processing/model specification parameters

as in the univariate analysis. The corresponding *t*-values were then used as input for the classifier and classification accuracies were computed using *n*-fold cross validation. To test whether decoding accuracies were significantly above chance, we entered the mean classification accuracy for each ROI, condition and participant into a one sample (one-tailed, FDR corrected) *t*-test against the classification accuracy expected by chance (50%).

3. Results

3.1. Univariate analysis

Retrospective power analysis using *NeuroPower* (Dumez et al., 2016; using a screening threshold of 0.05 and an alpha level of 0.005, as per our univariate analysis) demonstrated an average of 83% power across our conditions (OO, EE, EO, OE combinations for both same and different action pairs), suggesting that our design was reasonably powered for detecting BOLD signal changes (compared to baseline) during the action observation/execution conditions. Results of the repeated measures ANOVA revealed no significant three-way interaction and no significant two-way interaction between first event modality and congruency at either the cluster or peak voxel level. In other words, the modality of the first event prime did not modulate the uni-modal RS effect, nor did it modulate the cross-modal RS effect. Consistent with this finding, follow up analysis revealed no significant clusters of differential BOLD amplitude between $EO_{different}$ and $OO_{different}$ (i.e., the modality of the first event prime did not modulate the BOLD amplitude during action observation) and between $OE_{different}$ and $EE_{different}$ (i.e., the modality of the first event prime did not modulate the BOLD amplitude during action execution). Accordingly, we could be confident that our conjunction analyses testing for uni-modal RS and cross-modal RS included comparable effects for action observation (i.e., $EO_{different}$ can be considered comparable to $OO_{different}$) and action execution (i.e., $OE_{different}$ can be considered comparable to $EE_{different}$). These results support the validity of our analyses.

Criterion 1 – overlap between action observation and execution The conjunction analysis testing for common areas of increased BOLD response during both action observation and action execution revealed bilateral (sub)cortical signal increase in visual and/or motor areas. These included the IFG, the middle/superior frontal gyri, the pre/postcentral gyri, the inferior and posterior areas of the parietal lobe, the occipital lobe (V1), and both the exterior part and vermal lobules of the cerebellum (as shown in Figure 2). At the individual level, we observed common areas of increased BOLD response across the action observation and execution conditions in all participants ($p < .005$ uncorrected at the peak voxel level). Cluster level correction suggested common areas of increased BOLD response in 11 out of 12 participants ($p < .05$ FDR corrected). Taken together, our paradigm thus led to reliable BOLD signal changes in traditional motor areas, thereby justifying further analysis testing for uni-modal RS and/or cross-modal RS within these regions.

Criterion 2 – uni-modal RS The conjunction analysis testing for uni-modal RS in areas that responded during both action observation and action execution revealed significant uni-modal RS in a small group of voxels located in the right SMA (peak voxel coordinates [6, 11, 53], $t = 2.85$, $p = .003$ uncorrected, $k = 4$, $p_{cluster} = .847$ FDR corrected). This region did not survive cluster level correction (see Figure 2). However, when using a more liberal threshold ($p < .05$ uncorrected at both peak voxel and cluster level) to show the spatial specificity of this effect (see Kilner et al., 2009 for a comparable approach), analysis revealed two clusters. At a cortical level, these clusters were located within the right SMA (peak voxel coordinates [6, 11, 53], $t = 2.85$, $p = .003$ uncorrected, $k = 947$, $p_{cluster} = .004$ uncorrected) and the left middle frontal gyrus (peak voxel coordinates [-24, 38, 26], $t = 2.72$, $p = .004$ uncorrected, $k = 457$, $p_{cluster} = .031$ uncorrected) respectively. Figure 3 shows a visual representation of the results using this liberal threshold.

While uni-modal RS occurred during both action observation and execution trials (see Figure 3), follow-up analysis suggested that these effects were largely driven by RS effects during the observation condition (see supporting information). Specifically, when running separate conjunction analyses for the observation and execution conditions, we observed RS effects in the right SMA that were more pronounced during observation (OO) trials, although the peak voxel was now located more laterally in the surrounding white matter areas (see supporting information). Still, these analyses must be considered exploratory in light of the reduced trial numbers and the liberal significance thresholds applied to show the spatial specificity of these effects. At the individual level, six out of 12 participants showed significant uni-modal RS ($p < .005$ uncorrected at the peak voxel level, see Table 3). Our analysis did not reveal any uni-modal RF effects at either the peak voxel level or cluster level.

Criterion 3 – cross-modal RS The conjunction analysis testing for cross-modal RS in areas that responded during both action observation and execution did not reveal any informative regions at either the peak voxel or cluster level (see Figure 2). Similarly, when running separate conjunction analyses for the observation and execution conditions, we did not observe cross-modal RS effects. At the individual level, six out of 12 participants showed significant cross-modal RS ($p < .005$ uncorrected at the peak voxel level, see Table 3). Our analysis did not reveal any cross-modal RF effects at either the cluster or peak voxel level.

3.2. Multivariate pattern analysis

Searchlight based MVPA At a descriptive level, the searchlight analysis revealed above chance decoding results for both uni-modal (OO, EE) and cross-modal (EO, OE) decoding (see Figure 4). However, none of these regions survived correction for multiple comparisons. That is, our

searchlight analysis did not provide evidence for significant uni-modal or cross-modal decoding results when correcting for multiple comparisons across areas that were active during action observation/execution.

ROI based MVPA The conjunction map of the uni-modal (OO, EE) and cross-modal (EO, OE) conditions (see Figures 4, 5) revealed above chance decoding accuracy for a region within the right IPL (peak voxel coordinates [36, -49, 38], classification accuracy = 57%). This region was situated along the middle of the anterior arm of the intraparietal sulcus (aIPS). No other regions were identified where classification was consistently above chance. Within this inferior parietal ROI, univariate analysis revealed significant BOLD signal increases for each of the observation ($OO_{different}$, $EO_{different}$) and execution ($EE_{different}$, $OE_{different}$) conditions ($p \leq 0.001$ for each condition), suggesting that this region responded during observation/execution. We obtained significant decoding results for both uni-modal observation (OO), $t(11) = 2.25$, $p_{FDR} = .044$ and cross-modal observation (EO), $t(11) = 2.68$, $p_{FDR} = .044$. No significant decoding results were obtained for either uni-modal (EE), $t(11) = 0.78$, $p_{FDR} = .228$ and/or cross-modal (OE), $t(11) = 1.99$, $p_{FDR} = .051$ execution within this ROI. Decoding accuracies are presented in Figure 5.

4. Discussion

Repetition suppression paradigms have been considered a "gold standard" non-invasive method for measuring MN activity in the human brain. However, evidence has been mixed, in part due to critical differences in the application of the criteria necessary to infer MN activity. This has limited the degree to which the presence or absence of MN activity can reliably be inferred. To this end, we rigorously applied the previously defined criteria necessary to demonstrate MN activity against a well-validated *f*MRI RS paradigm and tested for regions with mirror properties using a

previously adopted univariate approach, and then subjected our criteria to a novel multivariate approach (MVPA). Univariate whole brain analysis revealed shared spatial convergence during observed and executed action and uni-modal RS within the SMA. However, no regions showed evidence of cross-modal RS, or both uni-modal RS and cross-modal RS, which would have been necessary to indicate MN activity.

In other words, when rigorously applying the necessary criteria for inferring MN activity, we failed to find evidence for regions with mirror properties in the human brain, using traditional univariate analysis. In contrast, MVPA revealed a region along the aIPS that met all three criteria for MN activity, suggesting that MVPA may provide a more sensitive technique for analysing RS data than univariate approaches. In the context of previously mixed findings, we argue that these findings question the sensitivity of RS paradigms of the type that have been adopted here, and in seminal works, for reliably detecting MN activity when rigorously applying the requisite criteria in conjunction with univariate analysis. Short of increasing sample sizes substantially, we recommend that future studies using RS paradigms to investigate MNs consider the use of MVPA.

4.1. Overlap between action observation and execution

As expected, our analysis testing for common areas of increased BOLD signal during both action observation and action execution revealed both contralateral and ipsilateral motor areas, including IFG, middle/superior frontal gyri, pre/postcentral gyri, inferior and posterior areas of the parietal lobe, as well as the cerebellum. We further observed overlap in visual areas, including the primary visual cortex. These findings support the well documented bilateral overlap in the neural response during both action observation and execution (Caspers et al., 2010; Gazzola & Keysers, 2009; Molenberghs et al., 2012). Importantly, given that these effects were obtained at both the

group level and at the individual level, we can be confident that the experimental design led to reliable BOLD signal increases in key visuomotor areas, which was a prerequisite for subsequent analyses testing for uni-modal RS and/or cross-modal RS within these regions.

4.2. Univariate analysis

4.2.1. Possible evidence for uni-modal RS

At a group level, our conjunction analysis testing for uni-modal RS effects in areas that responded (i.e., areas that showed an increased BOLD response) during both action observation/execution revealed a small cluster of voxels in the right SMA (BA 6). These voxels showed an attenuated BOLD response (at the peak voxel level) when the same action was repeatedly observed and/or executed. While the spatial location of this adaptation effect is consistent with previous *f*MRI RS studies showing RS effects in the SMA (e.g., Hamilton & Grafton, 2009), we must be circumspect in drawing inferences from this finding in light of the small cluster size, which as expected did not survive cluster level correction. Still, when using a more liberal significance threshold to show the spatial specificity of this effect (as per Kilner at al., 2009), our analysis revealed two clusters, located within the right SMA (BA 6) and the left middle frontal gyrus (MFG, BA 9). This analysis must be considered exploratory, however, in light of the liberal significance threshold used.

Broadly, these results are compatible with recordings from single neurons in neurosurgical patients (Mukamel et al., 2010), suggesting that neurons with mirror properties may exist outside classical mirror neuron regions (see also Keysers et al., 2010). At the same time, however, results from our whole brain analysis appear anatomically distinct from previously reported ROI based uni-modal RS effects in BA 6, which were found more laterally at the border of BA 6 and BA 44 (see Press

et al., 2012). However, in light of the spatial constraints imposed by ROI analysis and given that Press and colleagues did not apply the criteria for inferring MN activity as per the present study, it is difficult to compare the spatial distribution of these effects directly. Similar to Kilner and colleagues (2009), the uni-modal RS effect observed in this study was not modulated by the first event prime (even when using the more liberal threshold described above). In light of this non-significant interaction between first event prime and congruency, we can be confident that the observed attenuation in BOLD signal held constant, regardless of whether the first within-trial stimulus required participants to observe or execute an action. Finally, while uni-modal RS occurred during both action observation and execution trials, further analysis suggested that RS effects were largely driven by RS effects during the observation condition. This is largely consistent with previously reported uni-modal RS effects in posterior regions of the IFG (see Kilner et al., 2009; Press at al., 2012).

Taken together, our results highlight possible areas within the right SMA (and potentially the MFG) that responded during both action observation/execution and showed uni-modal RS. To comprehensively test for putative mirror neuron activity as per our criteria, we subsequently tested for cross modal RS effects at a whole brain level. As noted, only neurons that are active during both action observation/execution and show both uni-modal RS and cross-modal RS are considered to fulfil the necessary criteria for mirror neuron activity.

4.2.2. Insufficient evidence for cross-modal RS

At a group level, our conjunction analysis testing for cross-modal RS in the same areas that were active during both action observation and execution did not reveal significant effects at either the peak voxel or cluster level (even when a more liberal threshold was chosen as per our analysis

testing for uni-modal RS). That is, we did not find evidence for cross-modal RS anywhere in the brain, let alone in a region that was active during action observation/execution and showed uni-modal RS as would be necessary to reliably infer MN activity. Further, while six participants demonstrated significant cross-modal RS at the individual level, only four of these participants also showed uni-modal RS and no participant showed both uni-modal RS and cross-modal RS in the same region. Thus, our work did not demonstrate any regions that met the criteria necessary for inferring the existence of regions with mirror properties at the whole brain level.

At first, these results appear inconsistent with previous *f*MRI RS paradigms that reported mirror neuron activity within BA 6 and the IFG (Chong et al, 2008; de la Rosa et al., 2016; Kilner et al., 2009; Press et al., 2012). However, while each of these studies partially applied the key criterion necessary for inferring MN activity, none did so for all criteria at either the whole brain or region of interest level (see Table 1). As noted, this places substantial limitations on the ability to infer MN activity, making it difficult to compare earlier findings against the spatial distribution of regions with mirror properties (or lack thereof) reported here. In light of the methodologically and analytically rigorous application of previously pre-defined criteria for inferring MN properties used in this study, we thus argue that our findings have been instrumental in clarifying disparate evidence from earlier *f*MRI RS studies.

Similar to the present study, Lingnau and colleagues (2009) did not find evidence for the existence of mirror neurons across a comprehensive set of visual and motor ROIs. This study also applied the MN criteria as outline here (see Table 1) but used intransitive motor actions. Thus, our null findings add to this body of literature by showing that *f*MRI RS paradigms of the kind adopted here, and in earlier seminal work using comparable sample sizes (see Table 1), may not be sensitive for detecting MN regions when a univariate approach to analysis is taken and the requisite criteria

rigorously applied. This appears to be the case for both intransitive/pantomime actions (Dinstein et al., 2007; Lingnau et al., 2009) and object/goal-directed actions (as per the present study). Accordingly, we tested our RS data against the same criteria for detecting MN activity using MVPA.

4.3. Multivariate pattern analysis

Our searchlight analysis did not provide evidence for significant uni-modal or cross-modal decoding results when correcting for multiple comparisons across areas that were active during action observation/execution. However, visual inspection of the activation maps revealed a group of voxels along the right anterior intraparietal sulcus aIPS where classification accuracy was consistently above chance for both uni-modal (OO, EE) and cross-modal (EO, OE) decoding. Within this ROI, we obtained significant, yet modest (as noted decoding accuracy was only 7% above chance level), decoding results. That is, we were able to successfully decode RS trials from non-RS trials for both uni-modal (observe – observe) and cross-modal (execute – observe) observation. Thus, our analysis identified a region along the right aIPS that met all three criteria for MN activity: First, this region was active during action observation/execution (C1). Second, RS trials (i.e., trials where the action of the first within-trial stimulus was the same as the action of the second within-trial stimulus) produced patterns both within (C2) and across modalities (C3) that were distinct from the corresponding patterns produced by non-RS trials (i.e., trials where the action of the first within-trial stimulus was different to the action of the second within-trial stimulus).

Our finding that areas along the aIPS display MN specific activity patterns is largely consistent with previous MVPA research demonstrating that the aIPS show cross-modal activity patterns for transitive actions (Oosterhof et al., 2010). Further, our results extend previous work demonstrating uni-modal but not cross-modal decoding in the aIPS for intransitive actions (Dinstein

et al., 2008). Using MVPA, Dinstein et al. (2008) showed that activity patterns in the aIPS could discriminate between intransitive actions during action observation (i.e., the authors demonstrated within-modality decoding for pantomime actions during a rock-paper scissors game) but that this finding did not generalize to the cross-modal case. Thus, based on our findings, it appears that MN specific activity patterns in the aIPS may only occur when participants observe transitive/goal-directed actions. Finally, it is noteworthy that our ROI analysis highlighted significant decoding results for action observation but not for action execution. However, given that decoding results for the cross-modal execution condition approaching significance, and given the exploratory nature of our ROI analysis, we must be cautious in the interpretation of this result.

Still, while our MVPA results provide preliminary evidence for MN specific activity patterns along the aIPS, no significant uni-modal or cross-modal decoding results were obtained when correcting for multiple comparisons. A possible explanation for this finding might have been our choice of design for adopting MVPA. Indeed, previous studies using MVPA to infer MN activity typically tested for brain regions that responded with reproducible movement-selective response patterns during action observation and/or execution (Dinstein et al., 2008; Oosterhof et al., 2010). Thus, the classifier was trained to discriminate between different actions on responses to observed movements (i.e., uni-modal decoding) and then tested on responses to executed movements (i.e., cross-modal decoding) and vice versa. Successful decoding was then taken as evidence for the existence of groups of neurons that show both action specificity and cross-modality, two key traits of the MN system (Oosterhof et al., 2013).

However, our primary aim was to test the sensitivity of RS paradigms for detecting MN activity using the seminal design and univariate approach that has been most commonly adopted over the past decade by testing our RS data against those three criteria that are necessary for

inferring MN activity. A secondary aim was to determine whether MVPA analysis of this design may offer a more sensitive alternative to univariate analysis for detecting MN activity. Hence, to ensure direct comparability of our univariate and multivariate analyses in testing for RS effects, we used MVPA to identify regions that were active during action observation/execution and successfully discriminated RS trials from non-RS trials (both within and across modalities). That is, consistent with the criteria for inferring MN activity, we used MVPA to identify regions that showed a distinct neural response pattern on uni-modal (C2) and cross-modal (C3) RS trials and were active during action observation/execution (C1). Still, while our design/analysis was well placed to address the aim of the study, a valuable avenue for future research would be to adopt alternative MVPA designs for investigating the MN system (see Oosterhof et al., 2010).

4.4. Implications and future directions

This has been the first study to adopt the criteria for inferring MN activity to a popular RS paradigm and subsequently test for MN activity using both univariate and multivariate techniques. Given the lack of univariate effects reported here, it is possible that the RS paradigm adopted in our study (and in recent seminal RS studies aimed at investigating MN activity in humans), may not be sensitive for reliably detecting MN activity when rigorously applying the criteria for MN activity in conjunction with traditional univariate analysis. While one might argue that our design did not have sufficient power to detect cross modal RS effects (cross-modal RS), this seems unlikely given that a) we demonstrated the expected within modality RS effects (uni-modal RS) characteristic of RS; and b) even when using liberal significance thresholds, no cross-modal RS effects were found for either observed or executed movements. A more plausible explanation for the present findings is that traditional univariate analysis of RS paradigms may lack the necessary sensitivity for demonstrating RS effects consistent with MN properties (see also Kilner & Lemon, 2013) when rigorously applying

the criteria for MN activity, at least when modest sample sizes such as those adopted in our study and in comparable seminal works are involved (see Table 1).

The idea that techniques other than univariate RS may be better placed to study the action specificity of neural representation is not new, with several papers arguing that there may be mechanistic explanations (particularly with respect to the physiological basis of the RS mechanism) as to why mirror neurons do not show cross-modal RS effects that can reliably be detected using univariate analysis (Rizzolatti & Fabbri-Destro, 2010; Rizzolatti et al., 2014). Indeed, evidence from macaques suggests that mirror neurons in monkey area F5 may not necessarily attenuate their firing rate to the observation of repeated actions (e.g., Caggiano et al., 2013 and Kilner et al., 2013). Further, even when intra-modal-adaption occurs (as measured using local field potentials), it is unclear whether the attenuated spiking activity of neurons in a given area is necessarily related to *f*MRI adaption of the BOLD signal (for a more detailed discussion see Caggiano et al., 2013 and Kilner et al., 2013).

In response to these criticisms, recent reviews have advocated for the use of MVPA (see Oosterhof et al., 2013 and Barron et al., 2016 for a detailed discussion), prompting the exploratory analysis adopted here. MVPA is considered a promising alternative to investigating the MN system in light of its sensitivity in decoding subtle differences between stimulus evoked activity patterns (see Oosterhof et al., 2013 and Barron et al., 2016 for a detailed discussion). As outlined, ROI based MVPA on our RS data revealed a region along the aIPS that met all three criteria necessary for demonstrating MN activity. While this region did not survive correction for multiple comparisons, we argue that our findings nevertheless highlight that MVPA may provide a more sensitive technique for analysing RS data than traditional univariate approaches and may therefore hold advantages for

studying the human MN system. In light of these findings, we recommend that future work consider the use of MVPA for investigating the spatial distribution and properties of the MN system.

4.5. Conclusion

We rigorously applied the criteria necessary to demonstrate MN activity using a popular *f*MRI RS paradigm and tested for regions with mirror properties using both univariate and multivariate techniques. While traditional univariate analysis highlighted uni-modal RS effects within the SMA, no evidence for cross-modal RS effects consistent with mirror neuron activity was found anywhere in the brain. MVPA, on the other hand, revealed a region along the aIPS that met all three criteria for MN activity. Taken together, these results help to clarify disparate evidence from earlier RS studies, highlighting that *f*MRI RS paradigms of the kind previously adopted may not provide a reliable measure for detecting mirror neuron activity at a whole brain level when applying a univariate approach to analysis. Instead, MVPA may provide a more sensitive technique for analysing RS data and for studying the human MN system.

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Conflict of Interest

The authors declare no competing financial interests.

Author Contributions

IF, CH and PE designed the research. IF, CH, PE and MK developed the stimuli used in this study. IF, CH and SF performed the data acquisition and the neuropsychological testing of participants. IF, CH and KC performed the data analysis. IF and CH wrote the manuscript. All authors were involved in editing the manuscript.

Data Accessibility

Data are available via the Deakin University Research Repository (DRO) and/or may be requested from the corresponding author.

Abbreviations

aIPS	Anterior intraparietal sulcus
BOLD	Blood oxygen level-dependent
BOT-2	Bruininks-Oseretsky Test of Motor Proficiency
BA	Brodmann area
EPI	Echo planar imaging
FDR	False discovery rate
FWHM	Full width half maximum
FoV	Field of view
fMRI	functional magnetic resonance imaging

GLM	General linear model
HRF	Hemodynamic response function
IFG	Inferior frontal gyrus
IPL	Inferior parietal lobe
MN	Mirror neuron
MNI	Montréal Neurological Institute
MVPA	Multivariate pattern analysis
ROI	Region of interest
RS	Repetition suppression
SMA	Supplementary motor area
SVM	Support vector machine
umRS	Uni-modal repetition suppression
xmRS	Cross-modal repetition suppression

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Figure captions

Fig. 1 *f*MRI RS paradigm. Each trial consisted of a pair of stimuli presented one after the other. The pairs either contained two execution conditions, two observation conditions or one execution and one observation condition. The four trials shown are cross-modal trials. In the execution condition, a word indicated whether a button press or a wrist turn should be executed. For the button press, the participant moved their index finger from the default position (i.e., upper button, see top left trial shown in this figure) and pressed the lower button before returning to the default position. The four trials shown are examples of the following trial types: observe – execute same (OE_{same}), execute – observe different ($EO_{different}$), execute – observe same (EO_{same}), observe – execute different ($OE_{different}$)

Fig. 2 Voxels showing significant activation as per our conjunction analyses. Seven equidistant axial slices (MNI z-coordinates are shown at the top) were taken for each conjunction analysis. Color bars indicate t-values. (C1) Results of the conjunction analysis observation > baseline and execution > baseline; (C2) results of the conjunction analysis for uni-modal repetition suppression (umRS); (C3) results of the conjunction analysis for cross-modal repetition suppression (xmRS)

Fig. 3 Uni-modal repetition suppression at $p < .05$ (uncorrected). Box plots show the difference in beta values between the repetition of the same or different action across the clusters observed in the right supplementary motor area (top) and the left middle fontal gyrus (bottom). Crossbars indicate the location of cortical peak voxel activation for each cluster. The MNI z-coordinate for the peak voxel is shown in the bottom right corner. Negative values are consistent with repetition suppression

Fig. 4 Mean accuracy maps of the searchlight analysis for each condition (OO, EE, EO and OE). Individual maps were averaged and projected onto a common group template in MNI space. Seven equidistant axial slices (MNI *z*-coordinates are shown at the top) were taken for each condition. The conjunction map was obtained by averaging the accuracy maps from the different conditions. Decoding accuracy at chance is 50%

Fig. 5 ROI MVPA results. ROI classification accuracies for decoding at each condition (OO, EE, EO, OE). Dotted line represents chance decoding accuracy (50%). The axial slice on the right shows the location of the ROI in the inferior parietal cortex. Colours indicate the accuracy values from the conjunction map (see Figure 4). Crossbars indicate the centre of the ROI. The MNI z-coordinate for the centre is shown in the bottom right corner

Table 1.

Chronological overview of *f*MRI adaption studies testing for MN activity.

					Criterion		
					1	2	3
					Obs > Base Exe > Base	Obs > Base Exe > Base umRS	Obs > Base Exe > Base xmRS
	N	Age	Actions	Analysis			
Dinstein et al. (2007)	13	21 – 35 NH	Intransitive, pantomime	ROI	✗*	✓	✓
Chong et al. (2008)	17	18 – 35 RH, NH	Intransitive, pantomime	ROI and whole brain	✗	✗	✓
Lingnau et al. (2009)	12	22 – 37 RH, NH	Intransitive, pantomime	ROI	✓	✓	✓
Kilner et al. (2009)	10	25 – 45 RH, NH	Goal/object directed	ROI	✓	✓	✗
Press et al. (2012)	14	24 – 45 RH, NH	Goal/object directed	ROI and whole brain	✓	✗	✗
de la Rosa et al. (2016)	10	21 – 30 RH, NH	Goal/object directed	Whole brain	✓	✗	✓

Note: ✓ indicates that the criterion was met. ✗ indicates that the criterion was not met. RH = right handed, NH = neurologically healthy, MN = mirror neuron, ROI = region of interest, Obs = observation condition, Exe = execution condition, Base = baseline, umRS = uni-modal repetition suppression, xmRS = cross-modal repetition suppression. *Dinstein et al. (2007) used Imitation > Obs & Exe as their baseline condition.

Table 2.

Conjunction analyses used to test the criteria for MN activity.

	Criterion		
	1	2	3
	Obs > Base	Obs > Base	Obs > Base
	Exe > Base	Exe > Base	Exe > Base
		umRS	xmRS
Contrasts	EOd + OOd	EOd	OOd
	OEd + EEd	OEd	EEd
		OOs – OOd + EEs – EEd	EOs – EOd + OEs – OEd

Note: MN = mirror neuron, Obs = observation condition, Exe = execution condition, Base = baseline, umRS = uni-modal repetition suppression, xmRS = cross-modal repetition suppression, s = same, d = different.

Table 3.

Repetition suppression same < different.

	umRS					xmRS				
	Peak MNI coordinates					Peak MNI coordinates				
Participant	*x*	*y*	*z*	*p*-value	*t*-value	*x*	*y*	*z*	*p*-value	*t*-value
1	24	-49	62	0.021	2.03	24	-46	65	0.025	1.96
2	33	-43	5	0.011	2.30	-33	17	-1	0.039	1.76
3	-15	-76	35	0.010	2.32	-18	-46	38	0.007	2.49
4	-21	-49	-4	0.012	2.27	-12	-61	-49	0.028	1.91
5	24	-49	31	**<0.001**	3.44	-54	-46	-28	0.021	2.04
6	18	-19	14	**0.001**	3.17	-9	-58	-49	0.015	2.18
7	-15	-106	5	0.005	2.55	24	-52	-7	**<0.005**	2.62
8	27	-55	23	0.011	2.29	60	-37	29	**<0.005**	2.59
9	-33	-67	8	**0.001**	3.16	-3	-73	47	**<0.001**	4.77
10	42	-52	-10	**0.002**	2.90	-42	-25	35	**0.003**	2.74
11	54	-58	-10	**<0.001**	3.33	18	-76	2	**0.003**	2.78
12	0	-79	8	**0.004**	2.70	-3	-76	-1	**0.001**	3.02

Note: MNI = Montreal Neurological Institute, umRS = uni-modal repetition suppression, xmRS = cross-modal repetition suppression.