Activation patterns during action observation are modulated by context in mirror system areas.

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Abstract

The role of the mirror system in action understanding has been widely debated. Some authors have suggested that the mirror system plays an important role in action understanding (Rizzolatti and Sinigaglia, 2010), whereas others have claimed that direct evidence to support this view is lacking (Hickok, 2009). If mirror neurons have an active role in action understanding rather than passive visuomotor transformation during action observation, they should respond differently to the observation of actions depending on the intentions of the observer. In this fMRI study, twenty participants observed identical actions under different instruction contexts. The task was either to understand the actions, identify the physical features of the actions, or passively observe the actions. A multi-voxel pattern analysis revealed unique patterns of activation in ventral premotor cortex and inferior parietal lobule across the different contexts. The results suggest that ventral premotor and inferior parietal areas respond differently to observed actions depending on the mindset of the observer. This is consistent with the view that these regions do not merely process observed actions passively, but play an active role in action understanding.

Keywords: fMRI, perception of action, mirror system, action understanding, premotor cortex, inferior parietal lobule

Introduction

According to the 'direct-matching' hypothesis, people understand actions by directly mapping the visual representation of an action to an equivalent motoric representation of the action in their own motor system. The 'mirror system' has been suggested to be the underlying network responsible for this visuomotor transformation (Rizzolatti et al., 2001). Mirror neurons fire both when an action is performed, and when a similar or identical action is passively observed (Rizzolatti and Craighero, 2004). These neurons were discovered during single-cell recordings in monkey areas F5, PFG and AIP (Gallese et al., 1996, 2002; Rizzolatti and Sinigaglia, 2010). Mirror neurons have also been described in humans (Mukamel et al., 2010), and it has been hypothesized that a similar parieto-frontal 'mirror' mechanism is present in the human brain (for recent reviews see Cattaneo and Rizzolatti, 2009; Fabbri-Destro and Rizzolatti, 2008; Iacoboni and Dapretto, 2006; Rizzolatti and Fabbri-Destro, 2010; Molenberghs et al., 2011).

Several different brain mechanisms might underpin action understanding (Brass et al., 2007; Saxe, 2005, 2006), but Rizzolatti & Sinigaglia (2010) claim that the parieto-frontal 'mirror' mechanism is the only system that allows an individual to understand the action of others 'from the inside' by allowing the observer a first-person grasp of the motor goals and intentions of other individuals. According to this view, mirror neurons in higher motor areas play an important role in the cognitive understanding of actions (Blakemore and Decety, 2001; Rizzolatti and Sinigaglia, 2010). A number of single-cell studies in monkeys have provided evidence of a role for mirror neurons in action understanding. Umilta et al. (2001) found that a subset of mirror neurons in ventral premotor cortex respond to goal-directed actions even when the final part of the action is hidden from view. The authors concluded

that the missing parts of the actions were internally generated in the monkey brain and therefore involved in action recognition. Kohler et al. (2002) found that some mirror neurons in the ventral premotor cortex not only respond to the observation and execution of an action but also to the sound of that action, supporting the view that these neurons code the goal of an action, irrespective of input modality.

In a more recent single-cell study, Umilta and colleagues (2008) trained monkeys to grasp an object with 'normal' and 'reverse' pliers. With the normal pliers monkeys closed their hand to grasp the object whereas with the reverse pliers they opened their hand to achieve the same goal. Despite the different actions involved, mirror neurons in ventral premotor cortex were active in both conditions consistent with the coding of a common goal (grasping an object). These studies suggest that mirror neurons in monkeys do not simply represent the movement characteristics of an action, but in fact encode the *goal* of that action. In humans, fMRI studies have revealed very similar activations within the mirror system in response to robotic (Gazzola et al., 2007) and tool actions (Peeters et al., 2009), suggesting that this human mirror network also codes the goal of the action rather than the physical features of the action itself.

Repetition suppression refers to the observation that repeated presentations of a sensory stimulus consistently reduce blood-oxygen-level dependent (BOLD) responses relative to presentations of a novel stimulus. In humans, fMRI repetition suppression paradigms have been used to examine activity within the human mirror system (Chong et al., 2008; Kilner et al., 2009). These studies have also provided evidence that the goals of actions are represented within the inferior parietal lobule (Hamilton and Grafton, 2006) and ventral premotor cortex (Lestou et al., 2008), two regions assumed to contain mirror neurons. Further evidence for the

role of the mirror system in action understanding comes from a study by Calvo Merino et al. (2006), in which the authors had expert male and female ballet dancers observe gender-specific dance movements. This allowed for the control of visual knowledge, as all actions were equally known to all participants, but only half of the actions observed were within the participant's own expert motor repertoire. The observation of movements specific to one's own motor repertoire elicited greater activation in premotor and parietal areas relative to opposite-gender moves. The authors concluded that mirror circuits have a purely motor response over and above visual representations of action, and that we understand actions not only by visual recognition, but also motorically.

Further evidence for a crucial role of mirror areas in action understanding comes from a study by Pobric & Hamilton (2006), in which transcranial magnetic stimulation (TMS) of the pars opercularis impaired perceptual judgments about other people's actions, suggesting that this region of the ventral premotor cortex is necessary for the perception of action. Behavioral studies have also shown that the intention (Liepelt et al. 2008a) and the goal (Liepelt et al. 2010) of an observed action can influence motor simulation.

To understand an action, both the *goal* of the observed action and the *intention* of the person performing that action must be encoded (Kilner and Frith, 2008). Fogassi et al. (2005) showed that mirror neurons in the inferior parietal lobule of the monkey (see Bonini et al., 2010 for similar properties in area F5) not only encode the goal of an observed motor act but also discriminate identical motor acts (e.g., grasping) according to the context in which they are embedded (e.g., 'grasping to eat' versus 'grasping to place'). The authors concluded that because the discriminated motor act was part of a chain leading to the final goal of the action, this neuronal property allows the monkey to predict the goal of the observed action and, thus,

to 'read' the intention of the acting individual. Evidence for the role of the mirror system in intention understanding in humans comes from an fMRI study by Iacoboni and colleagues (2005). In this study actions were presented in different contexts (e.g., grasping a cup in the "before tea" context suggested grasping a cup to drink; by contrast, grasping a cup in the "after tea" context suggested grasping a cup to clean up). They found that the ventral premotor cortex was more active when participants watched actions that were imbedded in a context that facilitated understanding of the actor's intentions, compared with observation of actions without such a context. Unfortunately, in the study of Iacoboni et al. (2005) the stimulus features in the video clips were different between the conditions, making it unclear if the difference in activation was related to the context or the difference in visual presentation. Intention understanding in novel, non-stereotypic situations, on the other hand, does not appear to be mediated by a mirror neuron network that encodes observed actions "motorically", but rather by an inferential interpretative system in the superior temporal sulcus, posterior STS and anterior fronto-medial cortex, which analyse the visual features of the action (Brass et al., 2007; Kilner and Frith, 2008; Liepelt et al., 2008b).

Recently, evidence for the role of the mirror system in action understanding has been questioned. Hickok (2009) argued that no evidence from monkey data directly tests this theory, and evidence from humans makes a strong case against the position. According to Hickok (2009), if mirror neurons are crucial for action understanding, lesions in areas that contain mirror neurons, such as area F5 in monkeys and Brodmann area 44 in humans, should lead to impairments in action understanding; however, this does not appear to be the case. After listing eight problems for the mirror neuron theory of action understanding, Hickok (2009) concludes that, although it seems entirely possible that motor experience can augment

conceptual understanding in some situations, in others mirror-like activity appears to reflect sensory-motor associations that are devoid of meaningful conceptual content.

If the fronto-parietal mirror network has no active role in action understanding, as Hickok (2009) suggests, we would expect the pattern of activation in these regions during action observation to be the same whether participants try to understand the action or they simply observe the same action passively, with no task instructions. Contrary to previous fMRI studies in which stimulus features differed between the conditions (e.g., Iacoboni et al., 2005) or in which participants had to prepare different actions to identical video clips (Decety et al., 1997; Grèzes et al., 1999), we presented participants with a set of identical action-video clips to which identical actions had to be prepared. We only changed the context in which participants watched the actions in separate experimental conditions. In one condition, participants merely observed each successive action but were not required to make any explicit judgment about it. In a second condition, participants were instructed to make a judgment on the physical features of the action. In the third condition, participants had to make a judgment on the meaning of the action. We included a 6 s delay period between observing actions and making a judgment about the actions which allowed us to examine BOLD activation related purely to the perception of actions in the video clips completely independent of any brain activity associated with the response-period of the trial (see Methods section for details). If mirror areas are influenced by the intention of the observer through top-down modulation (Liepelt and Brass, 2010; Teufel et al., 2010), the ventral premotor cortex and inferior parietal lobule would respond differently to the observation of identical actions. On the other hand, if mirror areas only have a passive role in action observation, and automatically simulate the observed actions regardless of context, we expect these regions to show a similar pattern in all three conditions. Because mirror neurons only

represent a small proportion of the neurons in these areas (Gallese et al., 1996, 2002) we expected only subtle differences between our conditions. Therefore, in addition to a classical SPM analysis we also performed a more sensitive multi-voxel pattern analysis (Etzel et al., 2009; Haxby et al., 2001).

Methods

Participants. Twenty volunteers (mean age = 21.3 years; standard deviation = 1.45 years; 9 males) completed the experiment. All participants were right-handed, had normal or corrected-to-normal visual acuity, and had no history of mental or neurological diseases. They gave written informed consent in accordance with the Declaration of Helsinki. All experimental procedures were approved by the Medical Research Ethics Committee of The University of Queensland.

Experiment. Participants had to observe the same set of 40 video clips of hand actions under three conditions (Figure 1). In the "*Understand action*" condition participants had to try to understand the meaning of the observed video clip. In the *"Observe physical features"* condition participants had to watch the physical features of the action. In the *"Respond left or right"* condition participants had to passively observe the video clips. At the start of each trial a 2 s letter cue (U in the "*Understand action*" condition, O in the *"Observe physical features"* condition, R in the *"Respond left or right"* condition) indicated the condition. A single letter was used in each condition to minimize the difference in visual presentation between the three conditions. This was followed by a video clip, 1 s in duration. Stimuli were video clips of forty different pantomimed, goal-directed hand actions (e.g., clicking a pen; hammering a nail; scratching a surface; shooing a fly, etc.), taken from the study of Chong et

al. (2008). There were 40 movie clips in total, each clip appearing in each condition once during the experiment. The object toward which each action was directed (e.g., pen, hammer) was not present in any of the clips. Observation of an object can also activate the motor system but these neurons are known as canonical neurons (Murata et al., 1997) rather than mirror neurons. Therefore to avoid the activation of these canonical neurons we used pantomimed hand actions. Each action was carefully chosen to be as distinct from the others as possible and showed images of the action being performed by the right hand of an actor viewed from a third person perspective against a black background. Only the torso and right hand of the actor were visible. Each video clip was followed by a 6 s delay during which a fixation cross remained on the screen. A long delay was used to make it possible to analyze the data before the Go or NoGo cue (see fMRI analysis for details). After the delay a Go or NoGo cue (3 s duration) indicated whether participants should make a response. The NoGo cue (50 % of the time) was always indicated by the letter "N", but the Go cue differed depending on the condition. The Go-NoGo task design forced participants to wait until the cue, at the end of the delay period, before making any responses. It also allowed us to verify that our analysis of activity during the delay period following the action video was not influenced by the response-period of the trial, by showing no brain activity differences between between Go-NoGo trials during this interval.

A text cue with two options (e.g., 'knock door' or 'paint canvas' ?) indicated a Go trial in the "Understand action" condition and participants had to press a left or right button to indicate the side of the matching stimulus. The correct stimulus was randomly matched with an incorrect stimulus and the position of the correct stimulus (left or right) was counterbalanced across trials in all conditions. A picture with two different hand actions indicated a Go trial in the "Observe physical features" condition, and again participants had to press a left or right

button to indicate the correct response. During the "Respond left or right" condition, a text message ("Press Left" or "Press Right") was presented and participants had to press the response button indicated by the text. In the *Baseline* condition a fixation cross remained on the screen for the total duration of the trial (12 s). For all responses, participants used their right-hand only and pressed left or right buttons with their index and middle fingers, respectively. Each condition was presented in random order 8 times per run. Each participant completed 5 runs. To minimize learning, video clips were counterbalanced across runs so that each different observed action was only presented once in each condition across the entire experiment. Reaction times (RT) and percentage correct responses during the Go trials were recorded in the "Understand action", "Observe physical features" and "Respond left or right" conditions. A practice run, lasting around 5 minutes, and using a separate set of video clips, was used as training outside the scanner to familiarize participants with the task.

fMRI Acquisition. The fMRI data were obtained on a 3 T Siemens Trio MR scanner using a 12-channel head volume coil and a gradient-echo echo-planar imaging (EPI) sequence with the following parameters: 33 horizontal slices (3.5 mm slice thickness), repetition time (TR) 2 s; echo time (TE), 30 ms; 64×64 voxels at 3.3 ×3.3 mm in-plane resolution. The entire brain from the vertex to the cerebellum was covered by the 33 slices. The first three T_R periods from each functional run were removed to allow for steady-state tissue magnetization. A three dimensional, high-resolution T1-weighted image covering the entire brain was also acquired for anatomical reference (TR = 1900 ms, TE = 2.32 ms, 192 cubic matrix, voxel size = 0.9 cubic mm, slice thickness = 0.9 mm).

fMRI Preprossing. All data were preprocessed and analyzed using SPM8 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, London;

http://www.fil.ion.ucl.ac.uk/spm), implemented in Matlab (Mathworks Inc., USA). EPI volumes were realigned to the first image of each run for movement correction using a least-squares approach and six-parameter rigid body spatial transformations (Friston et al., 1995). A mean EPI volume was obtained during realignment, and the structural MRI was co-registered with that mean volume. The structural scan was normalized to the Montreal Neurological Institute (MNI) T1 template using nonlinear basis functions. The same deformation parameters were applied to the EPI volumes. The EPI volumes were spatially smoothed using a 8 mm FWHM isotropic Gaussian filter. The time series for each voxel was high-pass filtered to 1/128 Hz.

Because we were interested in activations associated with the perception of hand actions, rather than those linked to manual responses on Go trials, each of the three trial types (Understand, Observe and Respond) was modeled (Figure 2) by a series of 6 box-car functions with a duration of 2 seconds (Finite Impulse Response sets). The peak of the delayed hemodynamic response associated with the perception of the video clips was expected 5 seconds (Miezin et al., 2000) after the start of the video clip. Therefore the 4th box-car function (Figure 2) was selected for analyses using SPM. Note that the end of this 4th box-car was 1 s before presentation of the Go or NoGo cue; thus, differences in activations between conditions cannot be attributed to movement execution (see Cunnington et al., 2006 for a similar procedure).

fMRI analysis

Analyses based on the General Linear Model in SPM. For all active conditions, we contrasted activation while participants watched action videos with that obtained during the fixation baseline. To identify the brain network involved in the perception of actions across

all conditions we performed a null conjunction analysis (Nichols et al., 2005). Additionally, pairwise comparisons between each of the conditions were performed. As a control measure we also contrasted all Go trials with all NoGo trials to make sure differences between conditions were not related to different Go cues. Specific contrasts calculated in the first-level analyses were then compared in second-level random-effects group analyses using single-sample t-tests (Holmes and Friston, 1998). For all contrasts, significant activation was defined by a cluster-level probability threshold of $P_{FWE} < 0.05$ corrected for the whole brain search volume (with clusters defined by the voxel-level threshold $P < 0.001$).

Multi-Voxel Pattern Analysis (MVPA). We further investigated differences in activation patterns across the three experimental conditions ("Understand action", "Observe physical features" and "Respond left or right") using the Princeton MVPA toolbox (http://code.google.com/p/princeton-mvpa-toolbox/wiki/Main). We were particularly interested in two regions previously associated with the mirror system: Inferior Parietal Lobule (IPL) and Ventral Premotor Cortex and adjacent pars opercularis (vPM). Two regions of interest (ROI) were defined a priori using MRIcroN. The ROI in the left IPL had a 20 mm radius and was centered around a voxel with the following MNI coordinates: x = -48, y = -37, z = 31. This location (transformed from Talairach into MNI coordinates) was activated during observation and execution of actions in a previous fMRI study of the mirror system (Molenberghs et al., 2010). An homologous image was created for the right IPL and combined with the left IPL to make a bilateral ROI. The same procedure was used for the bilateral vPM ROI (-54, 14, 7). This loaction (transformed from Talairach into MNI coordinates) was also selected on the basis of being activated during observation and execution of actions in a previous fMRI study on the mirror system (Iacoboni et al., 1999). To ensure above-chance classification was not due to some artifact in the data such as

scanner drift, subject movement, or some other confound, we selected a control region in a bilateral temporal ROI (-47, -2, -23). This region was chosen for two reasons. First, the control region was located far away from the other two regions and therefore didn't overlap with them. Second, this temporal region has a role in higher level semantic processing (Visser et al., 2010). If no significant classification is found in this temporal region it supports the conclusion that above-chance classification in the other two regions is unlikely to be attributable to differences in semantic processing between the three conditions.

The ROIs were used as a mask to load in the raw EPI voxel data in MATLAB using the Princeton MVPA toolbox. We z-scored the data to normalize the response amplitudes of individual voxels relative to the average of the entire time course within each run. This minimised baseline differences across runs and reduced the impact of outliers. To maximise the sensitivity of the classification analyses, we first used an ANOVA to perform the feature selection and restricted our analysis to the most relevant voxels. As a regressor we used the information from the 4th TR after the start of each of the three relevant conditions ("Understand action", "Observe physical features" and "Respond left or right"). As mentioned above, this corresponded with the peak of the BOLD response associated with observation of actions in the video clips (Figure 2). The other time points were removed from the analysis. Cross-validation classification was performed with a backprop classifier, with no hidden layers, implemented in the toolbox. Classification was performed for each of the three conditions per ROI for each of the 5 runs using the information from the 4 other runs. Each run was set aside, in turn, as the test data; the remaining runs were then used to train the classifier. This procedure was repeated 5 times until all runs had been assigned once as the test data, allowing for an unbiased estimate of the overall classification performance. First we performed a three-way classification for each ROI between the three conditions. If the pattern

of activation between the three conditions is the same, the classifier should perform at chance level (33.33 %). Hit rates (= percentage correct classifications) across all three conditions were calculated per run and, per participant using the Princeton MVPA toolbox. Hit rates were then averaged across the five runs and calculated for all participants. Significant three-way classifications across participants were followed up by pairwise classifications. To compare the mean hit rate across all the participants with chance level, we used one-sample t tests (33.33 % for the three-way classifications and 50% for the pairwise classifications).

Results

Behavioral Results.

Response Times. Mauchly's test indicated that the assumption of sphericity had been violated (chi-square = 10.57, p <.05). Degrees of freedom were therefore corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.69). A one-way repeated measures ANOVA revealed a significant difference in RT between the three conditions, $F(1.39, 26.31) = 138.18$, $p < 0.001$. Post hoc tests (Bonferroni corrected) revealed that the RT in the "Understand action" condition (M = 1796 ms, SD = 309 ms) was significantly slower than the RT in the "Observe physical features" (M = 1546 ms, SD = 189 ms; p = 0.001) and "Respond left or right" (M=944 ms, SD=144ms; p < 0.001) condition. The RT in the "Observe physical features" condition was also significantly slower than that in the "Respond left or right" condition, $p < 0.001$.

Accuracy. A one-way repeated measures ANOVA revealed a significant difference in accuracy across the three conditions, $F(2, 38) = 17.67$, $p < 0.001$. Post hoc tests (Bonferroni corrected) revealed that participants made were less accurate in the "Understand action" (M =

89.5 %, SD = 8.3 %) and "Observe physical features" (M = 89.3 %, SD = 7.7 %) condition than in the "Respond left or right" (M = 99.3 %, SD = 1.8 %; p < 0.001) condition.

fMRI Results.

As expected no difference was found between Go and NoGo trials confirming that the analysis of the 4th box function during the delay period (see Figure 2 for details) was not influenced by any differences in the Go cue or responses, and reflected activity only associated with observation of the action video. All three conditions activated a similar parieto-frontal network compared with the Baseline condition (Figure 3). This network included the inferior parietal lobule and ventral premotor cortex, both of which are known to be activated during action observation. The pairwise comparisons revealed no significant differences between any of the three conditions at the whole brain level; nor were there any significant differences using a small volume correction when analyses were restricted to the IPL and vPM ROIs described in the MVPA methods.

Multi-Voxel Pattern Analysis (MVPA).

Because we only expected subtle difference between the conditions we performed a more sensitive MVPA. MVPA can detect small differences in the pattern of activation between different conditions (Etzel et al., 2009; Haxby et al., 2001). If the ventral premotor cortex and inferior parietal lobule respond the same to the observation of action video clips irrespective of context we would expect classification performance to be at chance level. We combined left and right regions of interest because a paired t-test showed that there was no difference between right and left vPM, t(19) = 0.31, p = 0.76 and left and right IPL, t(19) = 1.05, p = 0.31.

1) Ventral Premotor Cortex (vPM). MVPA performed within the vPM revealed above chance (33.3 %) classification (Figure 4A) between the three conditions across participants (t(19) = 3.1, p = 0.006; M = 36.5 %, SD = 4.7 %). Significant pairwise decoding (Figure 4B) above chance level (50 %) was further found between the "Understand action" and "Observe physical features" (t(19) = 2.3, p = 0.04; M = 53.3 %, SD = 6.5 %), "Respond left or right" and "Observe physical features" (t(19) = 2.7, p = 0.02; M = 53.4 %, SD = 5.7 %) and "Respond left or right" and "Understand action" (t(19) = 3.0, p = 0.007; M = 55.9 %, SD = 8.7 %) conditions.

2) Inferior Parietal Lobule (IPL). The three-way MVPA within the IPL also revealed a significant classification above chance (Figure 4A) across participants (t(19) = 3.2, p = 0.005; M = 37.6 %, SD = 6.1 %). Significant pairwise decoding (Figure 4C) was further found between the "Understand action" and "Observe physical features" (t(19) = 2.3, p = 0.03; M = 53.6 %, SD = 7.0 %), "Respond left or right" and "Observe physical features" (t(19) = 2.3, p = 0.04; M = 55.2 %, SD = 10.2 %) and "Respond left or right" and "Understand action" conditions (t(19) = 2.5, p = 0.02; M = 54.3 %, SD = 7.8 %).

3) Control region in the temporal lobe. The three-way MVPA within the temporal control region yielded no significant classification (Figure 4A) across participants (t(19) = -0.3, p = 0.78; M = 33.1 %, SD = 3.2 %). Pairwise decoding also showed no significant classification above chance across participants. To confirm that the pattern in the control region was significantly different from those of the two other regions, we performed a one-way repeated measures ANOVA which confirmed that the pattern of activation was significantly different between the three regions, F (2, 38) = 6.94, p = 0.003. Post-hoc tests (Bonferroni corrected)

showed that the pattern in BA44 (p = 0.015) and IPL (p = 0.007) was significantly different from that in the control region.

Discussion

In this study we set out to determine whether two cortical regions (vPM and IPL) commonly associated with the mirror system respond differently to the observation of actions depending on the intention of the observer. A parieto-frontal network was significantly active during the observation of pantomimed goal-directed actions, irrespective of the context. This is consistent with previous results showing that pantomimed goal-directed actions, even when the objects to which actions are directed are not present, automatically activate this mirror network (Buccino et al., 2001; Chaminade et al., 2005; Chong et al., 2008; Decety et al., 1997; Dinstein et al., 2007; Iacoboni et al., 1999; Molenberghs et al., 2010; Montgomery et al., 2007). The classical SPM analysis yielded no significant differences between the three contexts, but a more sensitive MVPA analysis found significant classification performance above chance level in the ventral premotor cortex and inferior parietal lobule across all of the three context conditions. A control region within the temporal cortex showed no significant classification above chance level, so we can rule out that the results for the vPM and IPL were caused by scanner drift, participant movement, or some other confound such as a difference in the degree of high-level semantic processing across the conditions.

Because only a relatively small proportion of the neurons (17 % in area F5 in monkeys; Gallese et al., 1996) in the vPM and IPL are mirror neurons, it is no surprise that the classical SPM analysis was not sensitive enough to pick up differences between the conditions. The MVPA analysis, on the other hand, revealed that these regions have an active role in action

understanding, as opposed to merely being involved in the passive encoding of observed

actions (Iacoboni et al., 2005). This is the first fMRI study that to use identical video clips in

all three context conditions, thus therefore we can rule out that distinct stimulus features

across the conditions were the cause of the different activation patterns. The significant

classification also cannot be attributed to differences in action execution or in the Go cue

because only fMRI data obtained *before* the execution phase (Figure 2) were used in the

classification. The RT results show that it took longer to respond in the "Understand action"

condition and accuracy results showed that participants were more accurate in the "Respond

left or right" condition. These results were expected because of the difference in difficulty

between the different Go cues. Note also that participants responded *after* the Go cue. There

were thus no implications of the RT and accuracy data for the imaging results, since we only

analysed BOLD information *before* the onset of the Go cue (see Figure 2 for details). In both

the "Understand action" and "Observe physical features" condition participants had to pay

attention to the actions, so differences in attention also cannot explain our results. Nor can

differences in action preparation account for our results, because in all three conditions

participants had to prepare identical actions (left or right button responses). The context, on

the other hand, was the only difference between the three conditions; therefore, we can

reasonably conclude that the different activation patterns in three action observation

conditions resulted from differences in the intention of participants while they were observing

actions.

Contrary to previous studies we did not investigate action understanding by manipulating the

context (Liepelt et al., 2009) or the goal (Iacoboni et al., 2005) of the observed action;

instead, we changed the mindset of observers by changing the kind of judgment they were

cued to make on identical video clips. The different patterns of activation in vPM and IPL are

in line with previous findings which have shown top-down modulation effects on mirror system activity (Liepelt and Brass, 2010; Teufel et al., 2010).

The current results suggest that when we try to understand an observed action we extract different information compared with than when we passively observe the same action. The fronto-parietal action-observation network is typically seen as passively encoding actions onto our own motor system regardless of the intentions of the observer, but our data show that there are subtle differences in this network depending on the context in which actions are observed. The mental state of an observer influenced the perception of the action in a top-down manner (Teufel et al. 2010). This is contrary to the classical bottom-up view of action understanding in which brain regions involved in the encoding of an action feed one-way information to different brain regions involved in the interpretation of that action. We can only speculate on the underlying mechanisms responsible for this different activation pattern but a possible explanation comes from a specific type of mirror neurons called ''logically related'' neurons (Iacoboni et al., 2005; Pellegrino et al., 1992). This subset of mirror neurons does not respond to the observation and execution of the same action, but they respond to the execution of a functionally related action (e.g. observation of grasping a peanut and execution of opening the mouth). These neurons therefore respond relative to the meaning of the observed action to the observer. If the observed action has no relevance to the observer, as in our Respond condition, these neurons will respond differently compared to when the action is relevant to the observer, as in the two other conditions. Even if both conditions have relevance to the observer but the goal is different, as for the "Understand action" and "Observe physical features" conditions, we would expect to see a different pattern of activation depending on the type of relevance to the observer. Contrary to a previous fMRI study (Iacoboni et al., 2005), we used identical video clips in all our

conditions, therefore our results are the first to show clear fMRI evidence for the role of the mirror system areas in action understanding.

In conclusion, we have shown unique patterns of activation in IPL and vPM depending on what information people have to extract from an observed action. These findings are consistent with previous studies (Iacoboni et al., 2005; Pobric & Hamilton, 2006; Umilta and et al., 2008) that have suggested that mirror regions have an active role in action understanding.

Acknowledgements

References

Blakemore, S.J., Decety, J., 2001. From the perception of action to the understanding of intention. Nature Reviews Neuroscience 2, 561-567.

Bonini, L., Rozzi, S., Serventi, F.U., Simone, L., Ferrari, P.F., Fogassi, L., 2010. Ventral Premotor and Inferior Parietal Cortices Make Distinct Contribution to Action Organization and Intention Understanding. Cerebral Cortex 20, 1372-1385.

Brass, M., Schmitt, R.M., Spengler, S., Gergely, G., 2007. Investigating action understanding: Inferential processes versus action simulation. Current Biololgy 17, 2117-2121.

Buccino, G., Binkofski, F., Fink, G.R., Fadiga, L., Fogassi, L., Gallese, V., Seitz, R.J., Zilles, K., Rizzolatti, G., Freund, H.J., 2001. Action observation activates premotor and parietal areas in a somatotopic manner: an fMRI study. European Journal of Neuroscience 13, 400-404.

Budell, L., Jackson, P., Rainville, P., 2010. Brain responses to facial expressions of pain: Emotional or motor mirroring? NeuroImage 53, 355-363.

Calvo-Merino, B., Grezes, J., Glaser, D.E., Passingham, R.E., Haggard, P., 2006. Seeing or doing? Influence of visual and motor familiarity in action observation. Current Biololgy 16, 1905-1910.

Cattaneo, L., Rizzolatti, G., 2009. The Mirror Neuron System. Archives of Neurology 66, 557-560.

Chaminade, T., Meltzoff, A.N., Decety, J., 2005. An fMRI study of imitation: action representation and body schema. Neuropsychologia 43, 115-127.

Chong, T.T.J., Cunnington, R., Williams, M.A., Kanwisher, N., Mattingley, J.B., 2008. fMRI Adaptation Reveals Mirror Neurons in Human Inferior Parietal Cortex. Current Biology 18, 1576-1580.

Cunnington, R., Windischberger, C., Robinson, S., Moser, E., 2006. The selection of intended actions and the observation of others' actions: A time-resolved fMRI study. Neuroimage 29, 1294-1302.

Decety, J., Grèzes, J., Costes, N., Perani, D., Jeannerod, M., Procyk, E., Grassi, F., Fazio, F., 1997. Brain activity during observation of actions - Influence of action content and subject's strategy. Brain 120, 1763-1777.

Grèzes, J., Costes, N., Decety, J.,1999. The effects of learning and intention on the neural network involved in the perception of meaningless actions. Brain 122, 1875-1887.

Dinstein, I., Hasson, U., Rubin, N., Heeger, D.J., 2007. Brain areas selective for both observed and executed movements. Journal of Neurophysiology 98, 1415-1427.

Etzel, J.A., Gazzola, V., Keysers, C., 2009. An introduction to anatomical ROI-based fMRI classification analysis. Brain Research 1282, 114-125.

Fabbri-Destro, M., Rizzolatti, G., 2008. Mirror neurons and mirror systems in monkeys and humans. Physiology 23, 171-179.

Fogassi, L., Ferrari, P.F., Gesierich, B., Rozzi, S., Chersi, F., Rizzolatti, G., 2005. Parietal lobe: From action organization to intention understanding. Science 308, 662-667.

Friston, K.J., Ashburner, J., Frith, C.D., Poline, J.B., Heather, J.D., Frackowiak, R.S.J., 1995. Spatial registration and normalization of images. Human Brain Mapping 3, 165-189.

Gallese, V., Fadiga, L., Fogassi, L., Rizzolatti, G., 1996. Action recognition in the premotor cortex. Brain 119, 593-609.

Gallese, V., Fadiga, L., Fogassi, L., Rizzolatti, G., 2002. Action representation and the inferior parietal lobule. Common Mechanisms in Perception and Action 19, 334-355.

Gazzola, V., Rizzolatti, G., Wicker, B., Keysers, C., 2007. The anthropomorphic brain: The mirror neuron system responds to human and robotic actions. Neuroimage 35, 1674-1684.

Hamilton, A.F.D., Grafton, S.T., 2006. Goal representation in human anterior intraparietal sulcus. Journal of Neuroscience 26, 1133-1137.

Haxby, J.V., Gobbini, M.I., Furey, M.L., Ishai, A., Schouten, J.L., Pietrini, P., 2001. Distributed and overlapping representations of faces and objects in ventral temporal cortex. Science 293, 2425-2430.

Hickok, G., 2009. Eight Problems for the Mirror Neuron Theory of Action Understanding in Monkeys and Humans. Journal of Cognitive Neuroscience 21, 1229-1243.

Holmes, A.P., Friston, K.J., 1998. Generalisability, random effects & population inference Neuroimage 7, S754.

Iacoboni, M., Dapretto, M., 2006. The mirror neuron system and the consequences of its dysfunction. Nature Reviews Neuroscience 7, 942-951.

Iacoboni, M., Molnar-Szakacs, I., Gallese, V., Buccino, G., Mazziotta, J.C., Rizzolatti, G., 2005. Grasping the intentions of others with one's own mirror neuron system. Plos Biology 3, 529-535.

Iacoboni, M., Woods, R.P., Brass, M., Bekkering, H., Mazziotta, J.C., Rizzolatti, G., 1999. Cortical mechanisms of human imitation. Science 286, 2526-2528.

Kilner, J.M., Frith, C.D., 2008. Action observation: Inferring intentions without mirror neurons. Current Biology 18, R32-R33.

Kilner, J.M., Neal, A., Weiskopf, N., Friston, K.J., Frith, C.D., 2009. Evidence of mirror neurons in human inferior frontal gyrus. Journal of Neuroscience 29, 10153-10159.

Kohler, E., Keysers, C., Umilta, M.A., Fogassi, L., Gallese, V., Rizzolatti, G., 2002. Hearing sounds, understanding actions: Action representation in mirror neurons. Science 297, 846-848.

Lestou, V., Pollick, F.E., Kourtzi, Z., 2008. Neural substrates for action understanding at different description levels in the human brain. Journal of Cognitive Neuroscience 20, 324-341.

Liepelt, R., von Cramon, D. Y., Brass, M., 2008a. What is matched in direct matching? Intention attribution modulates motor priming. Journal of Experimental Psychology: Human Perception and Performance 34, 578-591.

Liepelt, R., Von Cramon, D.Y., Brass, M., 2008b. How do we infer others' goals from non-stereotypic actions? The outcome of context-sensitive inferential processing in right inferior parietal and posterior temporal cortex. Neuroimage 43, 784-792.

Liepelt, R., Ullsperger, M., Obst, K., Spengler, S., von Cramon, D. Y., & Brass, M., 2009. Contextual movement constraints of others modulate motor preparation in the observer. Neuropsychologia 47, 268-275.

Liepelt, R., Brass, M., 2010. Top-Down Modulation of Motor Priming by Belief About Animacy. Experimental Psychology 57(3), 221-227.

Liepelt, R., Prinz, W., Brass, M., 2010. When do we simulate non-human agents? Dissociating communicative and non-communicative actions. Cognition 115, 426-434.

Miezin, F.M., Maccotta, L., Ollinger, J.M., Petersen, S.E., Buckner, R.L., 2000. Characterizing the hemodynamic response: Effects of presentation rate, sampling procedure, and the possibility of ordering brain activity based on relative timing. Neuroimage 11, 735-759.

Molenberghs, P., Brander, C., Mattingley, J.B., Cunnington, R., 2010. The Role of the Superior Temporal Sulcus and the Mirror Neuron System in Imitation. Human Brain Mapping 31, 1316-1326.

Molenberghs, P., Cunnington, R., Mattingley, J.B., 2011. Brain regions with mirror properties: a meta-analysis of 125 human fMRI studies. Neuroscience and Biobehavioral Reviews, 10.1016/j.neubiorev.2011.07.004.

Montgomery, K.J., Isenberg, N., Haxby, J.V., 2007. Communicative hand gestures and object-directed hand movements activated the mirror neuron system. Social Cognitive and Affective Neuroscience 2, 114-122.

Mukamel, R., Ekstrom, A.D., Kaplan, J., Iacoboni, M., Fried, I., 2010. Single-Neuron Responses in Humans during Execution and Observation of Actions. Current Biology 20, 750-756.

Murata, A., Fadiga, L., Fogassi, L., Gallese, V., Raos, V., Rizzolatti, G., 1997. Object representation in the ventral premotor cortex (area F5) of the monkey. Journal of Neurophysiology 78, 2226-2230.

Nichols, T., Brett, M., Andersson, J., Wager, T., Poline, J., 2005. Valid conjunction inference with the minimum statistic. Neuroimage 25, 653–660.

Peeters, R., Simone, L., Nelissen, K., Fabbri-Destro, M., Vanduffel, W., Rizzolatti, G., Orban, G.A., 2009. The Representation of Tool Use in Humans and Monkeys: Common and Uniquely Human Features. Journal of Neuroscience 29, 11523-11539.

Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., Rizzolatti, G., 1992. Understanding motor event : A neurophysiological study. Experimental Brain Research 91, 176-180.

Pobric, G., Hamilton, A.F.D., 2006. Action understanding requires the left inferior frontal cortex. Current Biology 16, 524-529.

Rizzolatti, G., Craighero, L., 2004. The mirror-neuron system. Annual Review of Neuroscience 27, 169-192.

Rizzolatti, G., Fabbri-Destro, M., 2010. Mirror neurons: from discovery to autism. Experimental Brain Research 200, 223-237.

Rizzolatti, G., Fogassi, L., Gallese, V., 2001. Neurophysiological mechanisms underlying the understanding and imitation of action. Nature Reviews Neuroscience 2, 661-670.

Rizzolatti, G., Sinigaglia, C., 2010. The functional role of the parieto-frontal mirror circuit: interpretations and misinterpretations. Nature Reviews Neuroscience 11, 264-274.

Saxe, R., 2005. Against simulation: the argument from error. Trends in Cognitive Sciences 9, 174-179.

Saxe, R., 2006. Uniquely human social cognition. Current Opinion in Neurobiology 16, 235-239.

Teufel, C., Fletcher, P.C., Davis, G., 2010. Seeing other minds: attributed mental states influence perception. Trends in Cognitive Sciences 14, 376-382.

Umilta, M.A., Escola, L., Intskirveli, I., Grammont, F., Rochat, M., Caruana, F., Jezzini, A., Gallese, V., Rizzolatti, G., 2008. When pliers become fingers in the monkey motor system. Proceedings of the National Academy of Sciences USA 105, 2209-2213.

Umilta, M.A., Kohler, E., Gallese, V., Fogassi, L., Fadiga, L., Keysers, C., Rizzolatti, G., 2001. I know what you are doing: A neurophysiological study. Neuron 31, 155-165.

Visser, M., Jefferies, E., Ralph, M.A.L., 2010. Semantic Processing in the Anterior Temporal Lobes: A Meta-analysis of the Functional Neuroimaging Literature. Journal of Cognitive Neuroscience 22, 1083-1094.

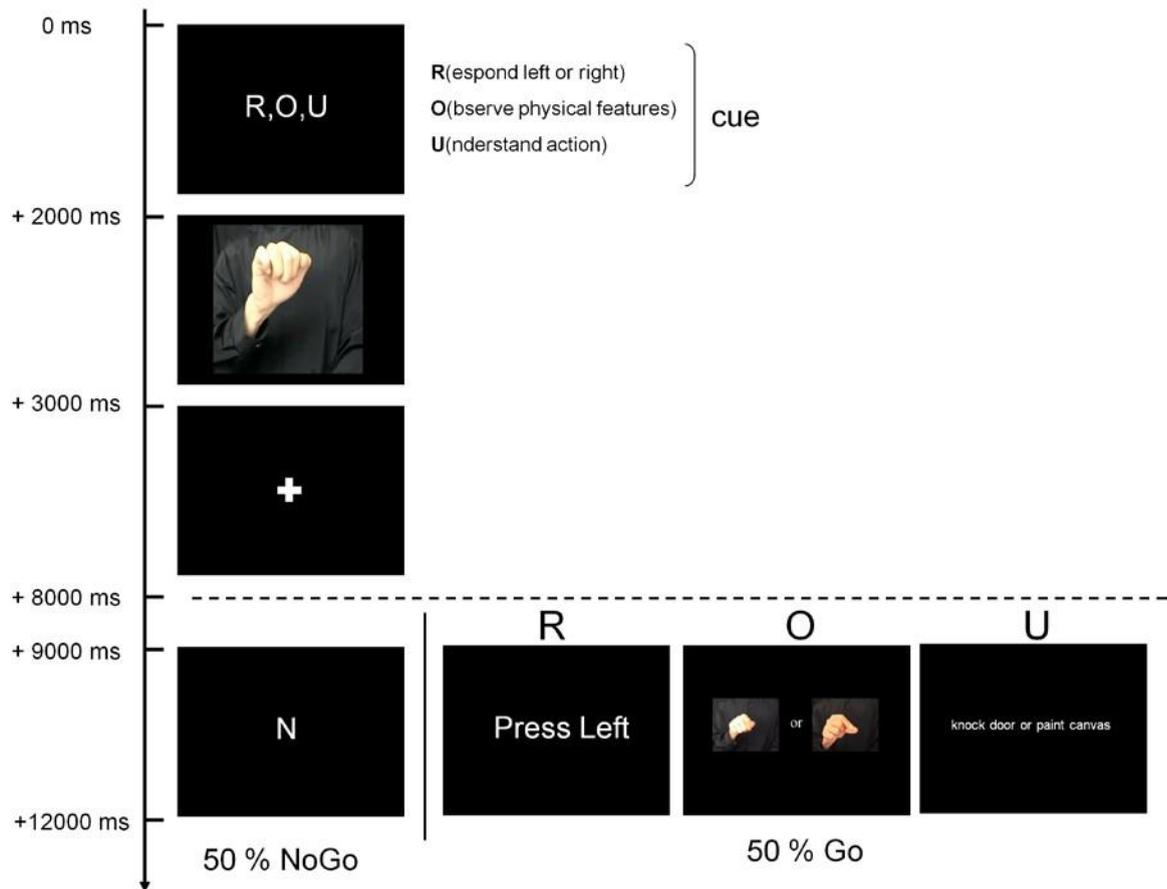**Figure 1 –** Sequence of events within a trial of the "**R**espond left or right", "**O**bserve physical features" and "**U**nderstand action" conditions. Pictures are from the videos presented in the different trials. Note that the hand actions during the perception phase were always depicted as dynamic displays for 1 s. In Go trials of the "Respond left or right" condition, participants had to press the button indicated by the text. During the "Observe physical features" condition participants had to choose the correct response between two still pictures of hand actions. In the "Understand action" condition participants had to understand the meaning of the action and indicate the correct response based on the two text options. Only events before the dashed line were included in the fMRI analysis (see Figure 2 for details).
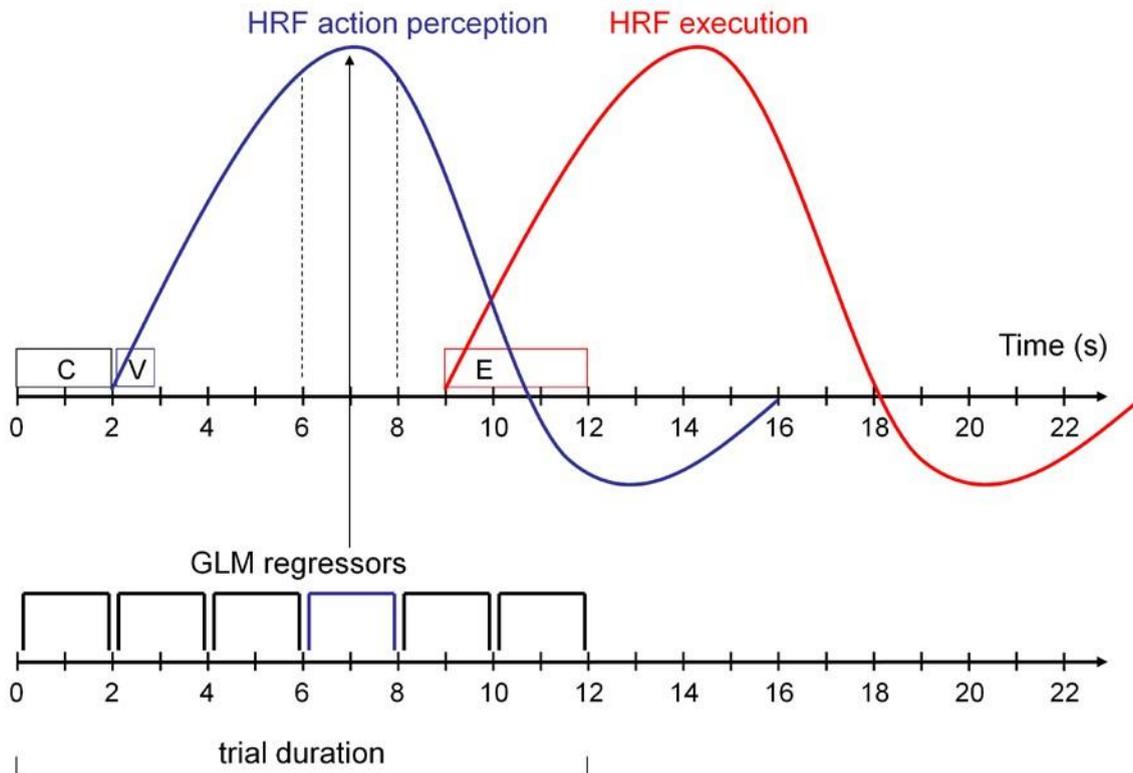
Figure 2 – Trials were modeled as a series of six box-car functions of 2 s each. Note that the 4 th box-car (shown in blue) corresponds to the peak of delayed BOLD activation from the observation of the video. This box-car ends 1 s before the presentation of the Go or NoGo stimulus. C = Context cue, V = Video clip, E = Execution cue.
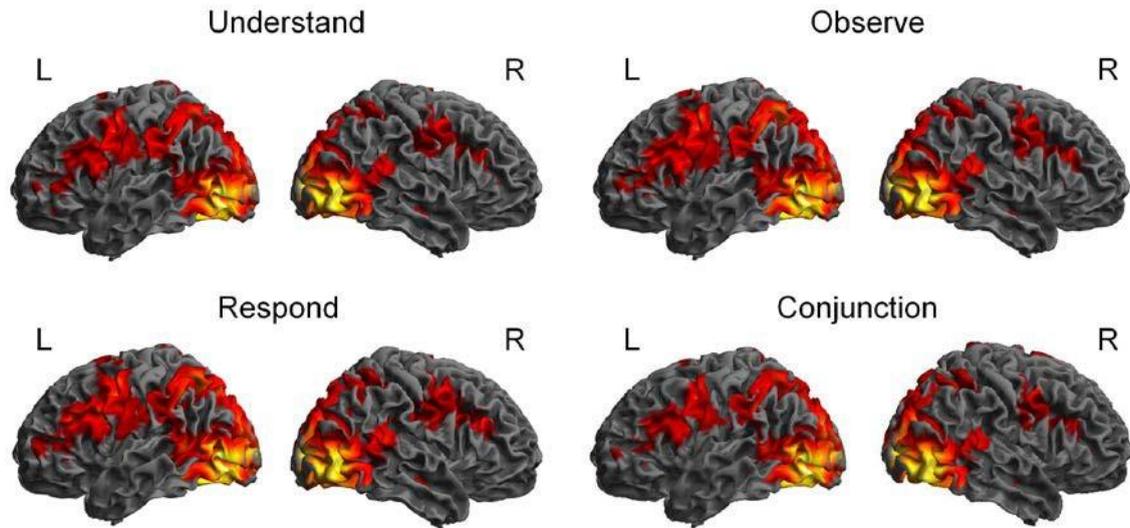
Figure 3 – Results from the fMRI analyses. Contrasts of each of the three conditions minus baseline displayed on a rendered brain using SPM8 thresholded at p < 0.001 uncorrected.
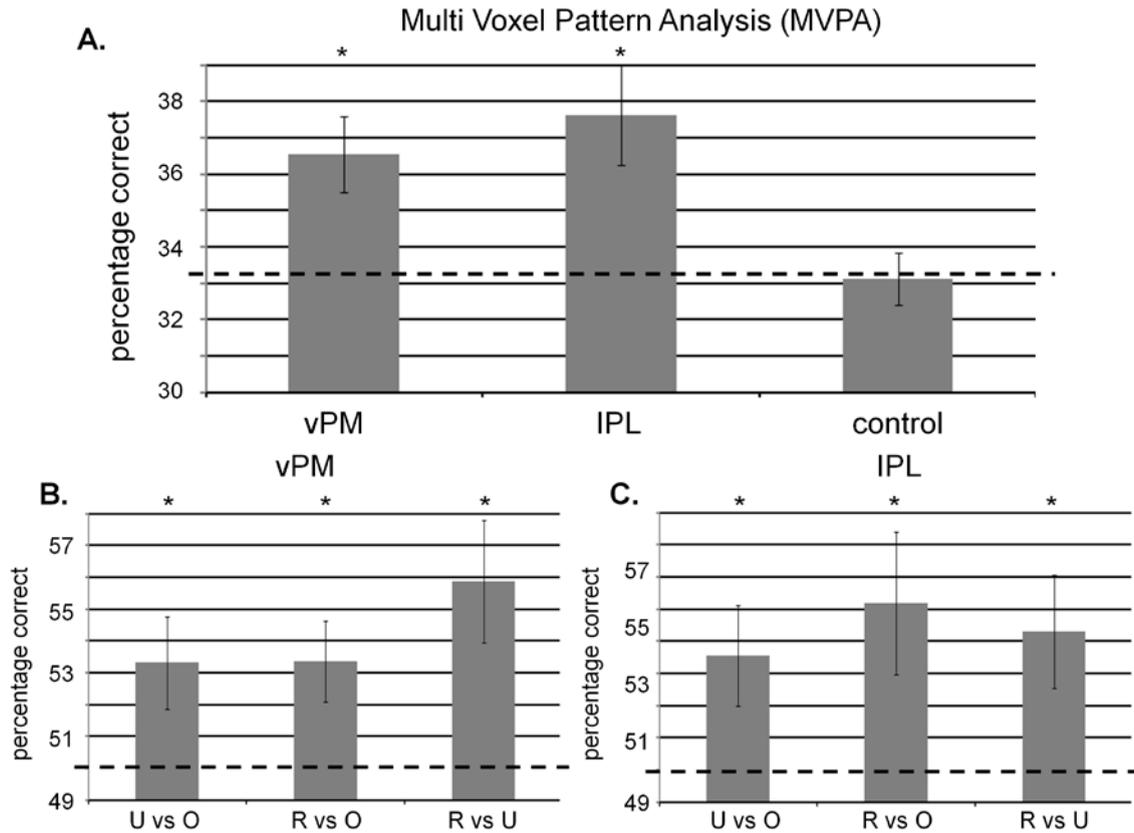
Figure 4 – Results from the MVPA analyses. Error bars represent the standard error. A. Three-way decoding performance in the three regions of interest. B. Pairwise decoding performance in the ventral premotor region of interest. C. Pairwise decoding performance in the inferior parietal lobule region of interest. The dotted lines indicate decoding performance at chance level (33.3 % in A and 50 % in B and C). * = significant ($p < 0.05$) decoding performance above chance level. vPM = ventral premotor cortex, IPL = Inferior Parietal Lobule, control = temporal control region.