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Motor resonance during action observation is gaze-contingent: A TMS study

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ABSTRACT

When we observe others performing an action, visual input to our mirror neuron system is reflected in the facilitation of primary motor cortex (M1), a phenomenon known as 'motor resonance'. However, it is unclear whether this motor resonance is contingent upon our point-of-gaze. In order to address this issue, we collected gaze data from participants as they viewed an intransitive action – thumb abduction/adduction – under four conditions: with natural gaze behaviour (free viewing) and with their gaze fixated on each of three pre-determined loci at various distances from the prime mover. In a control condition, participants viewed little finger movements, also with a fixated gaze. Transcranial magnetic stimulation (TMS) was delivered to M1 and motor evoked potentials (MEPs) were recorded from the right abductor pollicis brevis (APB) and right abductor digiti minimi (ADM). Results showed that, relative to a free viewing condition, a fixated point-of-gaze which maximized transfoveal motion facilitated MEPs in APB. Moreover, during free viewing, saccade amplitudes and APB MEP amplitudes were negatively correlated. These findings indicate that motor resonance is contingent on the observer's gaze behaviour and that, for simple movements, action observation effects may be enhanced by employing a fixed point-of-gaze.

1. Introduction

Humans have an innate ability to recognize the actions of others and to imitate those actions. These behaviours have been associated with the 'mirror neuron system' in the brain, a network of frontal and parietal areas first identified in the non-human primate brain by di Pellegrino et al. (1992). Di Pellegrino et al. found that 'mirror neurons' (MNs) in premotor areas discharged not only when a monkey performed an action, but also when it observed the same action being performed by an experimenter. Neuroimaging studies in humans have subsequently demonstrated that MN activity ultimately extends to the premotor cortex and primary motor cortex (M1), which encode the specific motor programme used to produce the observed action (Buccino et al., 2001; Grafton et al., 1996; Grèzes et al., 2003). As a result, mirror neurons activity is thought to play a pivotal role in the understanding and imitation of others' actions (Jeannerod, 2001; Rizzolatti et al., 2001).

The increase in excitability of M1 during action observation is termed 'motor resonance' (Fadiga et al., 1995) and has been demonstrated via direct application of transcranial magnetic stimulation (TMS) to M1. This motor resonance is highly distinct, in that the activation is specific to the muscles used to perform the action (Alaerts et al., 2009; Gangitano et al., 2001; Valchev et al., 2015), is time-locked to the unfolding action sequence (Alaerts et al., 2012), and is sensitive to the specific kinematics of the action (Borroni et al., 2011) – a specificity that is crucial for accurate motor learning through observation (Mattar and Gribble, 2005; Vogt and Thomaschke, 2007). Furthermore, merely observing a non-biological moving stimulus does not result in changes in corticospinal excitability (Lepage et al., 2010).

The specific functions of mirror neurons have been debated, with some authors questioning the involvement of the mirror network in action understanding (e.g., Csibra, 2007; Hickok, 2009; Jacob, 2008; 2009). The available evidence from neuroimaging and TMS studies, however, provides a compelling argument in support of the notion that motor resonance contributes to action understanding and imitation (e.g., see Decety and Grèzes, 1999; and Rizzolatti and Craighero, 2004).

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It is therefore not surprising that the mirror theory of action understanding has become one of the most prominent and mainstream hypotheses in the context of action processing and imitation. A comprehensive review of the involvement of mirror neurons in action understanding and a convincing rebuttal to the associated criticisms is provided elsewhere (see Rizzolatti and Sinigaglia, 2010).

Facilitation of M1during action observation has been observed not only for transitive actions (e.g., Sartori et al., 2012), but also for intransitive ones (Borroni et al., 2011; Burgess et al., 2013; Romani et al., 2005). Moreover, action observation (AO) has been shown to elicit learning-related changes in the brain that mirror those derived from physical practice. For example, Stefan et al. (2005) applied single-pulse TMS and recorded the consequent motor-evoked potentials (MEPs) from two thumb muscles (flexor pollicis brevis and extensor pollicis brevis). The direction of thumb movements evoked by TMS, along two movement axes (flexion/extension and abduction/adduction), was recorded at baseline. When participants engaged in either physical practice or observation of movements performed in a direction *opposite* to baseline, subsequent TMS-evoked thumb movements occurred in the entrained direction. In a later study, Stefan et al. (2008) asked participants to engage in a physical practice condition (thumb movements opposite to the direction of movements evoked by TMS pulses), and two conditions in which physical practice was combined with observation of synchronous movements that were either congruent or incongruent with the performed action. Both physical practice and the combination of physical practice with congruent movement observation enhanced motor memory formation, and increased corticospinal excitability of the trained muscle. Moreover, the combined condition was more effective than practice alone.

Alaerts et al. (2010) devised three TMS experiments to examine the relationships of kinematics, hand contraction state and intrinsic object properties to M1 excitability. Participants viewed an actor's hand picking up objects that varied in both actual and apparent weight; they also viewed the hand when it was not actually lifting the object, but either exerting an isometric force, or no force, thereby eliminating kinematic cues. Alaerts et al. observed that modulation of MEPs was congruent with the muscular force required, rather than with the observable properties of the objects that were being lifted. Thus, attention to both the kinematics of the observed action and the force requirements of that action may collectively determine the extent to which M1 is facilitated during action observation.

Researchers have found a strong link between eye movements and the mirror neuron system (MNS). Maranesi et al. (2013) used single- and multi-unit recording from F5 mirror neurons (ventral premotor cortex) in combination with gaze tracking to investigate the relationship between gaze behaviour and MN activity in macaque monkeys during both execution and observation of the same reaching-and-grasping action. Similar to previous findings in humans (e.g., Flanagan and Johansson, 2003), gaze behaviour tended to be predictive during action execution and during passive observation, in that gaze consistently moved toward the target object prior to the onset of the reaching movement. Maranesi et al. (2013) also identified a class of MNs as *gaze-dependant*; specifically, their discharge was greater when the monkey looked at the target than when it did not. Moreover, this discharge was not related to the time spent looking at the target, but it was related to the timing of the accompanying fixation. Prior to hand-target contact, the discharge was strongest for trials in which the gaze was proactive, as opposed to reactive, reflecting a tight coupling of effector and oculomotor control. However, the directionality of this relationship was ambiguous, as the issue of whether gaze was driving MN activity, or vice versa, could not be established.

Subsequent published reports have helped to clarify this issue. Leonetti et al. (2015) partially replicated an earlier TMS study by Borroni et al. (2011), in which participants viewed video clips of an avatar picking up a ball from a table. In the original study, participants viewed either a natural action (a pronated hand reaching out for, then grasping, the ball), or an entirely unnatural one, in which a supinated hand performed the same task. The associated MEPs for two agonistic muscles – abductor digiti minimi (ADM) and opponens pollicis (OP) – were time-locked to the unfolding of the action sequence, insofar as they were larger during the hand opening and grasping phases, respectively. Conversely, for the impossible movement, only ADM activity was significant, during both phases. Borroni et al. (2011) suggested that, while participants could see that the motion of the little finger was unnatural, the activation witnessed was still specific to the muscle that would be active in order to move the digit – ADM. However, when Leonetti et al. (2015) presented the same stimuli so that participants viewed them in their near peripheral vision, the pattern of MEPs was discernibly different. The ADM and OP were both significantly activated throughout the opening, grasping and lifting phases in a highly similar pattern for both natural and impossible movements. The authors noted that the reduced visual acuity in peripheral vision led to a perceptual error; the participants perceived the impossible movements of the little finger as those of the thumb. These findings suggest that point-of-gaze appears to affect motor resonance, and therefore perceptual degradation in the periphery may be an impediment to effective observational learning.

While the ability of the mirror neuron system to respond to subtle variations in kinematics and applied force is well-established, the contribution of human observers' point-of-gaze to motor resonance during action observation has not been considered. In the present study, we examine the effect of point-of-gaze manipulations on motor resonance as participants watched videos of continuous thumb adduction and abduction. We hypothesized that M1 motor resonance during observation of a simple thumb movement will be facilitated not only when point-of-gaze is relatively fixed, thereby reducing the loss of visual input associated with saccadic masking (Ross et al., 2001), but also when that fixation is located so as to focus overt visual attention directly on the location of biological motion. Participants observed the action under five different conditions: free viewing (i.e., normal viewing); with their gaze fixated on three different loci, each conferring different degrees of transfoveal motion; and a comparator condition in which they viewed little finger abduction and adduction with a fixed point-of-gaze, in order to assess the degree of muscle specificity of motor resonance. Single-pulse TMS was applied to M1 at a rate of 0.25 Hz and participants' eye movements were tracked throughout all conditions. This approach enabled us to determine the relationship between gaze behaviour and motor resonance, as manifested in the amplitude of MEPs recorded from the effector muscles.

2. Methods

2.1. Participants

Eighteen participants (3 females and 15 males; *M* age = 28.33 years, *SEM* = 1.03) took part. All were right-handed as assessed using the revised Edinburgh Handedness Questionnaire (Oldfield, 1971), *M* = 79.41, *SEM* = 6.21, and had normal or corrected-to-normal vision. Participants were naïve to TMS; none of them had any contraindication to TMS or neurological, psychiatric or other medical problems (Rossi et al., 2009; Wassermann, 1998). Participants gave their informed consent prior to taking part and did not report any discomfort or adverse effects during the TMS protocol. The protocol was approved by the research ethics committee of the lead institution and was carried out in accordance with the ethical standards of the 2008 Declaration of Helsinki.

2.2. Experimental stimuli and apparatus

All videos consisted of first-person perspective footage of a male actor's right hand, palm down on a desktop. This footage was used to extract a static image of the hand, which was used as a baseline

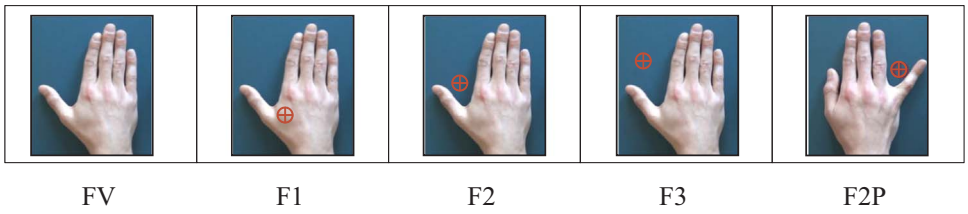

Fig. 1. Screenshots from the five experimental videos corresponding to free viewing (FV) and gaze-fixed conditions. F1, F2 and F3 corresponded to gaze-fixed conditions when observing thumb abduction/adduction. F2P corresponded to the gaze-fixed condition during little finger abduction/adduction – the equivalent of F2 for thumb motion.

reference condition, as well as to create five experimental video stimuli. These consisted of the actor performing continuous thumb or little finger abduction/adduction. The videos lasted for 1 min, started and ended with a 6 s grey screen, and each abduction and adduction movement was synchronized to a metronome set at 1 Hz, such that a total of 48 full movements were performed in each video. In the free viewing condition (FV), participants were able to view the image as they would normally. In the gaze-fixed conditions F1, F2 and F3, participants' visual attention was guided using a red fixation cross surrounded by a red circle, which subtended 2° of visual angle at the viewing distance of 60 cm, and was superimposed over the image. The fixation circle was located along an imaginary line that bisected the angle between that of the thumb at full abduction and the stationary forefinger, at one of three degrees of eccentricity from the first metacarpophalangeal joint (see Fig. 1). For condition F2P, in which the little finger moved instead of the thumb, the fixation cross was located over the proximal interphalangeal joint; this condition was included in order to assess the muscle specificity of the mirror response. The ability to accurately perceive and identify biological motion stimuli depends on whether the stimulus appears in the central or peripheral visual field, with performance deteriorating at increasing eccentricities from the fovea (Ikeda et al., 2005). Thus, our gaze-fixed conditions were designed to vary the amount of biological motion detected by the fovea. More specifically, in conditions F1 and F3, the intended point of fixation was located below and above the moving thumb, respectively, whereby motion could only be detected extra-foveally. In contrast, in conditions F2 and F2P the participant's gaze was directed onto a location that was constantly crossed by the moving thumb or little finger, respectively, thereby maximising the amount of biological motion detected by the fovea.

Videos were presented using Experiment Builder software (SR Research Ltd, Ontario, Canada), which also triggered the TMS pulses. The images were displayed on a 21-in. CRT monitor (100 Hz, screen resolution was set to 1024 × 768 pixels). Participants' eye movements were recorded using an SR Research EyeLink 1000 eye tracker (SR Research Ltd, Osgoode, Canada) (monocular, right eye; 1000 Hz).

2.3. TMS

Self-adhesive surface electrodes (Ag-AgCl) measuring 1 cm in diameter were placed in a belly-tendon montage over the abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles of the right hand to record motor-evoked potentials (MEPs) and a reference electrode was placed over the styloid process of the radius. Previous studies have shown that corticospinal facilitation during action observation can be specific to the muscles involved in the observed action (Alaerts et al., 2009; Valchev et al., 2015). Thus, since our stimuli consisted of thumb and little finger adduction and abduction movements, we selected the APB and the ADM because their main functions are to abduct (i.e. to move away from the hand) the thumb and the little finger, respectively (e.g., Palastanga et al., 2002). Electromyography (EMG) signals were recorded using Signal software (v. 6, Cambridge Electronic Design Limited, Cambridge, UK) and stored on a PC for offline analysis. EMG signals were band-pass filtered at 10–2000 Hz, digitized and displayed on a computer screen.

Transcranial magnetic stimulation was delivered using a Magstim 200 (Magstim Company Ltd., Whitland, UK) connected to a figure-of-eight coil (70 mm loop). The coil was positioned such that its centre was tangential to the scalp with the handle pointing at an angle of 45° relative to the mid-sagittal midline. In order to find the optimal scalp position (OSP) – the location on the scalp from which MEPs could be elicited in both the right ADM and the right APB – the coil was placed over the area of the left motor cortex corresponding to the 10–20 EEG position FC3 (American Clinical Neurophysiology Society, 2006) and was systematically moved, in both transverse and sagittal planes, in steps of approximately 1 cm. Thus, both muscles received TMS during all video conditions. Once the optimal stimulation site was determined, it was marked on the participant's scalp. The researcher continuously monitored the coil's position relative to this marker throughout the protocol.

Participants' resting motor threshold (rMT) was defined as the minimum level of stimulation required in order to elicit MEPs of at least 50 μV in magnitude, from at least 5 out of 10 consecutive TMS pulses (Rossini et al., 1994) in both targeted muscles. In order to elicit reliable MEPs during the experimental trials, stimulation intensity was set at 120% of the rMT. Stimulation intensities ranged from 40% to 66% of maximum stimulator output, M = 48.28, SEM = 1.88. During each experimental condition, the first TMS pulse was delivered at the onset of the video so as to trigger the start of the trial; MEPs elicited by this first pulse were excluded from analyses. Subsequent pulses were delivered during abduction at a frequency of 0.25 Hz, when the thumb reached the mid-point between maximal adduction/abduction (see Fig. 2). A total of 24 pulses were delivered in each experimental condition.

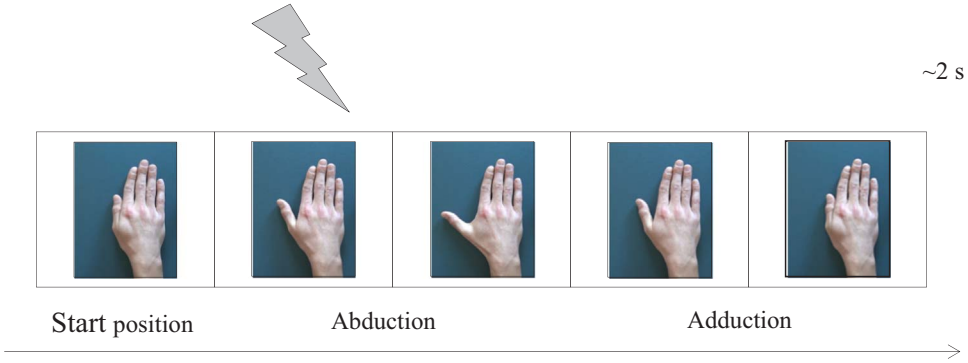

Fig. 2. Example of a single trial procedure of thumb abduction/adduction (2 s in duration) in a FV condition. TMS pulses were delivered during the presentation of thumb abduction at a frequency of 0.25 Hz.

2.4. Experimental design, task and procedures

Participants sat in a padded adjustable chair facing the monitor screen, with their forearms lying pronated on a table in front of them, (cf. Alaerts et al., 2009) and their chin positioned on the chin rest mounted on the table's edge, to avoid head movements. Viewing distance was 60 cm from the monitor. The participants' hands were also pronated on the table, within the participant's field of view and located at approximately 53° of eccentricity from the centre of the fovea in the vertical plane. EMG activity was monitored continuously and participants were reminded to relax their hand throughout the experiment.

The optimal stimulation site and rMT were determined prior to commencement of the experimental protocol by recording MEPs as per the procedures described in Section 2.3. The eye tracker was calibrated using a 9-point grid appearing on the PC monitor. Participants first watched a video of a static hand, which lasted approximately 2 min. This was done in order to assess the baseline level of corticospinal excitability; MEPs recorded during this baseline condition were then used to standardize the MEP amplitudes recorded during the experimental conditions. After the baseline condition, participants watched the video stimuli corresponding to the experimental conditions. These videos were organized into two blocks; each video was shown once in each block. Each video was preceded by an instruction screen. For the FV condition, the instructions were as follows: *In the following video, you will see a hand performing thumb movements. Please pay attention to the video throughout.* For the gaze-fixed conditions the instructions were the same as above, but with the addition of the following sentence: *Please maintain your gaze on the red fixation cross throughout the trial.*

The order in which the videos were presented within a block was randomized. There was a break of 10 min between blocks. Each testing session lasted 1.5 h. The experimenter regularly monitored the participants' attentiveness and alertness throughout the protocol.

2.5. Data processing and analysis

Eye movement data were analysed using Eyelink Data Viewer (SR Research Ltd., Ontario, Canada). Saccades were defined as eye movements with velocities and accelerations exceeding 30°/s and 8000°/s^2 respectively; eye movements with velocities and accelerations below these parameters were defined as fixations.

Circular areas of interest (AOIs) corresponding to the required fixation area (see Fig. 1) were created for each of the viewing conditions F1, F2, F3 and F2P. For the FV condition, a static AOI was superimposed over the entire hand, and a dynamic AOI was superimposed over the entire thumb. Preliminary analyses of the gaze data (average fixation duration and average saccade amplitude) identified one participant as a multivariate outlier; hence, this participant was removed from all subsequent analyses. In addition, the gaze data of two participants were discarded due to calibration error.

EMG data were analysed using the analysis features of the acquisition software (Signal v. 4.11, Cambridge Electronic Design Limited, Cambridge, UK). In order to screen the data for trials in which the background EMG exceeded an acceptable threshold, the root mean square of the background EMG during the 90 ms preceding the onset of the pulse was calculated. If the background EMG for a given trial was higher than 60 μV, the trial was excluded from the analysis. Post-experimental analyses revealed that none of the data met this criterion since EMG was continuously monitored and participants were reminded to relax their hand. Peak-to-peak amplitudes were measured for each MEP (mV) and then averaged across conditions. The averaged MEP amplitudes recorded in the various conditions during the first block of trials were compared to those recorded during the second block so as to determine whether there were any changes in MEP due to time. These analyses did not reveal any significant differences (all $p < 0.05$), indicating that there was no overall change in corticospinal excitability over time. Thus, MEP amplitudes were ultimately averaged across both blocks. Averaged amplitudes were normalized to the baseline reference condition (i.e., the static hand) and expressed as a percentage of that value as per the following equation: $X = (a - b)/b$ *100, where X is the normalized amplitude, a is the averaged amplitude recorded in a given condition, and b is the averaged amplitude recorded during the static condition.

Normality tests using Shapiro-Wilk were conducted on the normalized scores. Significant deviations from normality were found in several conditions, all $p < 0.05$; consequently, analyses of MEP amplitudes were performed using non-parametric tests (Friedman's ANOVA). Post-hoc tests using Wilcoxon Signed Ranks Tests were then used for significant interactions. Normality tests also revealed significant deviations from normality for average fixation duration and average saccade amplitude, all $p < 0.05$; subsequent analyses were therefore performed using non-parametric tests.

3. Results

3.1. MEP amplitudes

3.1.1. APB muscle

The MEPs recorded from the APB muscle revealed significant differences between the various conditions, $\chi^2(4) = 13.51$, $p = 0.009$. Post hoc tests were used to compare amplitudes in the free viewing (FV) condition to the amplitudes recoded in each of the thumb (F1, F2, F3) and little finger (F2P) gaze-fixed conditions. These tests revealed significant differences between FV and F2, $Z = -2.53$, $p = 0.011$ (Bonferroni-corrected threshold = 0.013). Specifically, APB MEP amplitudes recorded in condition F2 ($Mdn = 12.23$, $M = 22.16$, $SEM = 12.75$) were significantly higher than those recorded during FV ($Mdn = -5.18$, $M = 3.76$, $SEM = 7.95$). Differences in MEPs recorded between F2P ($Mdn = 9.43$, $M = 15.91$, $SEM = 6.00$) and FV only approached significance, $Z = -2.15$, $p = 0.031$; amplitudes recorded during conditions F1 ($Mdn = -2.29$, $M = 9.4$, $SEM = 12.41$) and F3 ($Mdn = 1.3$, $M = 14.63$, $SEM = 12.07$) were not significantly different from the amplitudes recorded in FV, both $p > 0.29$ (see Fig. 3).

An additional Friedman's ANOVA was conducted to assess differences in the amplitudes recorded across all VG conditions. This analysis revealed no significant differences, $p = 0.107$.

3.1.2. ADM muscle

A second Friedman's ANOVA revealed significant differences in MEP amplitudes recorded from the ADM in the different conditions, $\chi^2(4) = 17.04$, $p = 0.002$. Post-hoc tests revealed that ADM MEP amplitudes recorded during F2 ($Mdn = 5.95$, $M = 15.36$, $SEM = 6.61$) were significantly greater than those recorded during FV ($Mdn = -7.72$, $M = -1.70$, $SEM = 4.71$), $Z = -3.39$, $p = 0.001$. ADM MEP amplitudes recorded during F1 ($Mdn = -11.37$, $M = -6.73$, $SEM = 7.07$), and F3 ($Mdn = -2.34$, $M = -6.25$, $SEM = 4.67$) were not significantly

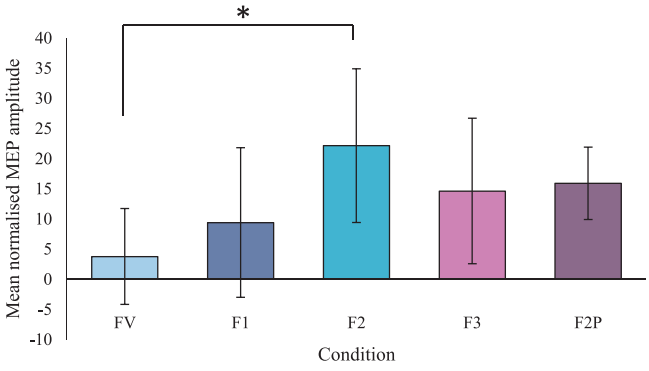

Fig. 3. Mean MEP amplitudes recorded from APB, expressed as a percentage of the baseline condition. Error bars represent standard error of the mean, * $p = 0.011$.

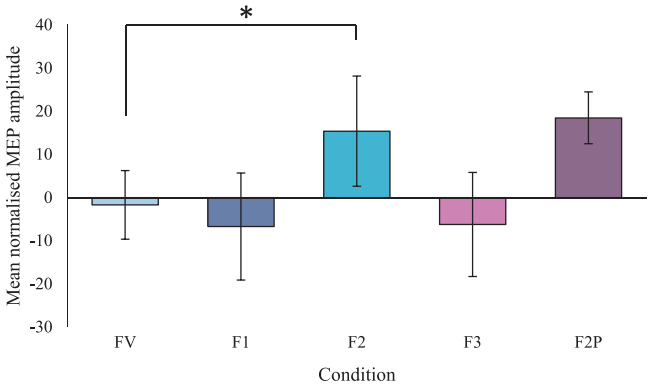

Fig. 4. Mean MEP amplitudes recorded from ADM, expressed as a percentage of the baseline condition. Error bars represent standard error of the mean. * $p = 0.001$.

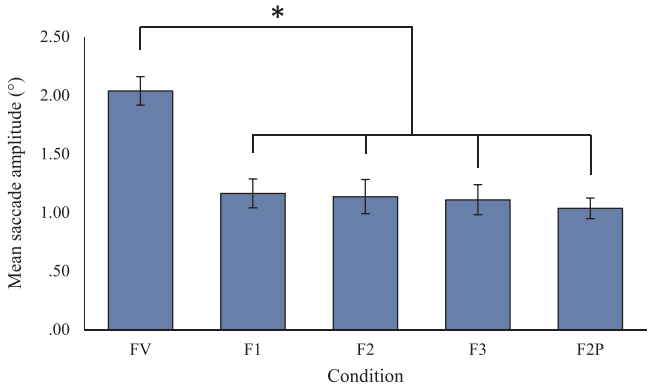

Fig. 6. Mean saccade amplitudes across viewing conditions. Error bars represent standard error of the mean. * $p \leq 0.001$.

different from the amplitudes recorded during FV, both $p > 0.05$ (see Fig. 4). Finally, amplitudes recorded during F2P (*Mdn* = 4.22, *M* = 18.45, *SEM* = 12.81) tended to be higher than those during FV, albeit this difference only approached significance, $p = 0.062$.

A further ANOVA was conducted to compare the normalized amplitudes recorded during all VG conditions. This analysis revealed significant differences, $\chi^2 = 12.46$, $p = 0.006$. Contrasts (Bonferroni corrected threshold = 0.0083) revealed that amplitudes recorded during F2 were higher than amplitudes recorded during F3, $Z = -3.05$, $p = 0.003$.

3.2. Gaze data

Since in the gaze-fixed conditions participants maintained their eyes on the visual guide, we expected gaze metrics not to differ across the four conditions. In contrast, in the free viewing condition participants were free to explore the visual display; hence we expected gaze behaviour to be more varied. In particular, we expected to find saccades of greater amplitudes in the FV condition than in the gaze-fixed ones. Separate Friedman's ANOVAs were used to compare the gaze metrics across all conditions accordingly. For all follow-up contrasts, the Bonferroni corrected threshold was set at 0.005.

For fixation duration, the results showed significant differences between conditions, $\chi^2 = 36.85$, $p < 0.001$. Contrasts showed that fixation durations were significantly shorter in the FV condition compared to F1, F3, F2P (all $Z = -3.52$, $p < 0.001$) and F2, $Z = -3.46$, $p = 0.001$. In contrast, no differences were found between the various VG conditions (Fig. 5).

For saccade amplitude, the ANOVA revealed significant differences, $\chi^2 = 24.85$, $p < 0.001$. Contrasts (Bonferroni corrected threshold = 0.005) revealed that amplitude was larger in the free viewing condition than during F1, $Z = -3.36$, $p = 0.001$; F2, $Z = -3.15$, $p = 0.002$; F3, $Z = -3.46$, $p = 0.001$; and F2P, $Z = -3.51$, $p < 0.001$. In contrast, saccade amplitudes did not differ between the various VG conditions (Fig. 6).

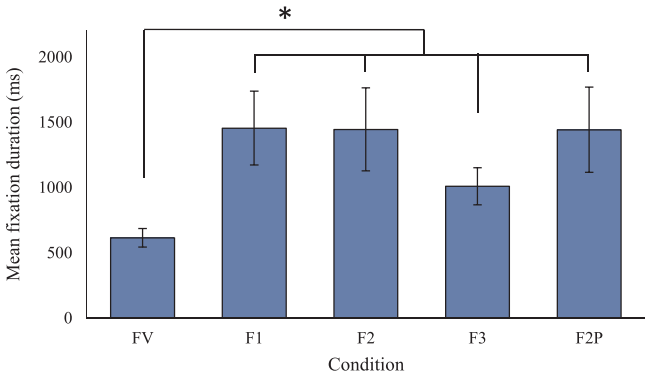

Fig. 5. Mean fixation durations across viewing conditions. Error bars represent standard error of the mean. * $p \leq 0.001$.

With regard to dwell times, analyses of the gaze data revealed that, for each of the gaze-fixed conditions, participants predominantly maintained their gaze on the fixation points as instructed, as per our AOI analysis (Fig. 7). Specifically, mean dwell time percentages for the specified loci ranged from 88.16% to 99.33% (*M* = 95.71, *SEM* = 0.91). In contrast, in the free viewing condition there were large interindividual differences in the percentage of dwell time spent exploring the two elements of the display – namely, the hand and the thumb. Specifically, dwell time on the hand ranged from 5.8% to 91.6% (*M* = 39.69, *SEM* = 7.35), while dwell time on the thumb ranged from 3.4% to 88.3% (*M* = 51.6, *SEM* = 7.51).

With regard to fixation duration and saccade amplitudes, separate Spearman's correlations were conducted in order to assess the relationships between these variables and MEP amplitudes, for both muscles, across all conditions. For APB, these analyses did not reveal any significant correlations, all $p > 0.11$. For ADM, no significant correlations between MEP amplitudes and saccade amplitude or fixation duration were found for conditions F1, F2 and FV, all $p > 0.1$. In contrast, MEPs recorded during condition F2P were positively correlated to the average duration of the fixations made in that condition, $r_s = 0.51$, $p = 0.044$.

As reported above, in the FV condition there was great interindividual variability in the percentage of time that participants spent looking at the hand and thumb. Thus, the relationship between the MEP amplitudes recorded during free viewing, and the gaze behaviour adopted by participants in the same condition may have been modulated by the gaze behaviour adopted by the participant. The relationship between gaze behaviour and MEP amplitudes recorded from the APB and the ADM in the FV condition was consequently subjected to a second-order partial correlation in order to control for the differences in the percentage dwell time for hand and thumb. When controlling for dwell time on the hand and thumb, average saccade amplitude was negatively correlated with APB MEP amplitude, $r_p(11) = -0.80$, $p < 0.001$. In contrast, no significant correlations were found for ADM.

4. Discussion

In the present study we investigated whether motor resonance in M1 during action observation is modulated by the observer's gaze behaviour. We compared MEP amplitudes from muscles of the thumb (APB) and little finger (ADM) when participants viewed video clips of thumb and little finger abduction/adduction under a number of conditions, in which the observer's gaze was either fixed in one of three predetermined loci affording various degrees of transfoveal motion, or when they were able to view the videos as they would normally (i.e., free viewing). We predicted that, by directing participants' gaze to a

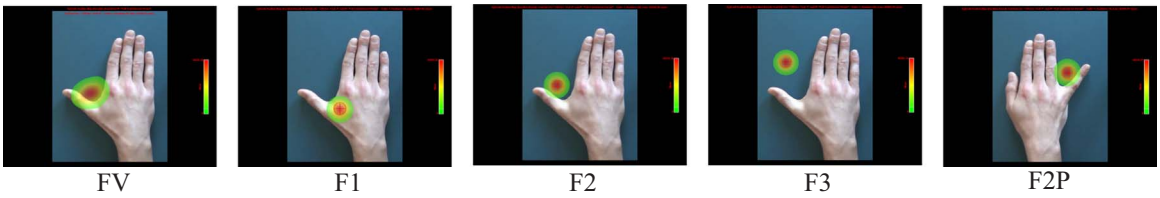

Fig. 7. Heat maps depicting one participant's gaze data, for each condition. Green = shortest dwell time; red = longest dwell time (max duration = 45,801 ms). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

location that maximized biological motion detection, we would observe greater facilitation of M1.

Our findings supported our predictions. The MEP amplitudes were greater when participants maintained their gaze on a point that maximized foveal detection of biological motion (Condition F2) when compared with the free viewing condition. Our gaze data further supported our hypothesis that eye movements would modulate motor resonance, in that MEP amplitudes were contingent on the observer's eye movements. Specifically, when participants were allowed to observe the action as they typically would (i.e., free viewing), MEP amplitudes were negatively correlated with the amplitudes of their saccadic eye movements. Additionally, when point-of-gaze was focused directly over the moving little finger, ADM facilitation increased with fixation duration. This finding, and the fact that the smallest MEP amplitudes were observed in the free viewing (FV) condition, are in line with our prediction that eye movements would inhibit information pickup and thereby reduce motor resonance. This finding supports our assertion that motor resonance during action observation in humans may be contingent on gaze behaviour; it is also consistent with previous research demonstrating gaze-dependency of premotor neurons (Maranesi et al., 2013) and degradation of motor resonance for peripherally-presented stimuli (Leonetti et al., 2015).

In natural contexts, gaze behaviour is comprised of fixations, in which the eye is maintained on a specific location and there is continuous perception of visual input, and saccades, eye movements of varying amplitude and velocity, during which there is a disruption of visual input. In healthy individuals, continuous perception and visual stability are achieved through a mechanism, known as *efference copy* or *corollary discharge*, which updates the retinal coordinates of visual stimuli across eye movements (e.g., Peterburs et al., 2013; Wurtz, 2008). Regardless, saccadic eye movements inevitably involve a suppression of visual input (Ross et al., 2001), particularly with regard to motion processing; in fact, displacement of a visual target goes undetected if it occurs during a saccade (Bridgeman et al., 1975; Shioiri and Cavanagh, 1989), and the perceptual threshold for detection increases with increasing saccade amplitude (Bansal et al., 2015). Thus, since mirror neurons are thought to be responsible for transforming visual information into motor representations and motor knowledge, it could be inferred that during saccadic suppression, the resulting inhibition of visual input may reduce MNS activity.

Alternatively, it could be speculated that our reported relationship between saccades and MEPs were due to intracortical mechanisms of *surround* or *lateral* inhibition. In the latter, the activation of a specific set of neurons is associated with decreased activity in adjacent neurons, to aid in the selection of neural responses and to focus neural activity (Beck and Hallett, 2011). This mechanism has been found to operate in both motor (e.g., Mink, 1996; Poston et al., 2012) and visual areas (Allman et al., 1985; Schwabe et al., 2010). It could therefore be argued that the activity in cortical regions associated with control of eye movements (e.g., frontal eye fields) may have induced inhibition of adjacent premotor areas, for example, resulting in reduced MEP amplitude. However, intracortical inhibition has typically been demonstrated to occur within relatively focused regions of the brain, ones that are functionally and anatomically related.

Another finding of note is the similarity in facilitation that we observed in both APB and ADM muscles during Condition F2 (see Figs. 3 and 4). We expected MEPs recorded from ADM to be greatest in the condition in which gaze was fixated on the little finger (F2P), compared to when point-of-gaze was located over the moving thumb (F2). On the contrary, our results showed that MEP amplitudes recorded from both ADM and APB were largest during the F2 condition. It is possible that, rather than reflecting motor resonance activated by the perception of action, the observed modulations in MEP amplitudes may actually reflect a generic increase in corticospinal excitability as a result of observing a moving stimulus. However, motion perception per se does not result in corticospinal excitability increases (see, for instance, Lepage et al., 2010). In addition, although a number of researchers have reported muscle-specific increases in MEP amplitude as a result of action observation (e.g. Alaerts et al., 2009; Valchev et al., 2015), others have found either a non-specific facilitation, or no facilitation at all. Loporto and colleagues (Loporto et al., 2013) showed participants videos of a static hand (baseline) or of the same hand performing either little finger or index finger adduction/abduction, and recorded MEPs from the FDI and the ADM. The authors found that MEP amplitudes showed facilitation from baseline only for the FDI during observation of index finger movements. In contrast, although ADM amplitudes recorded during observation of little finger movements were higher than those recorded during observation of index finger movements, they did not differ from baseline. Similar findings were reported by Ray et al. (2013), who found no facilitation in flexor pollicis brevis during observation of thumb flexion/extension. Moreover, Kaneko et al. (2007) reported both phase- and muscle-specific facilitation of the FDI during observation of index finger movements, but not in the ADM during observation of little finger movements. These findings are in line with our result, that ADM MEPs were not significantly facilitated during condition F2P. Furthermore, Lepage et al. (2010) asked participants to observe index finger adduction and abduction, and found facilitation in both the ADM and the FDI. This potentially reflects a rapid, automatic response to action observation, resulting in a crude, non-specific mapping of the observed muscle. This suggestion is supported by our findings, which show that ADM amplitudes were facilitated during observation of thumb movements.

A recent published report assessed the neurophysiological effects associated with of action observation suggesting that muscle specificity could be deduced for only 41% of the 85 studies reviewed (Naish et al., 2014). These findings suggest that the motor resonance effect may be muscle- and context-dependant to some degree, and that the muscle-specific aspect of MEP modulations during action observation may have been somewhat overemphasized. Future studies should assess which circumstances can elicit muscle-specific motor resonance by simultaneously recording MEPs from different muscles and using a variety of movements.

An alternative reason for the effects observed in the APB and the ADM may be found in the way in which we determined the optimal scalp position (OSP). Although the cortical representations of APB and ADM have been shown to overlap partially, the APB is located more laterally than the ADM (Pascual-Leone et al., 1994; Wilson et al., 1993), and the optimal coil orientation for stimulating the two muscles is different (Bashir et al., 2013). The *combined hotspot* used in our experiment involves finding a compromise location between the cortical

representations of the muscles of interest, and it is commonly used in TMS studies which target more than one muscle (e.g., Leonard and Tremblay, 2007; Marangon et al., 2013; Stinear and Byblow, 2003). Since in the present study the ADM was consistently less excitable than the FDI, we determined the OSP based on the amplitude of the responses observed in the ADM. Thus, it is possible that our OSP was inadvertently located more towards the centre of the cortical representation of the ADM, which may explain the observed similarity between the responses recorded from our two target muscles., Nonetheless, the combined hotspot method has been shown to yield responses that have a high inter-and intra-session reliability. Since these responses are based on stimulation parameters which take into account the responses of all the target muscles, this method may represent a more rigorous way of assessing the correct location for achieving consistent and reliable responses from all target muscles (Bastani and Jaberzadeh, 2012; see also Loporto et al., 2013).

Finally, it should be noted that normalized amplitudes recorded in the present study ranged from − 54.15 to 175.51 for the APB and from 54.70 to 186.69 for the ADM. The observed similarity between the MEP modulations in both the ADM and the APB may be explained by this high interindividual variability (Table 1 and 2). In an illustration of this phenomenon, Hétu et al. (2010) used TMS to investigate whether observing common everyday movements performed by proximal and distal upper-limb resulted in muscle-specific facilitation. Their participants watched videos of transitive hand and arm actions; TMS-evoked MEPs were recorded from the biceps and two hand muscles – opponens pollicis and first dorsal interosseus. Although their results showed a general muscle-specific effect of action observation, the authors reported high interindividual variability in the pattern of corticospinal facilitation. Whereas the majority of participants showed an increase in MEP amplitudes in the effector muscle, the magnitude of this effect varied greatly between individuals, to the extent that some participants exhibited no facilitation at all. Hétu et al. concluded that such variability reflected differences in observers' ability to precisely map the observed action onto their motor repertoire, which could explain our findings. Specifically, more than one-third of our participants ($n = 6$) exhibited ADM MEP amplitudes that were larger during observation of little finger movements than during observation of thumb movements, as expected. Hence, it is possible that our results simply reflect the fact that the majority of our participants lacked the ability to precisely map the observed action onto their motor system, thereby exhibiting a pattern of corticospinal facilitation that extended to the ADM muscle during observation of thumb movements.

The present study had some limitations. Although in some previous studies researchers have reported significant increases in corticospinal excitability as a result of observing intransitive actions (e.g., Burgess et al., 2013; Romani et al., 2005), such facilitation has typically been confined to the observation of goal-directed actions (e.g., Enticott et al., 2010). Therefore, it could be speculated that, had our participants observed a transitive action, we would have observed even greater facilitation. A second limitation is that participants were not instructed to observe the action with the intention to imitate; doing so elicits greater modulations in motor areas which are part of the putative human mirror neuron system (Buccino et al., 2004; Roosink and Zijdewind, 2010) than does passive observation of the same stimuli. However, our decision not to instruct participants to observe the action with this

Table 1
Normalized MEP amplitudes descriptives. Within participant variances and descriptive statistics for the normalized MEPs amplitudes recorded from the APB and the ADM.

		Condition																			
		F1				F2				F3				FV				F2P			
Muscle	Participant	*M*	*SD*	*SEM*	*Var*	*M*	*SD*	*SEM*	*Var*	*M*	*SD*	*SEM*	*Var*	*M*	*SD*	*SEM*	*Var*	*M*	*SD*	*SEM*	*Var*
APB	1	− 51	53	11	2840	− 19	61	13	3760	− 45	43	9	1854	− 19	55	11	3049	− 10	52	11	2704
	2	− 18	53	11	2786	39	59	12	3460	− 7	51	10	2577	2	65	13	4195	29	72	15	5241
	3	− 25	55	11	3078	− 31	44	9	1931	− 32	43	9	1838	− 28	59	12	3482	− 17	49	10	2369
	4	21	70	14	4928	93	88	18	7676	57	76	16	5820	31	101	21	10144	47	95	19	9016
	5	176	116	24	13,515	57	113	23	12,849	126	115	24	13,276	48	89	18	7852	62	91	19	8326
	6	− 16	51	10	2635	12	48	10	2345	93	103	21	10,580	6	68	14	4588	33	67	15	4537
	7	− 7	33	7	1121	31	70	14	4868	9	41	8	1644	7	69	14	4729	10	32	7	1017
	8	− 2	29	6	847	14	39	8	1543	6	39	8	1545	− 13	25	5	609	8	36	7	1321
	9	− 17	64	13	4102	− 44	25	7	619	− 54	29	6	819	44	144	29	20,611	9	103	21	10,586
	10	20	104	22	10,827	13	116	24	13,365	1	78	16	6104	20	106	22	11,151	32	136	28	18,615
	11	4	78	16	6124	2	52	11	2750	− 10	54	11	2888	− 15	37	8	1380	− 9	54	11	2896
	12	− 14	29	8	817	2	48	10	2268	− 6	33	7	1063	− 9	47	10	2189	5	35	7	1206
	13	7	62	13	3801	− 34	43	12	1850	− 10	70	14	4872	− 37	49	14	2397	13	76	15	5732
	14	57	139	28	19,413	− 1	60	12	3577	69	159	32	25,326	− 23	76	16	5778	− 2	100	20	10,031
	15	1	44	9	1922	0	36	7	1262	− 19	44	9	1907	− 29	29	6	860	− 6	46	9	2106
	16	55	185	42	34,108	74	203	42	41,349	6	83	17	6916	− 5	109	22	11,817	65	180	37	32,267
	17	− 33	62	13	3808	167	248	51	61,667	64	180	37	32,480	86	178	36	31,590	1	92	19	8409
ADM	1	− 27	30	6	906	2	37	8	1392	− 11	33	7	1082	− 17	34	7	1164	− 16	30	6	908
	2	− 19	36	7	1284	1	36	7	1320	− 13	43	9	1842	− 7	34	7	1131	1	30	6	926
	3	− 20	46	9	2151	2	58	12	3407	− 2	49	10	2404	− 18	57	12	3237	− 3	55	11	3031
	4	− 33	56	11	3083	20	91	18	8204	− 14	59	12	3485	− 8	71	14	4994	187	105	21	11022
	5	32	57	12	3273	7	46	9	2145	10	42	9	1730	5	57	12	3287	26	43	9	1830
	6	− 35	44	9	1893	3	46	9	2120	3	35	7	1215	− 12	75	15	5611	− 25	55	12	3041
	7	− 11	22	5	487	6	31	6	948	− 6	31	6	966	5	33	7	1113	4	21	4	445
	8	51	118	24	13,921	− 9	69	14	4817	− 32	31	6	972	− 8	57	12	3302	54	95	19	9049
	9	− 4	53	11	2794	32	84	17	7045	16	63	13	4016	21	71	14	5006	63	108	22	11,666
	10	23	83	17	6908	21	86	17	7326	14	36	7	1325	10	71	15	5095	− 17	46	9	2083
	11	− 25	45	9	2039	89	119	24	14,206	8	59	12	3493	− 11	59	12	3485	− 7	64	13	4125
	12	− 8	17	3	286	− 7	26	5	667	− 8	21	4	460	− 12	32	6	1006	7	17	3	284
	13	− 41	26	5	698	− 57	60	12	3574	− 55	51	10	2573	− 30	66	19	4399	24	99	20	9750
	14	9	82	17	6652	− 17	43	9	1839	− 2	74	15	5483	− 24	62	13	3901	− 19	86	18	7368
	15	4	53	11	2767	48	69	14	4800	2	46	9	2143	0	51	10	2562	13	34	7	1150
	16	37	122	28	14,841	44	115	23	13,223	16	68	14	4580	45	174	36	30,441	62	143	29	20,500
	17	− 50	35	7	1244	33	57	12	3249	− 32	59	12	3461	29	103	21	10,564	− 41	36	7	1292

Table 2
Raw MEP amplitudes descriptives. Within participant variances and descriptive statistics for the raw MEPs amplitudes recorded from the APB and the ADM.

		Condition																			
		F1				F2				F3				FV				F2P			
Muscle	Participant	*M*	*SD*	*SEM*	*Var*	*M*	*SD*	*SEM*	*Var*	*M*	*SD*	*SEM*	*Var*	*M*	*SD*	*SEM*	*Var*	*M*	*SD*	*SEM*	*Var*
APB	1	0.50	0.53	0.11	0.29	0.82	0.62	0.13	0.38	0.55	0.43	0.09	0.19	0.81	0.55	0.11	0.31	1.00	0.75	0.14	0.56
	2	0.52	0.34	0.07	0.11	0.88	0.37	0.08	0.14	0.59	0.32	0.07	0.10	0.65	0.41	0.08	0.17	0.63	0.33	0.06	0.11
	3	0.29	0.22	0.04	0.05	0.27	0.17	0.03	0.03	0.27	0.17	0.03	0.03	0.28	0.23	0.05	0.05	0.39	0.26	0.05	0.07
	4	0.89	0.51	0.10	0.26	1.41	0.64	0.13	0.41	1.15	0.56	0.11	0.31	0.96	0.74	0.15	0.54	0.73	0.52	0.10	0.27
	5	0.34	0.14	0.03	0.02	0.19	0.14	0.03	0.02	0.27	0.14	0.03	0.02	0.18	0.11	0.02	0.01	0.12	0.07	0.01	0.01
	6	0.05	0.03	0.01	0.00	0.07	0.03	0.01	0.00	0.12	0.07	0.01	0.00	0.07	0.04	0.01	0.00	0.06	0.04	0.01	0.00
	7	0.50	0.18	0.04	0.03	0.71	0.38	0.08	0.14	0.59	0.22	0.04	0.05	0.58	0.37	0.08	0.14	0.54	0.24	0.04	0.06
	8	1.33	0.40	0.08	0.16	1.55	0.53	0.11	0.29	1.44	0.53	0.11	0.29	1.18	0.34	0.07	0.11	1.36	0.38	0.07	0.14
	9	0.49	0.38	0.08	0.14	0.33	0.15	0.04	0.02	0.27	0.17	0.03	0.03	0.85	0.84	0.17	0.71	0.59	0.60	0.11	0.36
	10	0.24	0.21	0.04	0.04	0.23	0.23	0.05	0.05	0.20	0.16	0.03	0.02	0.24	0.21	0.04	0.04	0.20	0.20	0.04	0.04
	11	0.12	0.09	0.02	0.01	0.12	0.06	0.01	0.00	0.10	0.06	0.01	0.00	0.10	0.04	0.01	0.00	0.11	0.06	0.01	0.00
	12	0.60	0.20	0.06	0.04	0.71	0.33	0.07	0.11	0.66	0.23	0.05	0.05	0.64	0.33	0.07	0.11	0.70	0.24	0.04	0.06
	13	0.41	0.24	0.05	0.06	0.26	0.17	0.05	0.03	0.35	0.27	0.05	0.07	0.24	0.19	0.05	0.04	0.39	0.36	0.09	0.13
	14	0.53	0.47	0.10	0.22	0.33	0.20	0.04	0.04	0.57	0.54	0.11	0.29	0.26	0.26	0.05	0.07	0.34	0.30	0.05	0.09
	15	1.91	0.83	0.17	0.69	1.90	0.67	0.14	0.45	1.54	0.83	0.17	0.68	1.34	0.56	0.11	0.31	1.89	0.65	0.12	0.42
	16	0.34	0.41	0.09	0.17	0.38	0.45	0.09	0.20	0.23	0.18	0.04	0.03	0.21	0.24	0.05	0.06	0.22	0.21	0.04	0.04
	17	0.06	0.06	0.01	0.00	0.25	0.23	0.05	0.05	0.15	0.17	0.03	0.03	0.17	0.17	0.03	0.03	0.09	0.09	0.02	0.01
ADM	1	0.50	0.53	0.11	0.29	0.82	0.62	0.13	0.38	0.55	0.43	0.09	0.19	0.81	0.55	0.11	0.31	1.00	0.75	0.14	0.56
	2	0.52	0.34	0.07	0.11	0.88	0.37	0.08	0.14	0.59	0.32	0.07	0.10	0.65	0.41	0.08	0.17	0.63	0.33	0.06	0.11
	3	0.29	0.22	0.04	0.05	0.27	0.17	0.03	0.03	0.27	0.17	0.03	0.03	0.28	0.23	0.05	0.05	0.39	0.26	0.05	0.07
	4	0.89	0.51	0.10	0.26	1.41	0.64	0.13	0.41	1.15	0.56	0.11	0.31	0.96	0.74	0.15	0.54	0.73	0.52	0.10	0.27
	5	0.34	0.14	0.03	0.02	0.19	0.14	0.03	0.02	0.27	0.14	0.03	0.02	0.18	0.11	0.02	0.01	0.12	0.07	0.01	0.01
	6	0.05	0.03	0.01	0.00	0.07	0.03	0.01	0.00	0.12	0.07	0.01	0.00	0.07	0.04	0.01	0.00	0.06	0.04	0.01	0.00
	7	0.50	0.18	0.04	0.03	0.71	0.38	0.08	0.14	0.59	0.22	0.04	0.05	0.58	0.37	0.08	0.14	0.54	0.24	0.04	0.06
	8	1.33	0.40	0.08	0.16	1.55	0.53	0.11	0.29	1.44	0.53	0.11	0.29	1.18	0.34	0.07	0.11	1.36	0.38	0.07	0.14
	9	0.49	0.38	0.08	0.14	0.33	0.15	0.04	0.02	0.27	0.17	0.03	0.03	0.85	0.84	0.17	0.71	0.59	0.60	0.11	0.36
	10	0.24	0.21	0.04	0.04	0.23	0.23	0.05	0.05	0.20	0.16	0.03	0.02	0.24	0.21	0.04	0.04	0.20	0.20	0.04	0.04
	11	0.12	0.09	0.02	0.01	0.12	0.06	0.01	0.00	0.10	0.06	0.01	0.00	0.10	0.04	0.01	0.00	0.11	0.06	0.01	0.00
	12	0.60	0.20	0.06	0.04	0.71	0.33	0.07	0.11	0.66	0.23	0.05	0.05	0.64	0.33	0.07	0.11	0.70	0.24	0.04	0.06
	13	0.41	0.24	0.05	0.06	0.26	0.17	0.05	0.03	0.35	0.27	0.05	0.07	0.24	0.19	0.05	0.04	0.39	0.36	0.09	0.13
	14	0.53	0.47	0.10	0.22	0.33	0.20	0.04	0.04	0.57	0.54	0.11	0.29	0.26	0.26	0.05	0.07	0.34	0.30	0.05	0.09
	15	1.91	0.83	0.17	0.69	1.90	0.67	0.14	0.45	1.54	0.83	0.17	0.68	1.34	0.56	0.11	0.31	1.89	0.65	0.12	0.42
	16	0.34	0.41	0.09	0.17	0.38	0.45	0.09	0.20	0.23	0.18	0.04	0.03	0.21	0.24	0.05	0.06	0.22	0.21	0.04	0.04
	17	0.06	0.06	0.01	0.00	0.25	0.23	0.05	0.05	0.15	0.17	0.03	0.03	0.17	0.17	0.03	0.03	0.09	0.09	0.02	0.01

intention was due to the fact that the stimulus employed consisted of a very simple action, which was already present in the motor repertoire of our observers. Furthermore, since simple adduction/abduction movements represent such a common, everyday action, it is possible that, by instructing participants to observe the action with the intention to imitate, we might have inadvertently prompted them to look for additional information, potentially compromising our point-of-gaze manipulations.

Our findings extend previous work, by providing further evidence of a link between gaze and motor resonance. Specifically, they suggest that, during observation of single-joint actions such as those used in the current study, maintenance of a relatively fixed point-of-gaze facilitates M1 to a greater extent than does natural viewing. Larger MEPs can be taken as an index of motor expertise, in that the amount of motor resonance during the observation of an action is greater for previously learned actions that are already present in the observer's motor repertoire (e.g., Jola et al., 2012).

Thus, our findings raise the possibility that the pickup of information, and therefore observational learning, may be facilitated by adopting specific gaze strategies (see also Hétu et al., 2010). Specifically, learners may benefit from reducing eye movements, which can compromise the extraction of visual information, while at the same time maintaining their visual attention on loci that maximize motor resonance. Previous research has shown that such guidance can facilitate novices' perception of biological motion, consequently enhancing their perceptual and/or motor performance (D'Innocenzo et al., 2016; Jarodzka et al., 2013; Vine et al., 2012).

Practically, the notion of an optimal fixation point has implications for action observation in clinical and performance settings. For example, AO is increasingly being used as a means of motor and cognitive recovery from cerebral palsy, stroke and Parkinson's disease (Abbruzzese et al., 2015; Buccino, 2014; Ertelt et al., 2007). Research has consistently shown that action observation-based therapies improve motor function and increase activity in areas composing the observation-execution matching system; that is, the human correlate of the mirror neuron system (for a recent review, see Buccino, 2014). However, the effectiveness of protocols in which action observation is used to teach novel motor skills, or improve motor function, may depend on the learner's ability to maintain a suitable point-of-gaze. Consequently, by directing learners' gaze appropriately, we may maximize corticospinal facilitation and thereby accelerate motor skill acquisition/reacquisition.

To conclude, the present study contributes to the existing literature by providing evidence of a link between gaze and motor resonance, as indexed by MEP amplitudes. Motor resonance during action observation is thought to reflect the amount of learning and expertise with the observed action (Jola et al., 2012). Our results show that the amount of motor resonance in the observer's motor cortex can be maximized by adopting specific gaze behaviours during action observation. This is a novel finding, and one which suggests that approaches based on directing learner's gaze to increase motor resonance may allow us to effectively accelerate learning, or re-learning, of simple motor actions.

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