Title: Selective enhancement of motor cortical plasticity by observed mirror-matched actions

Authors: Martin V. Sale[a] and Jason B. Mattingley[a,b]

[a] Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia

[b] School of Psychology, The University of Queensland, Brisbane, QLD 4072, Australia

Corresponding author

Dr Martin Sale

Queensland Brain Institute

The University of Queensland

Brisbane

AUSTRALIA

Phone: +61 7 3346 3305

FAX: +61 7 3346 6301

Email: m.sale@uq.edu.au

Abstract

Watching others learn a motor task can enhance an observer's own later performance when learning the same motor task. This is thought to be due to activation of the action observation (or mirror neuron) network. Here we show that the effectiveness of plasticity induced in human motor cortex (M1) is also significantly influenced by the nature of prior action observation. In separate sessions, 17 participants watched a video showing repeated goal-directed movements (action observation) involving either the right hand (congruent condition) or the same video mirror-reversed to simulate the left hand (incongruent condition). Participants then received pulses of transcranial magnetic stimulation over the hand area of left M1 paired with median nerve stimulation of the right hand (paired associative stimulation; PAS). The resting motor-evoked potential (MEP) in right abductor pollicis brevis (APB) increased significantly 20 minutes after PAS, but only when participants had previously watched the congruent video. In this condition, all participants showed an increase in MEP amplitude at 20 minutes post-PAS. There was no change in MEP amplitude following PAS when participants watched the incongruent video. We conclude that prior action observation is a potent modulator of subsequent PAS-induced neuroplasticity, which may have important therapeutic applications.

Keywords: action observation; mirror neurons; neuroplasticity; transcranial magnetic stimulation; paired associative stimulation

1. Introduction

Several experimental paradigms have recently been developed that induce plasticity within the human cortex using non-invasive stimulation (Huang et al., 2005; Pascual-Leone et al., 1994; Ridding et al., 2001; Stefan et al., 2000). Plasticity refers to a change in central nervous system structure and function, and is critical for learning and memory (Sanes and Donoghue, 2000), and recovery from nervous system injury (Nudo et al., 1996). Research has focussed on improving functional recovery after brain injury (particularly stroke), with studies demonstrating improvement in function in stroke patients with such stimulation paradigms used on their own (Kim et al., 2006), or in conjunction with physical therapy (McDonnell et al., 2007). Unfortunately, the functional gains reported have generally been modest. This is probably due in part to individual differences in responsiveness to stimulation.

Many factors appear important in mediating plasticity induction in humans (for review see Ridding and Ziemann (2010)). One such factor is the history of prior cortical activity (Iyer et al., 2003; Muller et al., 2007; Stefan et al., 2006). One way of modifying cortical activity in the motor system is by observing others perform a matching movement. It is now well established that a specific set of neurons is activated during both action observation and action execution. Originally found in monkeys (Rizzolatti et al., 1996a), but also thought to be present in humans (Chong et al., 2008; Kilner et al., 2009; Rizzolatti et al., 1996b), mirror neurons are active when individuals perform a goal-directed movement and also when they observe another individual performing a matching goal-directed movement. Prior activation of such a network has been suggested to influence subsequent motor learning (Mattar and Gribble, 2005).

An ‘artificial’ paradigm has been developed which mimics the use-dependent plasticity associated with motor learning (Stefan et al., 2000). This paired associative stimulation (PAS) paradigm pairs a peripheral electrical stimulus delivered to a nerve innervating a muscle in the hand, with a pulse of transcranial magnetic stimulation (TMS) to the corresponding motor representation in the contralateral motor cortex. The changes induced with PAS are thought to reflect long-term potentiation (LTP)-like changes in synaptic efficacy (Stefan et al., 2002). The circuits activated by PAS are the same as – or at least very similar to – the circuits activated by motor learning (Ziemann et al., 2004). Importantly, PAS requires no muscle activation to induce plasticity in motor cortex, and could potentially offer advantages in neurorehabilitation (compared with motor training), particularly when voluntary muscle activation is not possible (due to hemiplegia), or even deleterious (dystonia).

We therefore investigated whether PAS-induced plasticity could be enhanced by prior action observation. Specifically, we hypothesised that action observation should enhance the effects of subsequent PAS-induced plasticity, but only when the observed action activates the same circuits as those modified during PAS.

2. Materials and Methods

2.1 Participants

Seventeen participants (mean age, 26.29 ± 1.39 years; 9 females) took part in the study. All participants were right handed (median laterality quotient = 0.84, range 0.30-1.00) as assessed by the Oldfield handedness questionnaire (Oldfield, 1971). All participants gave written informed consent prior to participation in the study, which was approved by the University of Queensland Medical Research Ethics Committee.

2.2 Overview of Experimental Procedure

Participants attended two experimental sessions, separated by at least one week. In each session they were required to watch an action observation video (15 minutes), after which plasticity was induced in the motor cortex using TMS and concurrent stimulation of the median nerve (the PAS procedure). In order to activate the same neural circuits as those stimulated during action observation, participants performed a simple action execution task during the PAS procedure. The two sessions were identical except that in one, participants watched a video in which a model performed actions with the same (right) hand as the participant during the subsequent PAS procedure, whereas in the other session the video showed the same actions performed with the opposite (left) hand (via mirror-reversal of a common video source). Cortical excitability was probed before action observation, as well as before and after plasticity induction, to quantify changes in plasticity in the different sessions. An overview of the experimental set-up is shown in Figure 1.

2.3 Experimental Arrangement

Participants were seated comfortably in an experimental chair with their arms comfortably resting on a table. Surface electromyographic (EMG) recordings from the abductor pollicis brevis (APB) muscle of the right hand were obtained using bipolar Ag-AgCl electrodes in a belly-tendon montage. EMG signals were amplified 1000 times, filtered (5 Hz – 500 Hz via a NeuroLog system (Digitimer, UK), digitized online (2 kHz/channel) with a data acquisition interface (BNC-2110; National

Instruments, USA) and custom MatLab software (Mathworks, USA) and stored on computer for offline analysis. The EMG signal from APB muscle was displayed on an oscilloscope to help participants maintain EMG silence when required.

2.4 Transcranial magnetic stimulation (TMS)

All participants completed a TMS safety screen (Keel et al., 2001), and were excluded if there was a family history of epilepsy, they were taking any neuroactive drugs or had undergone neurosurgery. Monophasic TMS was applied through a figure-of-eight coil (outer diameter of each wing 70mm) connected to a Magstim 200 magnetic stimulator (Magstim, Whitland, Dyfed, UK). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane at the optimal scalp site to evoke an MEP in the relaxed APB muscle of the right hand. With this coil placement, current flow was induced in a posterior to anterior direction in the brain. The optimal scalp position was marked with a pen, and the coil was held throughout the experiment by hand, with the position continually checked throughout the experiment.

2.5 TMS measures of motor cortex excitability

Motor cortex excitability was assessed at several time points during each experimental session (see Figure 1B for time-line of experimental assessments).

Mean peak-to-peak amplitude of the APB MEP at rest was calculated by averaging the individual peak-to-peak amplitudes of MEPs elicited by 20 separate TMS pulses (~0.2 s^{-1}). The stimulus intensity was expressed as a percentage of maximum stimulator output (% MSO). MEPs were evoked prior to action observation, following action observation (immediately prior to PAS) and 5 minutes following PAS. In order to investigate longer-lasting changes arising from PAS, we also probed cortical excitability 20 minutes following PAS in all experiments.

Resting motor threshold (RMT) was defined as the minimum stimulus intensity required to evoke an MEP in the relaxed APB of > 50 μV in 3 out of 5 consecutive trials (Carroll et al., 2001). RMT was assessed prior to action observation, and after the 5-minute post-PAS MEP measures were obtained.

2.6 Action observation

Participants watched one of two action observation movies in each experiment. The videos consisted of either “congruent” movements or “incongruent” movements. Congruency relates to the relationship between the hand observed in the videos and the hand muscle targeted by PAS/used to

perform actions (action execution) – see below. The other video was viewed during the second experimental session, and the order of videos watched was counterbalanced. Each video was 15 minutes long, and consisted of 90 short clips, each 10 seconds in duration. The 10-second clips showed a human hand and arm orientated in the first person picking up an orange ball 4 cm in diameter and placing it into a cylindrical tube (see Figure 1A). The hand picked up the ball using only the index finger and thumb. After the ball was placed in the tube, the hand returned to the original resting position. Participants' attention has been shown to be an important mediator of plasticity (Kamke et al., 2012; Stefan et al., 2004). Therefore, in order to maintain participants' attention during action observation, in a few trials (8-10/90) the ball was placed in the central cylinder rather than the right-most cylinder. Participants were instructed to pay attention to the hand and the task being performed, and to count the number of balls placed in the central cylinder. At the end of the 15-minute video, the number of balls placed into the central cylinder was reported. During observation of the movies EMG of APB was displayed online. Participants were instructed to keep their hands relaxed, and if EMG-related activity was detected, participants were reminded to relax their hand.

The "congruent" condition showed a model picking up the balls with his right hand, whereas the "incongruent" condition showed the same video that had been mirror-reversed (using iMovie version 8.0.6) so that it appeared that the model's left hand was performing the action.

2.7 Paired associative stimulation (PAS)

The PAS paradigm involves a series of paired peripheral and cortical stimuli. An electrical stimulus was delivered to the median nerve of the right wrist at an intensity equivalent to 200% of perceptual threshold, using a constant current stimulator (DS7 stimulator; Digitimer, UK) with bipolar surface electrodes separated by 30 mm, and with the cathode proximal. Stimuli were square waves with a pulse width of 200μs. The electrical stimulus was followed 25 ms later by suprathreshold TMS over the hand area of the contralateral (left) motor cortex. TMS intensity was established prior to PAS, and was adjusted to evoke an MEP in resting APB of 0.5-1.0 mV. For PAS, 90 paired peripheral and cortical stimuli were delivered at a frequency of 0.1 Hz (duration 15 mins). All experiments were performed at approximately the same time of day (~ 2pm) to minimise response variability due to circadian factors (Sale et al., 2007, 2008).

2.8 Action execution

The excitability of motor cortical neurons activated by TMS is enhanced during preparation for action of a visually guided grasping movement (Prabhu et al., 2007). Therefore, in order to preferentially target those networks activated during congruent action observation (and thus to establish congruency), the PAS paradigm was modified so that the effects of PAS were targeted to these neurons. Thus, an action execution task was performed immediately after each paired stimulus of PAS. Although we felt that combining PAS with subsequent action execution would help to target the same neuronal population during action observation and PAS, it is possible that action execution might have an independent effect on MEP amplitude, simply by virtue of the motoric component of the task. As such, we performed a series of control experiments to probe the relative influences of action observation, PAS, and action execution on MEP amplitudes. These control experiments are outlined in detail below. Prior to commencing PAS, a custom-made device was placed on the table in front of the participant (the same as that which was shown in the action observation videos, see Figure 1A). The device allowed for the controlled release of plastic balls, and tubes in which the balls were to be placed.

Participants were instructed to place the ball in one of two tubes as quickly as possible. The cue to grasp and move the ball was when the participants felt and/or heard the TMS pulse over contralateral M1 (as part of PAS procedure). Whilst participants were waiting for the cue to move, they were instructed to engage the motor command required to perform the movement. Importantly, PAS was performed whilst participants were preparing to execute the action, but *before movement initiation*. The tubes were at an equidistance of 43 cm from the participant, directly in front of him or her. The decision on where to place the ball was determined by the perceived weight of the ball. A small number of balls (8/90) were slightly heavier than the others. These balls were visually indistinguishable from the remaining balls, and were randomly placed within the sequence of balls to be picked up. The difference in weight between the balls was subtle, and required participants to pay attention to the weight of each ball. This was to try to maintain participants' alertness and attention during action execution. Participants were instructed to place the lighter balls into the tube positioned furthest to the right, and the heavier balls into the tube in the centre (see Figure 1A). It should be noted that the left-most tube was never used; it was included to replicate the visual scene displayed in the action observation video. After the ball was dropped into the tube, participants returned their hand into the resting position, and waited for the next TMS pulse. Between movements, participants were

instructed to place their hand in a relaxed position, with the right index finger and thumb resting on the ball (EMG activity was monitored online, and hand position was modified as required to maintain EMG silence between trials).

2.9 Control experiments

We conducted a series of control experiments to determine the relative influence of action observation, PAS and action execution on changes in MEP amplitude.

Control Experiment 1 – Action observation alone: Participants (n = 6) watched only the congruent video and MEPs (n = 20) were obtained prior to observation, and then re-assessed at 5-minute intervals (commencing 5 minutes after the video had ceased) up to 40 minutes following action observation. As in the action observation condition in the main experiment, participants' attention was maintained by asking them to count the number of balls placed in the central cylinder (see Action observation section above). Participants never had to execute movements in this condition, and they did not receive PAS. This experiment investigated whether prior action observation alone, in the absence of PAS and action execution, resulted in long-lasting changes in cortical excitability.

The remainder of the control experiments involved nine participants, who each attended three sessions, separated by approximately one week.

Control Experiment 2 – Action observation and action execution: This session was also similar to that of the main experiment, except that there was no PAS component. Participants watched the congruent video (and attended to the placement of the balls in the appropriate cylinder as in the main experiment) and then performed the action execution sequence (and monitored for the weight of the balls as in the main experiment). The TMS unit was triggered as for PAS (but not placed on the participant's head), which provided the participant with the auditory cue to move for the action execution. As with the main experiment, participants were asked to be prepared to move prior to the TMS pulse, but to maintain EMG silence until they heard the TMS click. MEPs (n = 20) were obtained before the video, after the video/before action execution, and 5-minutes and 20-minutes after action execution.

Control Experiment 3 – PAS and action execution: Participants received PAS and were required to perform the action execution sequence (again, attending to the weight of the balls and placing them in the appropriate cylinder), but they did not observe any hand-action videos. MEPs were obtained prior to PAS/action execution, and 5-minutes and 20-minutes after PAS/action execution.

Control Experiment 4 – Action observation and PAS: This session was similar to that of the main experiment, except that there was no action execution component. Participants watched the congruent video and then received PAS. MEPs (n = 20) were obtained before the video, after the video/before PAS, and 5-minutes and 20-minutes after PAS.

2.10 Statistical analysis

A two-way repeated measures analysis of variance (ANOVA) was performed on MEP amplitude data from APB with within-subject factors of Condition (two levels: congruent and incongruent) and Time (four levels: pre-observation, post-observation/pre-PAS, post-PAS 5 and post-PAS 20), to determine the effect of action observation and PAS on the extent of any MEP facilitation. A one-way repeated measures ANOVA assessed the effect of Intervention (two levels: pre-Action Observation and post-PAS) on APB resting motor threshold.

The analysis for the control experiments consisted of a one-way repeated measures ANOVA which assessed the effect of action observation on MEP amplitudes at various time points (ten levels: pre-Action Observation, post-Action Observation 0 mins, 5 mins, 10 mins, 15 mins, 20 mins, 25 mins, 30 mins, 35 mins and 40 mins). Finally, a two-way repeated measures ANOVA assessed the effect of the three different Interventions (three levels: action observation and execution, PAS and action execution, action observation and PAS,) on MEP amplitudes at three different time points (three levels: pre PAS +/- action execution, post-5 mins, post-20 mins).

For all analyses $P < 0.05$ was chosen as the significance level, and unless stated otherwise, all group data are reported as mean ± SEM. *Post hoc* tests were performed as appropriate and were adjusted for multiple comparisons.

3. Results

All participants completed the experimental sessions, and no adverse effects were reported.

3.1 Main experiment

TMS intensity used for test MEPs was not significantly different between incongruent and congruent sessions (45.9 ± 2.2% MSO vs. 45.2 ± 1.7 % MSO, respectively) (P = 0.94). The intensity of peripheral nerve stimulation during PAS was 5.4 ± 0.3 mA for the incongruent session, and 5.0 ± 0.3 mA for the congruent session, a non-significant difference (P = 0.75).

Resting motor threshold was not significantly different across sessions (P = 0.34), and was unchanged following PAS (P = 0.49). Motor cortical excitability was not significantly different prior to the action observation sessions (incongruent APB MEP amplitude = 0.65 ± 0.08 mV, congruent APB MEP amplitude = 0.73 ± 0.11 mV) (P = 0.60). These results indicate that the stimulus intensities used for PAS, and to assess MEP amplitude, were well matched across the two sessions.

Motor cortical excitability was unaffected immediately following observation of either the congruent or incongruent video ($F_{1,16} = 1.243$). During the congruent observation condition MEP amplitude was 0.73 ± 0.11 mV prior to observation, and 0.71 ± 0.12 mV following observation, a non-significant difference (P = 0.92). During the incongruent observation condition MEP amplitude was 0.65 ± 0.08 mV prior to observation, and 0.79 ± 0.10 mV following observation, also a non-significant difference (P = 0.33).

By contrast, there was a significant change in motor cortical excitability following PAS that was influenced by the congruency of prior action observation. When participants watched the congruent video, motor cortical excitability increased from pre-PAS levels. By contrast, when the incongruent video was watched, post-PAS MEPs remained unchanged. The changes in APB MEP amplitude following PAS are shown in Figure 2, plotted separately for the congruent and incongruent action-observation conditions. Repeated-measures ANOVA revealed a significant main effect of Time ($F_{3,48} = 7.059$, $P < 0.001$), as well as a significant Time x Condition interaction ($F_{3,48} = 4.241$, $P < 0.01$), indicating that the effect of time was influenced by which video had been watched previously. Post-hoc analyses indicated that the change in MEP amplitudes following PAS was restricted to the condition in which participants watched the congruent video. In this condition, MEP amplitudes assessed 5 minutes post-PAS increased from pre-PAS levels (> 100% pre-PAS MEP amplitude) in

11/17 subjects. At the group level, this was associated with a non-significant 24% increase in MEP amplitudes (P = 0.26) at this time point. MEPs assessed 20 minutes after PAS increased *in all 17 participants* compared with pre-PAS levels. Group analysis showed that MEPs increased significantly by 73% compared with pre-PAS MEPs (P < 0.001).

There was no significant change in MEP amplitudes in the incongruent condition. In this condition, MEP amplitudes assessed 5 minutes post-PAS were associated with a non-significant 35% increase in MEP amplitudes (P = 0.07), and MEPs assessed 20 minutes after PAS were associated with a non-significant 13% increase compared with pre-PAS MEPs (P = 0.29).

3.2 Control Experiments

Control Experiment 1 revealed that action observation alone did not cause long-term changes in motor cortical excitability, and thus could not be the sole contributor to the changes in MEP amplitudes we observed in the main experiment following PAS (Figure 3A). MEP amplitude was unchanged following action observation (P = 0.32). MEP amplitude prior to action observation was = 0.43 ± 0.06 mV. MEP amplitudes at 0, 5, 10, 15, 20, 25, 30, 35 and 40 minutes following action observation ranged from 0.34 ± 0.04 mV to 0.54 ± 0.05 mV.

When the effects of interactions between action observation, PAS and action execution were more extensively investigated, we found that long-term changes in MEP amplitudes were only evident when action observation was followed by PAS (Control Experiment 4; Figure 3B). ANOVA revealed a significant main effect of Intervention ($F_{2,32} = 6.399$, $P = 0.009$), as well as a significant Intervention x Time interaction ($F_{4,32} = 2.978$, $P = 0.034$), indicating that the effect of time was influenced by this particular combination of interventions. Post-hoc analysis revealed that MEP amplitude was greater 20 minutes after PAS compared with both the pre-PAS (71% increase) and 5-minute post-PAS (21% increase) levels, but *only when it was preceded by action observation*. Additionally, MEP amplitude 20 minutes after PAS (when preceded by action observation) was significantly greater than the same time point for the action observation and action execution condition (Control Experiment 2), and the PAS and action execution condition (Control Experiment 3).

4. Discussion

Here we have shown for the first time that the plastic effects induced in human motor cortex by a paired associative stimulation (PAS) paradigm are influenced by the congruency of previously

observed, repeated goal-directed movements. The network of neurons targeted with PAS was most effectively altered when participants observed a task that activated the same (or a spatially similar) network of neurons. Thus, for the congruent condition in which the model's hand actions were matched with those made subsequently by the participant, MEPs increased on average by 73%, (and in all participants) when measured 20 minutes after PAS. By contrast, when the incongruent video was observed, there was no significant change in MEP amplitudes for the group. Observing congruent actions more than doubled MEP amplitudes following PAS relative to observing incongruent actions, thus supporting our initial hypothesis that mirror-matched action observation enhances TMS-induced plasticity in the primary motor cortex.

To our knowledge, this is the first time that the action observation network has been engaged to enhance PAS-induced motor cortical plasticity. Mattar and Gribble (2005) showed that motor learning during a demanding training task is influenced by prior action observation. Their study was purely behavioural, however, and so they were not able to determine how action observation and subsequent training influence motor system activity. Moreover, Mattar and Gribble (2005) had their participants repeatedly practice visually guided arm movements in response to perturbations induced by a novel force environment. Performance was better when participants had previously observed a model performing actions in the same altered environment, and worse when they had observed actions in a different environment. By contrast, we used TMS and paired associative stimulation to *passively* induce plasticity in the primary motor cortex, during periods in which participants were *at rest* and preparing to perform a hand movement. Our results thus demonstrate that action observation can exert an influence on motor cortex even when plasticity is induced via external stimulation (TMS), and while the responding hand is at rest and merely preparing for action.

Results from the control experiments indicate that the repeated upper limb movements that occurred during action execution did not drive the increase in MEP amplitudes we found in the congruent condition of the main experiment. There was no change in MEP amplitude when action observation was followed by action execution without the subsequent PAS protocol (Control Experiment 2, Figure 3B). Crucially, observation of the congruent condition followed by PAS (and not combined with action execution) produced similar increases in MEP amplitude in a subset of participants compared to when PAS was combined with action execution and preceded by action observation (71% and 73% increase respectively). This provides compelling evidence that the large,

consistent changes in MEP amplitude we found in the main experiment were driven almost exclusively by the interaction between action observation and subsequent PAS. The motoric component of the main experiment appears to have had little or no measurable influence on increasing or decreasing the effects of PAS.

The changes in cortical excitability following PAS reported in the present study were only evident 20 minutes after PAS, and not at the 5-minute post-PAS time point. Previous studies investigating the time course of MEP changes following PAS have reported significant enhancement in MEPs 5 minutes after the intervention, which persists at 20 minutes (Castel-Lacanal et al., 2007; Stefan et al., 2000). We are not aware of studies that have reported a delay in the onset of MEP facilitation similar to what we have observed here. We speculate that the delayed effect might be unique to prior action observation, and its influence upon and interaction with subsequent PAS. One possibility is that action observation causes an enduring increase in motor cortical excitability, and that this contributes to elevated MEPs after PAS. In this context it is important to note, however, that in Control Experiment 1 action observation alone (i.e., without PAS or action execution) was *not* associated with an increase in MEP amplitudes at any time point following observation.

Previous studies have shown that action observation can enhance cortical excitability *during* action observation (Fadiga et al., 1995), but there is also evidence to suggest it can influence *longer-term* changes in motor cortical function (Stefan et al., 2005). Clearly, then, action observation can have a long-term influence on motor cortical plasticity. We hypothesise that this influence arises upstream from M1. It is well established that action observation activates the ventral premotor cortex (PMv) (Iacoboni et al., 1999), that the connections between PMv and M1 are enhanced with action observation (Koch et al., 2010), and that disruption of PMv with inhibitory rTMS can disrupt the contribution of action observation to use-dependent plasticity (Cantarero et al., 2011). Based on our findings, we speculate that action observation triggers a locally specific increase in functional connectivity between PMv and M1. This PMv-M1 pathway is subsequently re-engaged, more effectively, when participants receive PAS as they prepare to execute a motor command, but prior to action execution. By such a mechanism, the excitability of M1 neurons should be increased, thus rendering them more susceptible to PAS-induced plasticity.

Although the therapeutic potential of various repetitive TMS paradigms (including PAS) has been demonstrated in the clinical sphere (for review see Ridding and Rothwell (2007)), particularly in stroke

recovery (Kim et al., 2006; McDonnell et al., 2007), reported performance improvements have so far been modest. The reasons for this poor improvement in functional recovery are clearly multi-faceted. One reason must be the substantial inter- and intra-subject variability of effects induced by PAS. Some of the factors contributing to this variability include age, gender, time of day, genetic variations, and prior cortical activation (for review see Ridding and Ziemann (2010). Here we have demonstrated that prior cortical activation does not require active movement, but can be evoked merely by observing actions that selectively engage neuronal networks that will be the target of *subsequent* plasticity-inducing paradigms. Our control experiments confirm that such changes can occur without the need for any movement, i.e., as an entirely passive process. Moreover, we have shown that the actions observed need to be specific to the population of neurons undergoing plastic change. In future studies it will be important to determine whether the current protocol has utility as a therapeutic tool for improving motor function for patients with hemiplegia. We have shown that the plastic effects induced by PAS can be enhanced by prior action observation, and that the enhancement is specific to the action being observed. What remains unclear is whether the benefits of action observation that we have demonstrated in a cohort of young, healthy participants can be extended to older individuals and those with brain lesions.

In closing, we note that with congruent action observation our PAS protocol increased cortical excitability in every participant included in our study (N=17). Such a “strike rate” has not been shown before with PAS, or any other plasticity paradigm incorporating TMS. We therefore suggest that the approach adopted here may represent a novel, simple and effective way of enhancing neuroplasticity induction in human motor cortex.

Acknowledgements

This work was supported by a University of Queensland Postdoctoral Fellowship and a National Health and Medical Research Council Postdoctoral Training Fellowship awarded to M.V.S. Jason Mattingley was supported by an Australian Research Council (ARC Australian Laureate Fellowship (FL110100103). We thank Dr. David Lloyd for assistance with programming and technical advice.

References

Cantarero, G., Galea, J.M., Ajagbe, L., Salas, R., Willis, J., Celnik, P., 2011. Disrupting the Ventral Premotor Cortex Interferes with the Contribution of Action Observation to Use-dependent Plasticity. J. Cogn. Neurosci. 2, 3757-3766.

Carroll, T.J., Riek, S., Carson, R.G., 2001. Reliability of the input-output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. J. Neurosci. Methods 112, 193-202.

Castel-Lacanal, E., Gerdelat-Mas, A., Marque, P., Loubinoux, I., Simonetta-Moreau, M., 2007. Induction of cortical plastic changes in wrist muscles by paired associative stimulation in healthy subjects and post-stroke patients. Exp. Brain Res. 180, 113-122.

Chong, T.T., Cunnington, R., Williams, M.A., Kanwisher, N., Mattingley, J.B., 2008. fMRI adaptation reveals mirror neurons in human inferior parietal cortex. Curr. Biol. 18, 1576-1580.

Fadiga, L., Fogassi, L., Pavesi, G., Rizzolatti, G., 1995. Motor facilitation during action observation: a magnetic stimulation study. J. Neurophysiol. 73, 2608-2611.

Huang, Y.Z., Edwards, M.J., Rounis, E., Bhatia, K.P., Rothwell, J.C., 2005. Theta burst stimulation of the human motor cortex. Neuron 45, 201-206.

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

Iyer, M.B., Schleper, N., Wassermann, E.M., 2003. Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. J. Neurosci. 23, 10867-10872.

Kamke, M.R., Hall, M.G., Lye, H.F., Sale, M.V., Fenlon, L.R., Carroll, T.J., Riek, S., Mattingley, J.B., 2012. Visual attentional load influences plasticity in the human motor cortex. J. Neurosci. 32, 7001-7008.

Keel, J.C., Smith, M.J., Wassermann, E.M., 2001. A safety screening questionnaire for transcranial magnetic stimulation. Clin. Neurophysiol. 112, 720.

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

Kim, Y.H., You, S.H., Ko, M.H., Park, J.W., Lee, K.H., Jang, S.H., Yoo, W.K., Hallett, M., 2006. Repetitive transcranial magnetic stimulation-induced corticomotor excitability and associated motor skill acquisition in chronic stroke. Stroke 37, 1471-1476.

Koch, G., Versace, V., Bonni, S., Lupo, F., Lo Gerfo, E., Oliveri, M., Caltagirone, C., 2010. Resonance of cortico-cortical connections of the motor system with the observation of goal directed grasping movements. Neuropsychologia 48, 3513-3520.

Mattar, A.A., Gribble, P.L., 2005. Motor learning by observing. Neuron 46, 153-160.

McDonnell, M.N., Hillier, S.L., Miles, T.S., Thompson, P.D., Ridding, M.C., 2007. Influence of combined afferent stimulation and task-specific training following stroke: a pilot randomized controlled trial. Neurorehabil. Neural Repair 21, 435-443.

Muller, J.F., Orekhov, Y., Liu, Y., Ziemann, U., 2007. Homeostatic plasticity in human motor cortex demonstrated by two consecutive sessions of paired associative stimulation. Eur. J. Neurosci. 25, 3461-3468.

Nudo, R.J., Wise, B.M., SiFuentes, F., Milliken, G.W., 1996. Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. Science 272, 1791-1794.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97-113.

Pascual-Leone, A., Valls-Sole, J., Wassermann, E.M., Hallett, M., 1994. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain 117 (Pt 4), 847-858.

Prabhu, G., Voss, M., Brochier, T., Cattaneo, L., Haggard, P., Lemon, R., 2007. Excitability of human motor cortex inputs prior to grasp. J. Physiol. 581, 189-201.

Ridding, M.C., McKay, D.R., Thompson, P.D., Miles, T.S., 2001. Changes in corticomotor representations induced by prolonged peripheral nerve stimulation in humans. Clin. Neurophysiol. 112, 1461-1469.

Ridding, M.C., Rothwell, J.C., 2007. Is there a future for therapeutic use of transcranial magnetic stimulation? Nat. Rev. Neurosci. 8, 559-567.

Ridding, M.C., Ziemann, U., 2010. Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. J. Physiol. 588, 2291-2304.

Rizzolatti, G., Fadiga, L., Gallese, V., Fogassi, L. 1996a. Premotor cortex and the recognition of motor actions. Brain Res. Cogn. Brain Res. 3, 131-141.

Rizzolatti, G., Fadiga, L., Matelli, M., Bettinardi, V., Paulesu, E., Perani, D., Fazio, F., 1996b. Localization of grasp representations in humans by PET: 1. Observation versus execution. Exp. Brain Res. 111, 246-252.

Sale, M.V., Ridding, M.C., Nordstrom, M.A., 2007. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp. Brain Res. 181, 615-626.

Sale, M.V., Ridding, M.C., Nordstrom, M.A., 2008. Cortisol inhibits neuroplasticity induction in human motor cortex. J. Neurosci. 28, 8285-8293.

Sanes, J.N., Donoghue, J.P., 2000. Plasticity and primary motor cortex. Annu. Rev. Neurosci. 23, 393-415.

Stefan, K., Cohen, L.G., Duque, J., Mazzocchio, R., Celnik, P., Sawaki, L., Ungerleider, L., Classen, J., 2005. Formation of a motor memory by action observation. J. Neurosci. 25, 9339-9346.

Stefan, K., Kunesch, E., Benecke, R., Cohen, L.G., Classen, J., 2002. Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J. Physiol. 543, 699-708.

Stefan, K., Kunesch, E., Cohen, L.G., Benecke, R., Classen, J., 2000. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123 Pt 3, 572-584.

Stefan, K., Wycislo, M., Classen, J., 2004. Modulation of associative human motor cortical plasticity by attention. J. Neurophysiol. 92, 66-72.

Stefan, K., Wycislo, M., Gentner, R., Schramm, A., Naumann, M., Reiners, K., Classen, J., 2006. Temporary occlusion of associative motor cortical plasticity by prior dynamic motor training. Cereb. Cortex 16, 376-385.

Ziemann, U., Ilic, T.V., Pauli, C., Meintzschel, F., Ruge, D., 2004. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. J. Neurosci. 24, 1666-1672.

Figure Legends

Figure 1

Schematic representation of the experimental protocol. **(A)** Participants observed one of two action videos in each session showing repeated goal-directed movements, involving either the "congruent" hand or the "incongruent" hand (mirror-reverse of congruent hand). **(B)** Overview of the testing protocol, indicating the approximate timings for assessment of neurophysiological parameters and their relation to action observation and action execution with PAS. MEP – motor evoked potential; RMT – resting motor threshold.

Figure 2

Group (mean ± SEM) data from the main experiment, showing MEP amplitudes for APB before action observation (pre obs), after action observation but before paired associative stimulation (post obs/pre PAS), and at 5 minutes (post PAS 5 mins) and 20 minutes (post PAS 20 mins) following PAS. APB MEP amplitude was significantly greater 20 minutes following PAS compared with pre-PAS in the congruent condition ($^{*}p < 0.001$). APB MEP amplitudes at 20 minutes post-PAS were also significantly greater when participants had previously watched the congruent video than when they had previously watched the incongruent video ($^{\#}p < 0.05$).

Figure 3

Comparison of APB MEP amplitudes across the four control experiments. **(A)** Group (mean ± SEM) data showing APB MEP amplitude before (pre) and at 5 minute intervals after action observation alone (Control Experiment 1). There was no significant change in APB MEP amplitude at any of the time points following action observation. Filled black circles indicate time points that correspond to the post-PAS 5 and 20-minute time points from the main experiment. (**B**) Group (mean ± SEM) data showing APB MEP amplitude before (white bars), 5 minutes after (grey bars) and 20 minutes after (black bars) manipulation in Control Experiments 2, 3 and 4: Action observation and action execution (left columns); PAS and action execution (middle columns); Action observation and PAS (right

columns). In the action observation and PAS condition only, APB MEP amplitude was significantly greater 20 minutes after PAS compared with the pre- and 5-minute post-PAS time points (*$p < 0.007$). MEP amplitude at the 20-minute time point following action observation and PAS was significantly greater than the same time point for the other two interventions ($^{\#}p < 0.001$).