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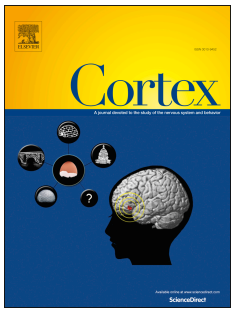

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Motor imagery alone drives corticospinal excitability during simultaneous action observation and motor imagery

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- Revised manuscript, 21th October 2019 –

- link to data, code and materials now included on p. 25 as requested, 23.1.2020.
I have also copied our CRediT statement there –

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Running title:
Corticospinal excitability during AO+MI

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Motor imagery alone drives corticospinal excitability during concurrent action observation and motor imagery

Abstract

We studied the motor simulation processes involved in concurrent action observation and motor imagery (AO+MI) using motor evoked potentials induced by transcranial magnetic stimulation. During congruent AO+MI, participants were shown videos of a model's hand performing rhythmical finger movements, and they imagined moving the same finger of their own hand in synchrony with the observed finger. During incongruent AO+MI, the imagery task involved a different finger from the observed one. As expected, congruent AO+MI yielded robust facilitatory effects, relative to baseline, only in the effector involved in the task. Incongruent AO+MI produced equally pronounced effects in the effector that was engaged in MI, whilst no corticospinal facilitation was found for the effector corresponding to the observed action. We further replicated that engaging in pure AO without MI does not produce reliable effects. These results do not support the proposal that observed and imagined action are both simulated at the level of the primary motor cortex. Rather, motor imagery alone can sufficiently explain the observed effects in both AO+MI conditions. This bears clear implications for the application of AO+MI procedures in sport and neurorehabilitation.

Keywords:

Motor simulation

Motor resonance

Transcranial magnetic stimulation

Motor evoked potentials

Neurorehabilitation

1. Introduction

Action observation (AO) and motor imagery (MI) are two covert forms of action processing that both engage motor cortical regions (Hardwick et al., 2018). Jeannerod (2001) suggested that AO and MI can both be regarded as forms of motor simulation, that is, both involve the unfolding of the related action representation in real time but in the absence of overt movement (see also Savaki & Raos, 2019). Whilst AO and MI have, until recently, been largely studied by separate research communities, there is now accumulating evidence demonstrating that humans can engage in AO and MI simultaneously (here called 'AO+MI', Vogt et al., 2013; Eaves et al., 2016). In the present study, we explored if AO+MI tasks might involve concurrent, separable motor representations of the observed and of the imagined action (here referred to as '*Dual Action Simulation'*, or DAS). That is, we tested the hypothesis that observed and imagined action are simulated in parallel (Vogt et al., 2013; Eaves et al., 2012, 2014, 2016).

Our motivation was twofold: on the one hand, we were seeking to contribute to the emerging, broader literature on multiple motor representations. Initial supporting evidence for the brain's capacity to simulate multiple motor actions comes from studies on joint action (e.g., Menoret et al., 2015; Richardson et al., 2018), and from the recent fMRI study by Cracco et al. (2018), who were able to decode each of two different, concurrently observed hand postures in premotor as well as posterior parietal cortices. On the other hand we were specifically interested in the neurocognitive mechanisms of AO+MI processes, where no such evidence is currently available. Whilst there is robust evidence for the involvement of motor cortical processing in pure AO (Rizzolatti & Sinigaglia, 2016; Hardwick et al., 2018; Naish et al., 2014) and in pure MI (Guillot et al., 2016; Hetu et al., 2013), it is currently not clear if, during AO+MI tasks, both the AO- and the MI-component involve separable motor simulation processes. A better understanding of the neurocognitive architecture of AO+MI processes is indeed highly desirable with a view on optimising applications of AO+MI procedures in motor rehabilitation and sports training (Vogt et al., 2013; Eaves et al., 2016). Before we expand on research design and hypotheses, we briefly summarise the existing research on AO+MI.

The available studies indicate robust facilitatory effects of AO+MI instructions on motor cortical processing relative to pure AO or pure MI (Eaves et al.,

2016). Whilst the majority of studies focussed on immediate effects of AO+MI instructions on neurophysiological parameters such as the BOLD signal in functional Magnetic Resonance Imaging (fMRI), event-related desynchronisation in electroencephalography, or the amplitude of motor evoked potentials (MEPs), researchers have also begun to study the behavioural effects of AO+MI instructions on motor learning (Binks et al., 2018; Marshall et al., 2019; Romano-Smith et al., 2018; Scott et al., 2018). Interestingly, the initial 'wave' of neuroimaging studies on AO+MI (Berends et al., 2013; Macuga and Frey, 2012; Nedelko et al., 2012; Villiger et al., 2013) was undertaken with a clear motivation to assess the suitability of AO+MI procedures in motor rehabilitation, where either AO or MI procedures are typically still applied in an isolated, non-integrated manner (Vogt et al., 2013). However, none of the available studies on AO+MI aimed to test the DAS hypothesis.

Alternative accounts to the DAS hypothesis of AO+MI are certainly conceivable. Whilst we had little doubt that the MI-component of AO+MI would involve motor cortical structures, the same might not be true for the AO-component. Specifically when imagined and observed action are not identical, the latter might either be largely ignored, or when it is task-relevant, it might merely be used as an external visual guide for MI, rather than activating a separate motor representation. This alternative *'visual guidance hypothesis'* of the AO-component of AO+MI would appear plausible on a number of grounds: First, in a series of neuroimaging studies, Lingnau and colleagues have recently shown that the categorisation of observed actions is primarily achieved by occipito-temporal cortex and not by motor cortical regions (e.g., Lingnau and Downing, 2015; Wurm et al., 2017). Second, although there is a large body of evidence available for the involvement of motor cortical structures during AO (Naish et al., 2014; Rizzolatti & Sinigaglia, 2016), we would pertain that a good part of the related research on the 'action observation network' might have been contaminated by participants spontaneously engaging in MI during AO (Vogt et al., 2013; DiGruttolla, 2018). Third, it is unclear if, and at which levels of the motor cortical system, separate simulations of the observed and the imagined action can be maintained over a time window of several seconds, as is typical in practical applications of AO+MI in sports and neurorehabilitation.

The aim of the present study is to provide a first empirical assessment of the DAS and of the (alternative) visual guidance hypotheses of AO+MI which served as a fallback. Our approach is to establish separate neural markers for the observed and the

imagined action, and to study to what extent either marker shows enhanced activity during AO+MI. A shortcoming of most existing studies is their exclusive usage of congruent AO+MI, where the observed and imagined actions are essentially the same, making it difficult to then establish separate neural markers for each component. Here we contrast *congruent and incongruent AO+MI conditions* (cAO+MI and iAO+MI, for short), where in the latter, participants observe one action (e.g., movement of the index finger) and imagine a different action (e.g., movement of the little finger). We use Motor Evoked Potentials (MEPs) recorded from two effectors, induced via single-pulse Transcranial Magnetic Stimulation (TMS) as separate neural markers for the AO- and MI-components.

In addition to the cAO+MI and iAO+MI conditions, we also included a *Baseline condition* in which participants observed a static hand, as well as a *pure AO condition* ('AO') where participants watched a movement of the index or little finger and were asked to disengage from MI. We aimed to facilitate possible motor simulation processes of the observed action in three ways: First, in all AO+MI conditions participants were asked to synchronise their imagined finger movement, over a number of movement cycles, to the movement of the observed (different) finger, as to strengthen visuo-motor encoding of the latter. Second, participants were asked to distribute their attention evenly between the AO and MI components of AO+MI. Third, in all conditions involving action observation, we used an oddball task where participants were asked to detect occasional deviant finger movements, as to encourage processing of the movement type, rather than only its timing.

We made the following predictions:

- *cAO+MI:* the effector engaged in AO+MI should exhibit increased MEP amplitudes, whilst for the non-engaged effector, MEP amplitudes should be substantially lower and near baseline level.
- *iAO+MI:* Here the DAS hypothesis predicts that MEPs for the MI- and the AO-task components are both enhanced to a similar extent, relative to baseline levels, since each task should engage separate motor simulation processes. In contrast, the visual guidance hypothesis predicts that MEPs would be primarily enhanced for the MI-component, whilst MEPs for the AO-component would be significantly lower. That is, according the visual

guidance hypothesis the results of iAO+MI should essentially mirror those of cAO+MI.

- *cAO+MI vs. iAO+MI:* When contrasting the two AO+MI conditions directly, the DAS hypothesis can be assessed via two further tests: First, the differences in MEP amplitudes between engaged and non-engaged effector in the cAO+MI condition should be stronger than the differences between MI- and AO-engaged effectors in the iAO+MI condition (i.e., an interaction prediction). Second, the DAS hypothesis would predict that MEP amplitudes of the engaged effector in the cAO+MI condition should be yet stronger than those of the MI-engaged effector during iAO+MI, since during cAO+MI the two simulation processes should converge onto the same effector. However, since such a result might be counteracted by ceiling effects on corticospinal excitability, we only regarded the latter prediction as supplementary.
- *Pure AO.* Given that previous studies where pure AO was contrasted with other instruction conditions, and notably with AO+MI, often obtained weak or no effects of pure AO against baseline (e.g., Cengiz et al., 2018; Wright et al., 2014, 2018), and that we explicitly discouraged participants from MI during the pure AO condition, we had no strong grounds to predict enhanced MEPs in this condition relative to baseline, other than our inclusion of an oddball detection task and the legacy of earlier positive findings (Naish et al., 2014) that were, however, likely confounded by spontaneous MI. As such, the pure AO condition was not central to the present study, and it was mainly included for control purposes.

2. Materials and methods

2.1. Participants

Thirteen healthy volunteers (ten females) aged 19-26 years took part (mean age 20.9 years). According to the Edinburgh Handedness Inventory (Oldfield, 1971), twelve participants were right-handed and one was ambidextrous (but identified herself as right-handed). Five additional participants were excluded from the study based on preestablished criteria, namely: inaccessible motor hand area (n=2); MEP data from

the two recorded muscles either not obtainable or not comparable (n=2), and TMS system failure (n=1). A sample size of n=13 yields 71% power to detect a moderate-to-large effect size of d=0.68 in a within-groups contrast. The latter was the lowest effect size found in a precursor study by Wright et al. (2014) who studied differences in CSE between baseline and AO, MI, and congruent AOMI conditions involving an index finger movement.

Before the experiment, participants gave their written informed consent and completed the Lancaster University TMS screening form, which identified that none of the participants showed any contraindication to TMS. All participants reported having normal or corrected-to-normal vision and no neurological/psychiatric disorders. Participants' responses to an abbreviated version of the Movement Imagery Questionnaire-3 (MIQ-3; S.E. Williams et al., 2012) yielded an average score of 4.3 (*SD* = 0.99, range = 3.6 to 6). This indicated overall 'neutral' abilities in performing kinaesthetic MI ("not easy nor hard"). The experimental procedures were approved by the Lancaster University ethics committee.

2.2. Design

The experiment comprised four basic conditions: congruent action observation and motor imagery (cAO+MI), incongruent action observation and motor imagery (iAO+MI), pure action observation (AO), and observation of a static hand ('Baseline'; see Fig. 1A). In the first three conditions, participants watched either a rhythmical abduction/adduction movement of the index or the little finger of a model's right hand. This resulted in six blocks with different action observation tasks. In addition, we included two separate, identical Baseline blocks. The experiment was divided into two sessions with a short pause in between, and each session included all eight blocks, resulting in a total of 16 blocks to be completed by each participant.

2.3. Stimuli and apparatus

Participants were seated in a comfortable chair in a quiet room with their left hand on their lap and their right hand resting on the table in front of them in pronate orientation (see Fig. 1B). The to-be-observed finger movements were presented on a

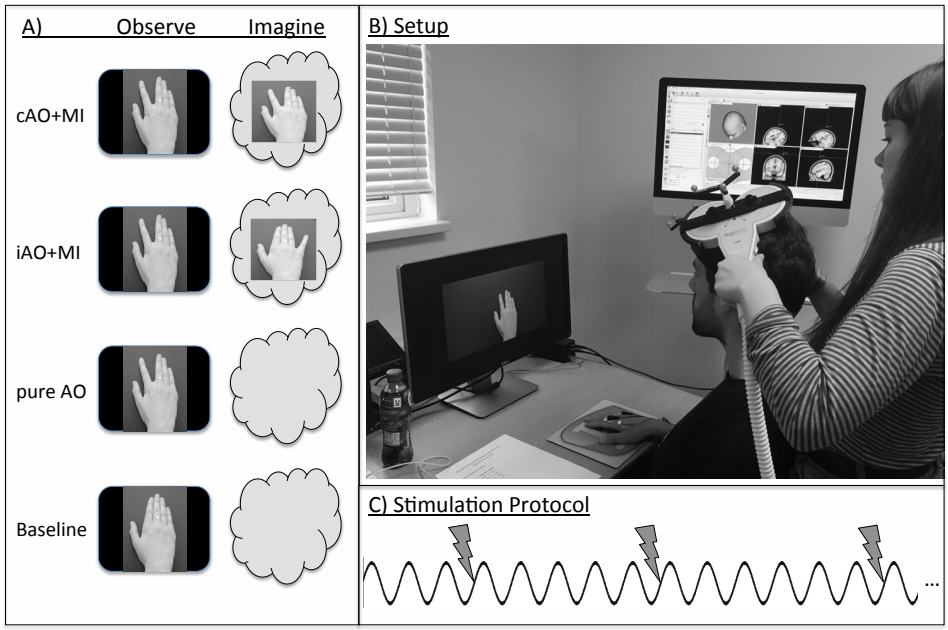

Fig. 1- Experimental conditions and setup. Panel A illustrates the four experimental conditions, congruent AO+MI (cAO+MI), incongruent AO+MI (iAO+MI), pure AO (not involving MI), and Baseline (observation of a static hand). The three action observation conditions involved display of a rhythmically moving index finger, or little finger (not shown) over 90 s. During cAO+MI, participants engaged in MI of the same finger of their own hand as that shown on the display, and during iAO+MI, they engaged in MI of a different finger (i.e., AO of index and MI of their little finger as illustrated, or AO of the little finger and MI of their index, not shown). Panel B shows the experimental setup, and Panel C illustrates the stimulation protocol with TMS pulses being delivered every 5 to 7 cycles (see Sections 2.3 and 2.5).

23.5-inch LCD display (resolution: 1920 × 1080 pixels), which was positioned at approximately 80 cm viewing distance. The display was controlled by an Apple 'Mac mini' computer (Apple, CA, USA) running a dedicated stimulus presentation programme written in Matlab (version 2017a, MathWorks, Inc.) and using the Psychophysics Toolbox (version 3, Brainard et al., 1997).

The video stimuli were recorded using a Panasonic Lumix G digital video camera (resolution: 1280 × 720 @ 50 Hz) and showed the dorsal surface of a female right hand performing rhythmical abduction/adduction movements of either the index finger or the little finger at 1 Hz over 90 cycles. That is, each block lasted 90 s. A small white fixation point was attached to the proximal phalanx of the model's middle finger. The hand was displayed in egocentric, vertical orientation, and its location was

clearly distinct from the participant's own hand location (Fig. 1B). During recording, the model synchronised her outwards (extension) movements to a metronome set to 60 bpm so that the beats coincided with extension peak velocity. We used three standard videos and five videos containing two or three aberrant events for the oddball detection task. The three standard videos showed regular movements of either the index or little finger, or a static hand. In the latter (Baseline) video, every 1000ms the white fixation point turned red for 100ms. This was designed to allow participants to anticipate the possible time points of TMS stimulation in a similar way as with the videos containing finger movements. The videos for the oddball detection task contained either two or three aberrant movements in place of a standard movement cycle at quasi-random time points. These were either a single lifting movement of the index or little finger, or a single 'hop' movement where the finger both lifted and abducted/adducted. A single video was used for the baseline oddball detection task, where the colour of the fixation point changed to blue or green, rather than to the standard red, for 100 ms. Participants were asked to name the individual aberrant movements or colours when they occurred.

The Apple Mac mini computer was also used to trigger the delivery of TMS pulses at or just after peak velocity of finger extension. For doing so, an equidistant series of expected time points of peak velocity was first created for each video. This was then carefully inspected in real time for temporal accuracy and, where there were notable deviations from the regular beat in the video recording, this was either replaced by another recording, or the related sample was adjusted.

2.4. Procedure and tasks

The experiment consisted of TMS- and EMG-setup (see Sections 2.5 and 2.6), a practice session, and two experimental sessions, all of which were run consecutively over approximately 90 minutes.

2.4.1. Practice session

First, participants were asked to overtly perform rhythmical abduction/adduction movements of their index or little finger in synchrony with that of the model's hand. We then asked them to overtly move their index finger along with an observed movement of the little finger as to introduce and practice incongruent movements. In

a second step, participants were trained in both the motor and kinaesthetic aspects of MI, where they were asked to imagine actively initiating each movement, as well as to imagine the kinaesthetic and tactile sensations involved. With this in mind, the experimenter went through an abbreviated version of the MIQ-3 (S.E. Williams et al., 2012) for reasons of both practice and a brief assessment of kinaesthetic MI abilities. Stinear et al. (2006) had shown that this form of imagery, but not visual imagery, elicits corticospinal facilitation. Third, participants were asked to practice the congruent AO+MI (cAO+MI) condition, followed by the incongruent AO+MI (iAO+MI) condition, with a balanced mix of videos showing movements of the index or little finger. During *cAO+MI*, participants' task was to imagine moving the same finger of their right hand as they saw moving on the display in front of them. During *iAO+MI*, participants imagined moving the 'opposite' finger to the observed finger, that is, when they watched a video of an index finger movement, they imagined moving their own little finger (Fig. 1B). In both AO+MI conditions, participants were asked to synchronise their imagined finger movement with the observed finger movement as to ensure that observed and imagined abduction movements, as well as the respective adduction movements, occurred simultaneously. Importantly, we also instructed participants to divide their attention evenly between the observed and the imagined action. Fourth, the oddball detection task was introduced (see Section 2.3), which we used in all conditions involving action observation, in order to facilitate the detailed processing of the observed movements. Fifth, we introduced the *pure AO* condition, where participants were asked to passively observe the displayed finger movement and to disengage from any MI, and finally the *Baseline* condition, where participants watched the picture of a static hand, along with the related oddball colour detection task. In all conditions, participants were asked to keep their gaze on the fixation point in the video, as to control for differential attentional foci across participants which may modulate corticospinal excitability (CSE; Carson and Collins, 2017).

2.4.2. Main experimental sessions

Each of the two consecutive main sessions comprised the eight blocks of the experimental design with a duration of 90 s per block, short pauses between the blocks, and a pause of five minutes between the two sessions when participants filled in the Edinburgh Handedness Inventory (Oldfield, 1971). Two different pseudo-

random block orders were used for the first and ninth participant, and the block orders for the remaining participants followed latin squares. Participants initiated each block by pressing the space bar on the computer keyboard with their left hand. At the end of each block, they were given feedback on whether they had correctly identified all oddball events. Throughout the study participants were reminded to attend equally to observed and imagined movement and to keep both in sync, given that phase consistency between sensorimotor representations evoked by AO and MI is thought to facilitate CSE (Sakamoto et al., 2009).

2.5. Transcranial magnetic stimulation

Single-pulse TMS stimulation was triggered by the Stimulus presentation programme at peak velocity of finger extension every 5 to 7 movement cycles in a jittered fashion to reduce participants' anticipation (Fig. 1C). The first pulse was delivered randomly only at the 3rd, 4th, or 6th cycle to allow participants to settle into each task. A total of n=16 pulses was delivered in each block, equating to n=32 MEPs per experimental condition and observed finger, and n=64 MEPs for the Baseline condition which was run in two blocks per session. No TMS pulse was delivered during an oddball movement or in the cycle following this.

TMS was applied using a figure-of-eight coil (70 mm diameter) connected to a DuoMAG MP magnetic stimulator (DEYMED Diagnostic, Czech Republic), which delivered monophasic pulses to the hand representation of the primary motor cortex (M1) contralateral to the right hand. The coil was held tangential to the scalp with the handle pointing posterior-laterally at 45° to the midline, resulting in a posterior–anterior-induced current flow under the junction of the two coil wings. This is regarded as the best orientation to activate indirect trans-synaptic corticospinal neurons (Brasil-Neto et al., 1992), which increases the responsiveness of MEP amplitudes to factors which may influence CSE levels, such as motor simulation (Loporto et al., 2013). The motor hotspot was identified as the scalp site from which MEPs with the most robust and comparable peak-to-peak amplitudes were recorded simultaneously from the first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles. This was found by repeatedly stimulating the approximate location of the hand representation of the M1 and adjusting the coil position and orientation in small steps at a stimulator output of 50-60%. Once the motor hotspot was identified, it

was marked as a target using a stereotaxic neuronavigation system (BrainSight, Rogue Research Inc, Montreal, Canada). The neuronavigation system enabled the accurate replication and maintenance of coil positioning over the motor hotspot throughout the experiment, as even slight coil movements can significantly influence MEP amplitudes (Sandbrink, 2008). The next step was to establish each participant's resting motor threshold (rMT). The rMT was defined as the lowest stimulation intensity that elicited peak-to-peak MEP amplitudes of at least 50μv in 5 out of 10 consecutive trials from the FDI and ADM (Rossini et al., 2015). This was achieved by starting at the percentage intensity used to localize the motor hotspot, which was decreased in 1%-5% increments until the rMT was determined (Rothwell et al., 1999). During the experiment, the stimulation intensity was set to 120% of each participants' rMT. The mean rMT was 48% (±5) and the mean test intensity was 57% (±6) of the maximum stimulator output.

2.6. Electromyographic recording

TMS-evoked MEPs were measured using pairs of Ag/AgCl self-adhesive surface electrodes (24-mm diameter), which recorded the ongoing electromyographic (EMG) signal of the FDI and ADM muscles. We chose these two muscles, as TMS pulses over the hand representation of the M1 can elicit MEPs in both muscles simultaneously. Electrodes were attached in a tendon-belly montage. Two active electrodes were attached over the right FDI and ADM, two reference electrodes attached over the tendon of FDI and ADM, and a ground electrode was attached over the ulnar styroid process of the right wrist as an electrically neutral site. EMG activity was monitored and recorded using TruTrace EMG with a sampling rate of 12.5 kHz (DEYMED Diagnostic, Czech Republic). The EMG signal was amplified and band-pass filtered (DC–2000 Hz) with an adaptive notch filter of 50Hz to remove power line contamination. As the presence of EMG activity in the muscles prior to receiving a TMS pulse is known to increase subsequent MEP amplitudes (Watkins et al., 2003), pre-stimulus background EMG activity was continuously monitored throughout each experimental block, and participants were frequently reminded to keep their right hand relaxed.

2.7. Data analysis

The MEP time series were exported to a data extraction routine written in Matlab, and the mean peak-to-peak amplitudes of MEPs recorded from the FDI and ADM were then calculated. Means were based on n=6 MEPs per level of the design and session. The mean Baseline amplitudes of the two muscles were only moderately different, FDI: 2.20 ± 0.15 (*SEM*) mV, and ADM: 1.96 ± 0.11 mV. As expected, inter-participant variability was large, and so the mean MEP amplitudes were normalized using the *z*-score transformation separately for each participant and muscle to enable comparison for all analyses. All data satisfied our inclusion criterion of standardized residuals < ±3.0. All variables were then considered normally distributed based on visual inspection of Q-Q plots.

All statistical analyses were performed using SPSS (version 24, IBM Corp.). Effect sizes were reported as partial eta-squared (η^2_ρ), and the level of significance was set to $\alpha<0.05$. For comparisons with more than two levels, degrees of freedom were adjusted using the Greenhouse-Geisser method.

3. Results

3.1. Baseline blocks

Before collapsing the two separate Baseline blocks within each session where participants were shown a static hand, we tested for possible effects of Muscle (FDI or ADM), Session (1st or 2nd), and Block (1st or 2nd). No significant main effects or interactions were found (all $F_s < 2.95$, all *p's* > .11), thus it was deemed justified to collapse the MEPs across the two Baseline blocks within each session. This resulted in a single, robust Baseline condition with separate means for Muscle and Session.

3.2. Plan of analysis

The main focus in the present study is the comparison between the cAO+MI and iAO+MI conditions: according to the DAS hypothesis, MEPs for the (separable) MI-

and AO-components of iAO+MI should show similar magnitudes, whereas MEPs in cAO+MI should be significantly larger for the effector engaged in AO+MI than for the non-engaged effector. In contrast, the visual guidance hypothesis, which served as a fallback, predicts that the results of iAO+MI should mirror those of cAO+MI, that is, the AO-component of iAO+MI should generate significantly smaller MEPs than the MI-component.

For the statistical analysis, we used a three-factorial repeated measures ANOVA with the factors Muscle, Session, and Condition with subsequent focussed comparisons (Section 3.4.: 'Main analysis'). The latter factor comprised five conditions, namely the four cells of the cAO+MI and iAO+MI conditions plus the Baseline. Crucially, the DAS hypothesis predicts an interaction between congruency (cAO+MI vs. iAOMI) and Engaged effector. This was tested using a contrast-contrast interaction (Rosenthal & Rosnow, 1985) which comprised the four cells of the cAO+MI and iAO+MI conditions. In addition, we ran two sets of selected pairwise comparisons between conditions (Section 3.4.1.): In the first set, we tested if the MI-engaged effector in cAO+MI showed enhanced MEP amplitudes relative to Baseline, and we contrasted the MEP amplitudes of the MI-engaged effector in iAO+MI against those in cAO+MI: here the DAS hypothesis would predict yet larger MEPs in the latter condition, since AO- and MI-components should converge. The second set of pairwise contrasts focussed on the two remaining cells of the AO+MI conditions: Here the DAS hypothesis predicts that the AO-engaged effector in iAO+MI should show enhanced MEP amplitudes relative to the non-engaged effector in cAO+MI. Since the pure AO condition mainly served control purposes, we analysed effects in a separate ANOVA.

3.3. Results overview

The z-scores for the mean peak-to-peak MEPs across all experimental conditions are shown in Figure 2. Essentially, there is a clear division between two strong MEP

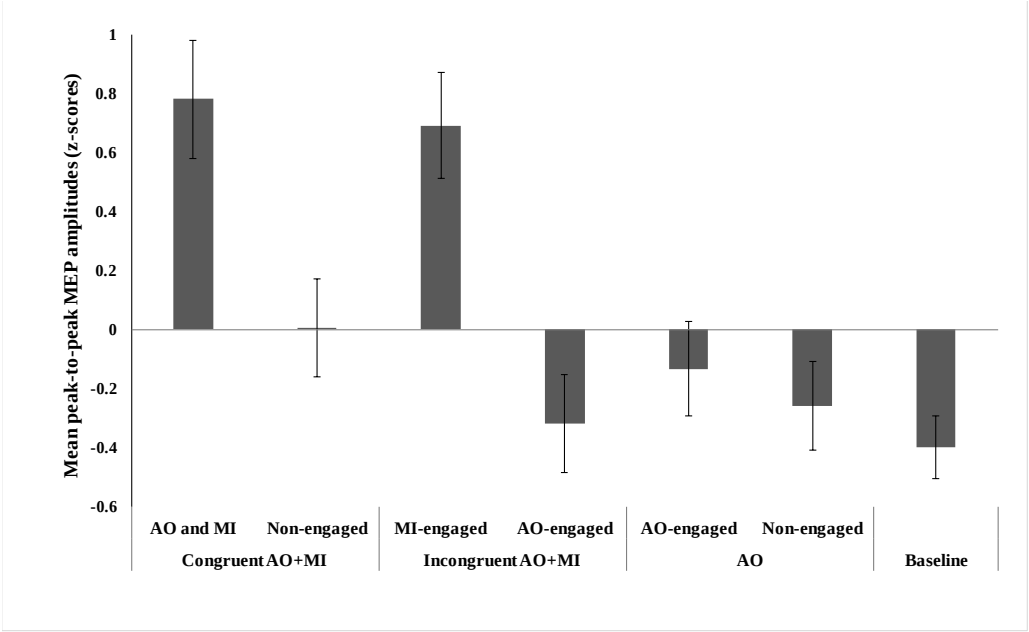

Figure 2 - Main results. Mean z-transformed MEP amplitudes (with *SEM*) for each experimental condition, collapsed across the two sessions and across FDI and ADM muscles. Abbreviations: AO: action observation; MI: motor imagery; AO+MI: simultaneous AO and MI. In the congruent AO+MI condition, participants engaged in MI of the same effector as the one they observed moving, whilst their other effector was non-engaged. In incongruent AO+MI, participants observed movement of one effector (AO-engaged) and imagined moving their other effector (MI-engaged). The pure AO condition involved observation of one moving effector (AO-engaged). The Baseline condition involved observation of a static hand. A figure showing the results separately for each muscle is provided in the Supplementary materials.

amplitudes for the effector that was engaged in MI, whilst all other MEPs were substantially lower. As predicted, in cAO+MI the MEP amplitudes were markedly enhanced for the effector engaged in cAO+MI, compared the non-engaged effector. Unexpectedly, however, this result was mirrored by the iAO+MI condition, where MEPs for the effector that was engaged in MI were markedly stronger than MEPs for the AO-engaged effector.

For ease of exposition, Figure 2 does not show the results separately for the FDI and ADM muscles since no related significant main effects or interactions with this factor were found. For the interested reader, a figure including the factor Muscle

is provided in the Supplementary materials. Note that we distinguish between 'effector' (as independent variable) that could be engaged or non-engaged in AO, MI, or AO+MI tasks, and recorded 'muscle' (FDI and ADM, which were always recorded simultaneously).

3.4. Main analysis

For the main analysis we employed a three-factorial repeated measures ANOVA comprising the factors Muscle (FDI or ADM), Session (1^{st} or 2^{nd}), and Condition with five levels: engaged and non-engaged effector of the cAO+MI condition, MI-engaged and AO-engaged effector of the iAO+MI conditions, and the Baseline. Importantly, the ANOVA indicated a highly significant overall effect Condition, $F_{(2.9,\ 34.8)} = 13.23$, $p < .001$, $\eta^2_\rho = .52$. In contrast, the main effects of Muscle and Session were not significant, $F_{(1,\ 12)} = 0.83$, $p = .38$, $\eta^2_\rho = .07$, and , $F_{(1,\ 12)} = 2.49$, $p = .14$, $\eta^2_\rho = .17$, respectively, and none of the interactions approached significance (all Fs < 0.51; all ps > 0.69).

The DAS hypothesis was tested directly in a focussed contrast-contrast interaction (Rosenthal & Rosnow, 1985) comprising the factors Congruency (congruent vs. incongruent AO+MI condition) and Engaged effector (note that this factorial structure, as apparent in Fig. 2, was 'flattened' in the above ANOVA). Regarding the latter factor, in the cAO+MI condition the factor levels were 'effector engaged in AO+MI' vs. 'non-engaged effector', and in the iAO+MI condition the levels were 'MI-engaged effector' vs. 'AO-engaged effector'. Against the prediction of the DAS hypothesis, this contrast-contrast interaction was not significant, $F_{(1,\ 12)} = 0.90$, $p = .36$, $\eta^2_\rho = .07$. That is, the pattern of results in the iAO+MI condition did not differ significantly from that in the cAO+MI condition. Furthermore, the effects of Engaged effector were even more robust numerically in the iAO+MI condition, $F_{(1,\ 12)} = 30.7$, $p < .001$, $\eta^2_\rho = .719$, than in the cAO+MI condition, $F_{(1,\ 12)} = 11.27$, $p = 0.006$, $\eta^2_\rho = .484$. These results fail to provide any support for the DAS hypothesis, whilst they are compatible with the visual guidance hypothesis.

3.4.1. Pairwise comparisons between conditions

In two sets of planned contrasts we further tested selected conditions of the above ANOVA against the Baseline. In the first set, the planned comparison between the effector engaged in cAO+MI and the Baseline condition was highly significant, $F_{(1, 12)} = 25.48$, $p < .001$, $\eta^2_\rho = .68$, as expected. Interestingly, the contrast between the MI-engaged effector in cAO+MI vs. iAO+MI was not significant, $F_{(1, 12)} = 0.33$, $p = .57$, $\eta^2_\rho = .03$. As can be seen in Fig. 2, MEP amplitudes for the MI-engaged effector in iAO+MI indeed approached these of the engaged effector in cAO+MI. Also this result tentatively violates the DAS hypothesis.

In the second set of contrasts, the planned comparison between the non-engaged effector in cAO+MI against Baseline was significant, $F_{(1, 12)} = 6.40$, $p = .03$, $\eta^2_\rho = .35$, indicating slightly facilitated MEP amplitudes even in the non-engaged effector during cAO+MI. Finally, the comparison between the non-engaged effector in cAO+MI and the AO-engaged effector iAO+MI conditions was not significant, $F_{(1, 12)} = 1.63$, $p = .23$, $\eta^2_\rho = .12$. MEP amplitudes in the latter condition were at Baseline level and numerically even below the MEPs in the non-engaged effector in cAO+MI (see Fig. 2). Again, this result fails to support the DAS hypothesis.

3.5. Pure AO

Effects of the pure AO condition were analysed in a separate, three factorial repeated measures ANOVA which mainly served control purposes. This included the factors Muscle, Session, and Condition (AO-engaged effector, the non-engaged effector of the pure AO condition, and Baseline). In short, no reliable effects for pure AO were found: the main effect of Condition was not significant, $F_{(1.88, 22.55)} = 1.76$, $p = .20$, $\eta^2_\rho = .13$, neither were the remaining main effects of Muscle and Session significant, or any of the interactions (all Fs < 1.83; all ps > .19). Whilst Fig. 2 suggests a trend towards enhanced MEPs for the effector engaged in AO to exceed Baseline levels, the related contrast analysis indicated that this was not statistically reliable, $F_{(1, 12)} = 3.15$, $p = .10$, $\eta^2_\rho = .21$). Finally, also the contrast between AO-engaged and non-engaged effector was not significant, $F_{(1, 12)} = 1.04$, $p = .33$, $\eta^2_\rho = .08$.

In summary, in addition to the striking facilitatory effects of MI across cAO+MI and iAO+MI conditions, only one further statistically reliable effect was found, namely enhanced MEPs for the non-engaged effector in cAO+MI against Baseline.

4. Discussion

The objective of this study was to test two competing hypotheses: (1) the hitherto untested Dual Action Simulation (DAS) account of AO+MI as proposed by Eaves et al. (2012, 2016) and Vogt et al. (2013), and (2) the visual guidance hypothesis of AO+MI. Our results provide clear support for the visual guidance hypothesis that MI is the dominant driver of corticospinal facilitation in AO+MI tasks. Conversely, the data provide no support for the DAS hypothesis. The results for iAO+MI are novel, and the findings for the other conditions are highly consistent with the available research literature. This gives us confidence in the present methodology and in the specific results obtained for iAO+MI.

4.1. Congruent AO+MI

As predicted, we found large MEPs for the engaged effector during cAO+MI, and the difference in MEP amplitudes between the engaged effector and Baseline was highly significant. This result was specific for the engaged effector, as indicated by a highly significant effect of engaged vs. non-engaged effector in this condition. These findings are entirely consistent with previous research on the effects of combined cAO+MI on CSE (e.g., Ohno et al., 2011; Sakamoto et al., 2009; Tsukazaki et al., 2012; Wright et al., 2014, 2018), as well as with the related neuroimaging studies (Berends et al., 2013; Macuga and Frey, 2012; Nedelko et al., 2012; Taube et al., 2015; Villiger et al., 2013). Although the MEP amplitudes for the non-engaged effector were markedly below those for the engaged effector, they were still reliably above Baseline levels (Section 3.4.1). A tentative explanation for this unexpected finding is related to task complexity. This has been shown to increase general levels of excitability in M1 (Kuhtz-Buschbeck et al., 2003; Mouthon et al., 2015; Roosink and Zijdewind, 2010), and task complexity was most likely higher in the cAO+MI condition than in the Baseline condition.

4.2. Incongruent AO+MI

To our knowledge, the present study provides the first neurophysiological data on iAO+MI. The MEP amplitudes during iAO+MI closely mirrored those during cAO+MI, in that MEPs for the MI-component of iAO+MI approached those for the focussed effector in cAO+MI, whilst MEPs for the AO-component of iAO+MI were significantly lower than for the MI-component, and here they were at Baseline level. These main findings of the present study fail to support the DAS hypothesis, whilst they are fully compatible with the visual guidance hypothesis, which served as a fallback in the present study.

Unexpectedly, there was a trend towards even lower MEP amplitudes for the AO-component of iAO+MI relative to the non-engaged effector in cAO+MI (Fig. 2). This low MEP amplitude is of particular interest, given that the DAS hypothesis would have predicted a substantially higher amplitude. The present result cannot be explained in terms of task complexity, which should be at least comparable, or even higher in iAO+MI than in cAO+MI. More likely, the particularly low CSE for the AO-component of iAO+MI might reflect that access to lower-level motor processing was inhibited for the observed action: Whilst the iAO+MI condition required sustained visual attention towards oddball movements, participants needed to engage in MI of a different effector, and inhibiting any motor processing of the observed action would have helped task completion. It should be clear, however, that this is plainly a post-hoc interpretation of an unexpected trend in the data, and that separate, dedicated studies would be required to explore such putative inhibitory processes further. The present result is, however, well in line with the current literature on inhibition of surrounding effectors during MI (see Naish et al., 2014; Aoyama et al., 2017; Bruno et al., 2018).

4.3. Motor imagery is the main driver in AO+MI

Previous research clearly indicated that pure MI can enhance CSE (see Wright et al., 2014; Mouthon et al., 2015), and that it can engage primary and secondary motor regions (Hardwick et al., 2018; Hetu et al., 2013). Whilst TMS studies that included both MI and AO conditions typically reported similar MEP magnitudes (e.g., Clark et al., 2004; Leonard and Tremblay, 2007; Roosink and Zijdewind, 2010; J. Williams et

al., 2012; Wright et al., 2014), the present contrast between the strong MEP-amplitudes for the MI-component of iAO+MI against both Baseline and the pure AO condition is possibly the most robust difference reported so far in a single study. Given the sensitivity of MEP amplitudes to the time point of TMS pulse delivery (Borroni et al., 2005; Cengiz et al., 2018), and the difficulty of precisely stimulating certain timepoints in an imagined trajectory, we would suggest that previous studies likely underestimated CSE levels for pure MI, due to the likely greater temporal variability of TMS pulses in MI than in AO, relative to the aimed-for landmark in the imagined or observed movement. The present study minimised this problem since the MI was visually guided.

Once again, our findings leave little doubt that MI was the main driver in iAO+MI. Distributing the MI- and AO-components across different effectors allowed us to assess respective levels of CSE relatively independent of each other. The finding that MI-related MEP amplitudes were not different across iAO+MI and cAO+MI conditions further suggests that MI was likely also the main driver in cAO+MI: apparently, CSE during cAO+MI was not enhanced by the concurrent, congruent action observation, relative to the MI-component during iAO+MI. This does not mean that such an enhancement might not be found for other tasks. Also, other approaches might allow assessing AO- and MI-components of cAOMI more directly in future. At this point in time, however, the best available working assumption is that the visual guidance hypothesis applies to incongruent as well as congruent AO+MI.

4.4. Dual action simulation – quo vadis?

The DAS hypothesis assumes motor simulation for both components of AO+MI, not only for the MI-component. However, across conditions we found (1) that pure AO did not generate reliably stronger MEP amplitudes relative to Baseline or to the unfocussed effector in the pure AO condition, (2) that the AO-component in iAOMI was also at Baseline level, and (3) that CSE was not enhanced by action observation in the cAO+MI condition, relative to the MI-component in iAO+MI, as just discussed. These null-results were obtained despite several design features introduced to facilitate motor simulation of the observed action, namely the usage of an oddball detection task, instructions to pay equal attention to both components of the AO+MI tasks, and the requirement to temporally coordinate imagined and observed

movements. The latter two measures only applied to the two AO+MI conditions, thus we had good reasons to expect corticospinal facilitation for the AO-component in these tasks, even in case that the pure AO condition would not be effective.

Whilst the above null-results might appear to stand in contrast with the legacy of related studies on pure AO beginning with Fadiga et al. (1995; for review see Naish et al., 2014), a number of recent TMS studies directly contrasted AO+MI and pure AO conditions and these also yielded null-effects for pure AO (e.g., Cengiz et al., 2018; Wright et al., 2014, 2018; see also the excellent discussion in Wright et al., 2014). Importantly, the convergent results of these studies and the present one were obtained despite considerable variation in procedure (e.g., unlike Wright et al., 2014, we used a fully counterbalanced order of conditions, ongoing rhythmical movements, an oddball detection task, a crossed effector design where each effector could be either focussed or unfocussed, and neuronavigation for coil positioning). These results nicely corrobate Vogt et al.'s (2013) concern that "spontaneously performed AO+MI is an important and largely ignored confound in many related behavioural and neuroimaging studies" (p. 10). That is, in many of the earlier studies testing putative 'pure' AO, effects might have been unduly boosted by spontaneous and unnoticed AO+MI. In support of this proposal, DiGruttola (2018) interviewed their participants after a session of pure AO, and about half of the participants reported that they had spontaneously engaged in concurrent MI (i.e., in AO+MI). In contrast, in our study participants were instructed to disengage from MI during the pure AO condition (or in iAO+MI, MI was directed to a different effector), and AO-effects disappeared.

As a consequence, we can only reiterate the pledge to reassess the large body of behavioural and neuroimaging work on putative pure AO regarding confounding spontaneous concurrent MI (Vogt et al., 2013). For example, Hardwick et al.'s (2018) recent meta-analysis of neuroimaging studies indicated a considerable overlap between activations during AO and MI: such a finding is rather unsurprising in case that the included studies on presumed 'pure AO' might have involved spontaneous AO+MI. In contrast, when AO and MI instructions are more carefully controlled, differences between these forms of motor simulation are likely to become more apparent (e.g., Vogt et al., 2016).

Finally, the body of recent work by Lingnau and colleagues (e.g., Lingnau and Downing, 2015; Wurm et al., 2017) indicates a primary role of lateral occipitotemporal regions (and not motor regions) in action categorisation. These

findings further support the notion that AO and MI, when properly instructed and controlled for confounds, might turn out to be rather different kettle of fish.

In our view, the above considerations do in no way invalidate the potential benefit of AO+MI instructions in neurorehabilitation and sports training over and above pure MI training. Indeed, visual guidance of MI should particularly help in situations where novel skills are acquired, or where re-acquisition requires sustained practising. However, the specific explanatory framework of DAS is put into question by the present results. The present results thus stand in contrast to the support for multiple motor representations provided by studies on joint action (Menoret et al., 2015) and observation of multiple actions (Cracco et al., 2018). In addition, Colton et al. (2018) recently demonstrated that observing an unexpected, incongruent finger movement whilst imagining a short sequence of moving one's own fingers can induce action slips, that is, overt execution of either the observed or the imagined action. Thus, under appropriately designed conditions, observed actions are indeed capable of 'inserting an action intention' and to facilitate motor execution, - which can also be interpreted as evidence for multiple motor representations. One reason why we did not find such effects in the present study might be that our AO+MI tasks involved concurrent AO and MI over a relatively long time period, compared to the momentary and unpredictable appearance of the action stimuli in Colton et al.'s (2018) study. Surely, further research is needed to identify the boundary conditions for possible DAS processes during AO+MI tasks, joint action, and observation of multiple actions more fully.

4.5. Limitations and future research

Assessing corticospinal excitability via MEPs provides a restricted window into motor cortical processing, namely to the primary motor cortex and potentially its inputs from fronto-parietal circuits. As such our findings do not exclude that concurrent action representations during AO+MI might be found at higher levels of the motor hierarchy. A more encompassing assessment of the DAS hypothesis can thus be expected from whole-brain neuroimaging methods such as fMRI or Magnetoencephalography (MEG). One interesting question here is whether activated areas during pure MI or pure AO might show greater overlap than during iAO+MI tasks where these representations might spatially segregate.

A second limitation is the relatively simple finger movement task that has been employed here due to its suitability for joint TMS-stimulation of two separately controllable muscles, and to its widespread usage in previous research. More complex actions, such as prehensile or manipulative actions, might yield different results to those presented here.

A further limitation might be seen in the number of participants used (n=13), and the related limited power to detect relatively small effects. In particular, we would concede that with a substantially larger sample, we might have found a significant effect of pure AO against the Baseline, where this was not significant in the present study. However, we would firstly note that our design was certainly sufficiently sensitive to demonstrate effects of MI, and given that MI and AO are comparable tasks in that both refer to motor processes without involving overt execution, we see no a priori grounds why our design should have favoured CSE during MI over CSE during AO. Second, our finding of weak CSE during pure AO replicates the related null-effects in the studies by Cengiz et al. (2018) and Wright et al. (2014; 2018). Taken together, these studies corrobate the observation by Vogt et al. (2013) that earlier research which reported significant CSE effects of pure AO might have overestimated putative effects of pure AO since spontaneous AO+MI was not controlled for (see Section 4.4). In contrast, in the present study participants were asked to disengage from MI in the pure AO condition, and CSE was only marginal. Surely, more research would be needed to identify possible conditions under which robust CSE effects of pure AO conditions, unconfounded by spontaneous MI, might be found. Third, whilst an effect of pure AO might have been detectable with a substantially larger participant sample, such a result would by no means invalidate our main finding, namely the - admittedly unexpected - robust asymmetry of the MI- and AO-components during iAO+MI.

Finally, one could argue that the temporally extended, rhythmical nature of the present AO+MI task might have been suboptimal to engage motor representations of the observed action. Whilst this becomes very apparent when contrasting the present task with that used by Colton et al. (2018, see above), we would argue that the present task bears stronger similarities with applications of AO+MI in sports and neurorehabilitation, where displays of physical exercises are typically also fairly predictable (e.g., Scott et al., 2018).

5. Conclusions

Exploring a dual action simulation account of congruent AO+MI processes is hampered by the likely overlap of neural populations of the putatively involved simulations of the observed and imagined action. Here we employed an incongruent AO+MI task to overcome this limitation. Corticospinal excitability was found to be markedly unbalanced for the two components of iAO+MI, which were assessed via separate effectors. The results indicate that MI is likely the main, if not the only driver in AO+MI tasks. The lack of support for a dual action simulation account does in no way put into question the potential relevance of AO+MI procedures in neurorehabilitation and sports training. For these applications of AO+MI, the present study highlights the crucial role of motor simulation of one's own action via MI, where concurrent AO most likely functions as an external visual scaffolding of MI, and not as a separate and potentially competing motor simulation. Action observation therapy (Buccino et al., 2014) might well work on its own, but evidence is accumulating that spontaneous MI (i.e., AO+MI) might be the unrecognised driver of its therapeutic effects.

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Declarations of interest

The authors declare no competing financial interests.

Open practices

Materials and data for the study can be found here:
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CRediT Author statement

All aspects of the Contributory Roles Taxonomy were shared between the three authors, with Rosie Meers and Stefan Vogt being particularly involved in the Formal analysis, Visualisation, and Writing the Original Draft and Revision, and Helen Nuttall providing Resources and Methodology regarding Transcranial Magnetic Stimulation procedures.

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Credit Author Statement

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