Running head: Response Preparation During Imagery and Observation

When unintended movements “leak” out: A startling acoustic stimulus can elicit a prepared response during motor imagery and action observation

Dana Maslovat, Romeo Chua, & Nicola J. Hodges,

School of Kinesiology, University of British Columbia

HTTPS://WWW.SCIENCEDIRECT.COM/SCIENCE/ARTICLE/PII/S0028393213000304?CASA_TOKEN=JGJVSEOJB1MAAAAA:BDYXLZFIA7XSUBEKJEMSEC2EP5UEZHP3KRAIKHE4TOMK0SC49BIS_AYLQHRNFPQBCFKBPYN78T8

THIS IS A PREPRINT VERSION. FOR FINAL PDF SEE LINK ABOVE

HTTPS://DOI.ORG/10.1016/J.NEUROPSYCHOLOGIA.2013.01.016

Maslovat, D., Chua, R., & Hodges, N. J. (2013). When unintended movements “leak” out: a startling acoustic stimulus can elicit a prepared response during motor imagery and action observation. *Neuropsychologia*, *51*(5), 838-844.

Email: nicola.hodges@ubc.ca

Abstract

Covert forms of practice, such as observation and imagery, have been shown to involve neurophysiological activation of the motor system, and a functional equivalence between covert and overt processes involved in action execution has been proposed (Jeannerod, 2001). We used a startling acoustic stimulus (SAS), which has been shown to trigger prepared movements involuntarily at short latencies via an increase in cortical activation, to probe the similarity of these processes and elicit movement responses in imagery and observation trial. Startle trials were interspersed with control trials while participants (n=16) performed or imagined a right hand key lift or observed a model perform the key lift. During physical movement trials, intended movements were triggered by the SAS at a short latency (RT = 78 ms) in comparison to control trials (RT = 110 ms). During imagery and observation, unimanual partial movements (assessed by force changes and muscle activation) were elicited by the SAS, with a greater incidence recorded in the imagery condition (29 % of trials) as compared to the observation condition (21 % of trials). Examination of the magnitude of the reflexive startle response (an index of motor preparation) during imagery and observation revealed similarities to physical movement trials. We conclude that covert and overt movements involve similar preparatory processes and neural pathways and propose that movements do not normally occur during imagery and observation due to low level neural activation.

Keywords: motor imagery, observation, response preparation, startle reflex, neural activation

1. Introduction

In an attempt to better understand movement preparation and execution, researchers have examined the processes involved in overt (physical) movements as well as covert "actions" such as motor imagery and action observation. There is considerable evidence implicating the involvement of the motor system during imagery and observation (see Fadiga, Craighero, & Olivier, 2005; Jeannerod & Frak, 1999 for reviews), and the discovery of a common neural network involved in both covert and overt movements, known as the mirror neuron system (MNS) (see Iacoboni, 2005; Jeannerod, 2001; Rizzolatti & Craighero, 2004; Rizzolatti & Fabbri-Destro, 2010 for reviews), has provided additional support for motor system activation during imagery and observation.

A variety of neurological measures have led to the suggestion that the involvement of the motor system is related to specific preparation of the observed or imagined movement. For example, brain activation patterns during observation and imagery of gymnastic movements are similar and highly related to the actual execution of the actions being viewed or imagined, suggesting a common neural system for these processes (Munzert, Zentgraf, Stark, & Vaitl, 2008). Transcranial magnetic stimulation induced motor evoked potentials (which are thought to index movement preparation) are comparable during observation, imagery and actual hand movements (Clark, Tremblay, & Ste-Marie, 2003; see also Kumru, Soto, Casanova, & Valls-Sole, 2008), and the observation and imagery of hand movements has also been shown to generate a lateralized readiness potential (a measure of the preparation of a specific unilateral response) consistent with the laterality of the hand being imagined or observed (Kranczioch, Mathews, Dean, & Sterr, 2009; Touzalin-Chretien & Dufour, 2008). There is also evidence for a desynchronization of EEG mu rhythms during observation of precision grip movements, a process that occurs during active movement and is thought to be involved in motor preparation (Muthukumaraswamy & Johnson, 2004; Muthukumaraswamy, Johnson, & McNair, 2004). The similarity in neural activation patterns between action, observation, and imagery has resulted in the hypothesis that the motor system is part of a simulation network that can be activated during overt, observed, and imagined movements (Jeannerod, 2001).

While it is clear that there is activation of various levels of the motor system during imagery and observation, it is not yet understood if this activation is indicative of identical processes occurring during covert and overt practice (see Holmes & Calmels, 2008 for a review). In fact, differences have been noted between imagined, observed, and executed movements in cortical activation (Carrillo-de-la-Peña, Galdo-Álvarez, & Lastra-Barreira, 2008; Macuga & Frey, 2012; Munzert, et al., 2008; Solodkin, 2004), spinal activation (Baldissera, Cavallari, Craighero, & Fadiga, 2001), autonomic nervous system levels (Demougeot, Normand, Denise, & Papaxanthis, 2009), as well as time to complete a movement (Rodriguez, Llanos, Gonzalez, & Sabate, 2008). In addition to the uncertainty regarding the equivalence between preparatory processes involved in imagery, observation and physical movement, it is also unclear as to why activation of the motor system does not produce overt movement during covert actions. Two possible explanations have been offered for the absence of motor output during imagery and observation (Hohlefeld, Nikulin, & Curio, 2011; Jeannerod, 2001). One suggestion is that motor output is blocked from reaching the motor neuron pool by an inhibitory mechanism generated in parallel to the motor activation. Alternatively, it has been proposed that motor activation during covert actions is at a reduced or subliminal level such that it is insufficient to fire motor neurons. Support for the inhibition hypothesis has been provided by a case study involving a patient with bilateral lesions in the parietal lobe (an area associated with movement initiation and inhibition) who unknowingly executed “imagined” movements (Schwoebel, Boronat, & Branch Coslett, 2002). Similarly, increased activation has been found in inhibitory brain areas during observation (Brass, Derrfuss, & von Cramon, 2005; Brass, Zysset, & von Cramon, 2001). However, single neuron recordings in monkeys have failed to show “gating” of premotor cortex output, leading to the conclusion that movement inhibition is likely not the mechanism when movements are not executed, indirectly supporting the subliminal activation hypothesis (Kaufman, et al., 2010). Indeed, low level EMG activation during imagery lends direct support for the subliminal activation hypothesis during covert preparation (Bonnet, Decety, Jeannerod, & Requin, 1997; Guillot, et al., 2007; Wehner, Vogt, & Stadler, 1984; see Guillot & Collet, 2005 for a review). It is also possible that both inhibition and low level activation operate at the spinal level such that subthreshold corticospinal activation and movement inhibition occur in parallel (Jeannerod, 2001; see also Prut & Fetz, 1999).

The purpose of this experiment was to probe response preparation processes during movement execution, imagery and observation in order to further understand the mechanisms underpinning covert actions. The methodology we used involved the use of a startling acoustic stimulus (SAS, >124 dB) which can elicit a prepared action at a much shorter latency, bypassing the usual voluntary initiation processes (see Carlsen, Maslovat, & Franks, 2012; Carlsen, Maslovat, Lam, Chua, & Franks, 2011; Valls-Solé, Kumru, &

Kofler, 2008 for reviews). The use of a SAS is a novel methodology used to examine under what conditions advance preparation does, and does not occur. For example, during a simple reaction time (RT) task when pre-programming would be advantageous, replacing the auditory “go” signal with a loud startle tone has shown to trigger such diverse movements as arm extension (Maslovat, Carlsen, Chua, & Franks, 2009; Maslovat, Carlsen, Ishimoto, Chua, & Franks, 2008), stepping (MacKinnon, et al., 2007), sit-to-stand (Queralt, et al., 2008), and head rotation (Oude Nijhuis, et al., 2007). Conversely, a lack of triggering by the startling stimulus is typically attributed to a lack of advance preparation in such paradigms as choice RT (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004), “Go-No Go” RT (Carlsen, Chua, Dakin, et al., 2008), and dual-task interference (Maslovat, et al., in review). We hypothesized that if motor imagery and action observation engage advance preparatory processes similar to that seen during overt actions a loud startle stimulus may be capable of eliciting such unintended, yet prepared responses.

The use of a SAS also allows for a better understanding of why movements do not occur during imagery and observation. It is suggested that the SAS increases cortical activation levels via a reticulo-thalamo-cortical pathway (Carlsen, et al., 2012). This pathway causes an automatic, involuntary release of a prepared movement, provided that preparatory processes have increased the activation of cortical circuits to a sufficiently high level. If movement inhibition is the cause of a lack of neural activation reaching the motor neuron pool, the involuntary subcortical initiation pathway associated with the SAS should bypass movement inhibitory processes, resulting in triggering of a response similar to that seen during movement execution trials. Alternatively, if low-level activation is the cause of a lack of observed movement, the SAS would increase activation levels such that at least a subset of motor neurons would reach threshold levels, causing a small amount of neural activation to “leak out” to the muscles in the form of a smaller or partial response. Thus the behavioural response to the SAS not only provides evidence for response preparation during imagery and observation, it also allows for discrimination between the proposed alternatives for a lack of observed movement during covert preparation.

In addition to the direct measure of response preparation (shown by the triggering of a full or partial response), the use of a SAS allows for an indirect measure of action preparation through examination of the reflexive response to the SAS. The magnitude of activation in startle indicators (e.g., activation of sternocleidomastoid muscles in the neck) is assumed to be related to the level of excitability of cortical and subcortical motor centres and an index of the degree of preparation undertaken by the participant (Kumru, et al., 2006; Maslovat, Carlsen, & Franks, 2012). For example, reflexive startle effects are of a greater magnitude when advance preparation can occur in a simple reaction time paradigm, as compared to a choice reaction time paradigm when the required response is unknown (Maslovat, Carlsen, et al., 2012). Similar results have been shown in anticipation timing tasks in that as the time nears for the participant to prepare and initiate a response, the startle response amplitude increases (Carlsen, Chua, Inglis, Sanderson, & Franks, 2008; Carlsen & Mackinnon, 2010). Collectively, these experiments provide evidence that the size of the reflexive startle response is related to movement preparation and thus can be used as a proxy measure of cortical and subcortical excitability. We hypothesized that if response preparation during covert actions occurred in a similar manner to overt movement, the magnitude of activation in the startle reflex indicators would be similar for trials in which a movement was and was not required (i.e., during imagery and observation).

2. Methods

2.1 Participants

All participants were naïve to the hypothesis under investigation and this study was conducted in accordance with ethical guidelines established by the University of British Columbia. Twenty-five right-handed volunteers with no obvious upper body abnormalities or sensory or motor dysfunctions participated in the study after giving informed consent. However, only data from sixteen right-handed volunteers (7 male, 9 female; M = 21.3 yrs, SD = 1.9 yrs) were employed in the final analysis. Six participants did not show consistent activation in our startle indicator muscle during physical movement trials in which a SAS was presented, and thus were excluded from the analysis (see Carlsen, et al., 2011, for more detail regarding the exclusion criteria for participants). It was critical to ensure a startle response was elicited as engagement of the startle reflex circuitry generally indicates sufficient subcortical activation to produce response triggering (Carlsen, Dakin, Chua, & Franks, 2007).

An additional three participants were excluded for not meeting our baseline trial criteria. To begin the experiment, prior to any knowledge of the required tasks, participants depressed 2 telegraph keys with both their right and left hands and were presented with an unexpected startling stimulus (see Section 2.3 - Experimental Design for more details). To ensure any unilateral responses we subsequently observed in the experiment were not due to the startle reflex but rather reflected lateralized preparatory processes, we excluded three participants that reacted to the startling stimulus with a unilateral keylift. The remaining participants either showed no response (n=12) or a bilateral response (n=4) to the baseline startle trial whereby both arms showed either a full or partial key lift. Although our stringent inclusion criteria resulted in a fairly high rejection rate, it is important to note that exclusion of participants was based on their reaction to the SAS, rather than any differences in imagery and observation ability.

2.2 Apparatus and Task

Testing sessions occurred with two participants seated in height-adjustable chairs across a table from each other. One participant was a confederate to the researchers (unknown to the other participant) and was used for the entire study. A 22-inch computer monitor (Acer X233W, 1152 x 864 pixels, 75 Hz refresh) was on a table beside each participant. In front of each participant were two telegraph keys (Western Union Design, #808k1) requiring 2 N to close (i.e., simply resting the hand on the switch was sufficient to close it), on which participants placed their hands to depress the switch (Figure 1). Strain gauges (JP Technologies, PA06-250BA-120) on each telegraph key were wired to an instrumentation amplifier (Northwood Instruments, Model IA-02) that provided a voltage proportional to force (precision of 0.011N; range of 0-10N).[1] Participants were asked to keep their fingers straight such that opening of the switch was achieved through wrist (rather than finger) extension.

(INSERT FIGURE 1 ABOUT HERE)

Surface EMG data were collected from both participant and confederate for the muscle bellies of the following superficial muscles: right and left extensor carpi radialis

---

[1] Note that the telegraph keys were programmed such that a keylift movement resulted in an increase in voltage in both the switch and strain gauge so that the upward movement and change in voltage occurred in the same direction.

longus (ECR - agonist), and right and left sternocleidomastoid (SCM - startle indicator) using preamplified surface electrodes connected via shielded cabling to an external amplifier system (Delsys Model DS-80). Recording sites were prepared and cleansed in order to decrease electrical impedance. The electrodes were oriented parallel to the muscle fibres, and then attached using double sided adhesive strips. A grounding electrode was placed on the ulnar styloid process (left for participant, right for confederate).

EMG burst and force onsets were defined as the point at which the recorded response first began a sustained rise two standard deviations above baseline levels (mean of 100 ms of EMG/force activity preceding the go signal), which were visually verified during data marking. Initial movement onset was defined as the first point at which contact with the switch was broken. We measured EMG, force and switch activity for the left and right hand of the participant for 300 ms following the “go” signal for trials involving physical movement, imagery and observation. We considered a full response in either arm to involve breaking contact with the switch, whereas a partial response involved a decrease in pressure on the switch force transducer accompanied by EMG activation but no switch release. A customized LabView® computer program controlled stimulus and feedback presentation, and initiated data collection at a rate of 1 kHz (National Instruments, PC-MIO-16E-1) 500 ms before the presentation of the “go” signal and terminated data collection 2000 ms following the “go” signal.

2.3 Experimental Design

All trials began with a warning tone consisting of a short beep (80 +/-2 dB, 100 ms, 100 Hz), followed by a “go” signal presented 3000 ms later which could either be a control stimulus (80 +/-2 dB, 100 ms, 1000 Hz) or startling stimulus (124 +/-2 dB, 40 ms, 1000 Hz, <1 ms rise time). All auditory signals were generated by a customized computer program and were amplified and presented via a loudspeaker placed directly behind the head of the participant. The acoustic stimulus intensities were measured using a sound level meter (Cirrus Research model CR:252B) at a distance of 30 cm from the loudspeaker (approximately the distance to the ears of the participant).

To confirm any unilateral responses observed in the experiment were due to preparatory processes, we began by assessing the participant's response to control and startle trials when no movement preparation was instructed. We presented three trials whereby the participant was asked to sit quietly with both right and left telegraph key depressed. As previously outlined, the "go" signal for the first trial was a startling stimulus and provided an indication of the participant's reflexive startle response when there were no instructions to prepare a movement (with those participants showing a unilateral response excluded from the data analysis). For the next two trials a control stimulus was presented whilst participants were instructed to close their eyes for one trial (to mimic the imagery condition) and observe the confederate's left hand for one trial (to mimic the observation condition), with the order counterbalanced. No responses, in either arm, were observed for any of the participants during these two control trials.

Next, participants were told they would be performing a key-lift reaction time task in competition with each other to examine the effects of different types of practice. The confederate always performed a left hand key-lift, while the participant always performed a right hand key-lift. We chose this arrangement so that participants would be observing a mirror-image of themselves performing the movement, which has been suggested to share the same representation for simulated movements (Anquetil & Jeannerod, 2007). Participants performed five testing blocks of 10 trials each which started and ended with physical practice blocks and alternated between imagery and observation on blocks 2 and 4. For all physical practice trials, a reward bonus (CDN $0.25 per trial) was offered for the participant that had the fastest reaction time (RT). This monetary incentive was introduced to encourage the participant to be maximally engaged in the task for all trials. RT was displayed on the computer screen for five seconds following each physical practice trial with no feedback provided to either the participant or confederate for the imagery and observation trials.

The first block of trials consisted of control trials (i.e., no startle trials) of physical practice for both participants (for trial details and order of testing see Table 1). For the second block of trials, only the confederate performed physical practice while the participant either performed imagery or observation (order counterbalanced with block four; all participants performed both imagery and observation). Before the block of imagery trials, participants were asked to perform a single imagery trial and rate their ability to imagine the movement on a visual and kinesthetic imagery scale of the Movement Imagery Questionnaire – Revised (MIQ-R; Hall & Martin, 1997). During each block of imagery or observation trials, three startle trials were presented, interspersed with seven control trials. The third block of trials consisted of control trials of physical practice for both participants, while the fourth block consisted of either imagery or observation trials, again with three startle trials. Participants then performed a final block of physical practice with three startle trials. For the blocks that involved startle trials (blocks 2, 4 and 5), the first SAS was always presented on the second trial with the other two SAS pseudo-randomly presented in the last eight trials such that there were never two consecutive startle trials. Thus, all participants performed 30 trials of physical practice, 10 trials of observation, and 10 trials of imagery. There were 10 startle trials in total, one as a control (no movement), three during each of imagery and observation conditions, and three during the last block of physical practice.

(INSERT TABLE 1 ABOUT HERE)

2.4 Dependent Measures and Analyses

For the physical movement trials, a total of 18 of the 480 trials were discarded (3.8 %). Reasons for discarding trials included the switch not being fully depressed to start the trial (9 trials), displacement reaction time shorter than 50 ms (which was assumed to be due to anticipation, 5 trials) or longer than 300 ms (1 trial), lifting of both keys (2 trials) or no response (1 trial). These trials were identified during data marking procedures and were not repeated in the experiment; however, the low error rates ensured a sufficient

To confirm that the startle response triggered the movement at short latency during overt preparation (i.e., physical practice), we compared premotor RT (PMT; time from the stimulus onset to agonist EMG onset) for startle and control trials in the final physical testing block via a paired-samples t-test.

During the imagery and observation trials, no responses (full or partial) were seen in the left hand. Therefore, when a response was observed it was always lateralized to the right (expected) hand. Full responses in the right hand were rare for startle and control trials in the imagery and observation conditions (see Section 3 - Results). Thus we combined full and partial responses for analyses. We analyzed the percentage incidence of responses, subjected to an arcsine square root transform to correct for violations to normality, via a 2 Condition (imagery, observation) x 2 Stimulus (control, startle) repeated measures analysis of variance (ANOVA).

We quantified the reflexive startle activation by integrating the rectified raw SCM EMG (averaged between the left and right side as we were not interested in any laterality differences) for the time period of 50-150 ms following the “go” signal (SCM iEMG). We chose these time periods as they reliably capture the onset and offset of SCM activation when startled (Brown, et al., 1991; Maslovat, Kennedy, Forgaard, Chua, & Franks, 2012). SCM iEMG were analyzed using a 3 Condition (movement, imagery, observation) repeated measures ANOVA.

Alpha level for the entire experiment was set at .05, partial eta squared (η_p^2) and Cohen’s d values were reported as measures of effect size, and where appropriate statistically significant effects were further analyzed via Tukey’s honestly significant difference (HSD) test to determine the locus of the differences.

3. Results

During physical movement trials the startling stimulus had the expected effect of significantly reducing premotor RT between control (M = 110 ms) and startle trials (M = 78 ms), $t(15) = 6.91$, $p < .001$, $d = 3.57$. This short latency startle reaction time is consistent with previous experiments that have suggested the startling stimulus triggers a prepared response through a faster, subcortical pathway, as compared to voluntary movement initiation (Carlsen, et al., 2012).

For the imagery trials, all participants reported being able to imagine themselves perform the movement, as confirmed by their self-reported ratings on the MIQ-R for both kinesthetic (M = 5.3, SD = 1.1) and visual (M = 5.6, SD = 1.2) imagery (max. = 7).

Full responses were rarely seen and only occurred during imagery trials (4/48 startle trials, 3/112 control trials) and thus were combined with partial responses (from here on in all responses are referred to as partial, unilateral responses). We collapsed full and partial responses even though full responses were predicted to result from bypassed inhibition while partial responses were thought to be indicative of subliminal activation. However, the hypothesis of low-level activation would predict a full response if enough neural activation “leaked out” to the motor pool, especially on startle trials (where four of the seven responses were observed). Given the vast majority (85%) of responses were partial in nature, our data favour the subthreshold activation explanation, and thus we do not believe combining full and partial responses significantly affected our results or conclusions. An exemplar partial unilateral response is shown in Figure 2 for a startle trial during the imagery condition. Note the significant increase in force production and agonist EMG activation on the right side consistent with a prepared response, and the bilateral activation in the startle indicator confirming a startle reflex was elicited.

(INSERT FIGURE 2 ABOUT HERE)

Partial unilateral responses were consistently found in the imagery and observation conditions, with a greater incidence of responses on startle trials, and for imagery as compared to observation trials. This was confirmed statistically by a main effect for condition, $F(1, 15) = 6.77$, $p = .020$, $\eta_p^2 = .31$, and a main effect for stimulus, $F(1, 15) = 5.26$, $p = .037$, $\eta_p^2 = .26$. Overall, a unilateral response for imagery was seen on 29 % of the startle trials (14/48) and 17 % of the control trials (19/112), and for observation trials on 21 % of the startle trials (10/48) and 3 % of the control trials (3/112). The incidence of right hand responses during imagery (top panel) and observation (bottom panel) by participant is shown for control and startle trials in Figure 3.

(INSERT FIGURE 3 ABOUT HERE)

A larger magnitude of activation in the reflexive startle response indicator was seen during imagery than observation. This was confirmed by a main effect of condition in the SCM iEMG analysis, $F(2, 30) = 3.37$, $p = .048$, $\eta_p^2 = .18$ and a Tukey's HSD post-hoc test confirmed that this effect was due to a significantly larger activation during imagery ($M = 5.42$ mV*ms) as compared to observation ($M = 3.34$ mV*ms), with neither condition different to physical practice ($M = 3.74$ mV*ms). In Figure 4 we have plotted these activation differences via an ensemble rectified average for all participants of averaged left and right SCM for each condition, normalized in time to the startling stimulus.

(INSERT FIGURE 4 ABOUT HERE)

4. Discussion

The purpose of the current study was to determine whether motor imagery and action observation result in prepared movements that can be released (or partially released) in response to a startling stimulus. This would allow for inferences about the processes involved in these conditions with respect to their functional similarity with physical movements and assumed overlapping preparatory processes. We also quantified participants' reflexive response to the startling stimulus as an indirect measure of movement preparation. Additionally, we hoped that the involuntary increase of neural activation caused by the startling stimulus would help determine why imagined and observed movements are not typically initiated.

We found that the startling stimulus triggered a response in the imagery condition on 29 % of the trials (9/16 people) and in the observation condition on 21 % of the trials (6/16 people). The presence of partial unilateral responses for startle trials in both imagery and observation conditions provides support for the idea that a specific motor plan was prepared and stored during these conditions. This conclusion is consistent with previous work showing lateralized brain activation during imagery and observation (Kranczioch, et al., 2009; Touzalin-Chretien & Dufour, 2008). However, what we have now demonstrated is behavioural evidence supporting a functional similarity between covert and overt actions and movement preparation processes. Further evidence for motor preparation was found by examination of the activation levels of the startle indicator. Both imagery and observation trials showed similar activation magnitudes as compared to physical practice trials, providing additional (albeit indirect) support for similar motor preparation during imagery, observation and execution.

In addition to finding evidence for specific motor preparation that directly results in overt movement, we also used the startle methodology to entertain explanations for why overt movements do not occur during imagery and observation. We hypothesized that the SAS could either bypass movement inhibition, thus resulting in a full response, or increase neural activation such that a subset of neurons would reach firing threshold, resulting in a partial response. As the vast majority of observed responses were partial in nature, our results are consistent with the explanation of subthreshold activation during imagery and observation. This explanation is also supported by the presence of partial responses on control trials (Figure 3), and is consistent with previous research showing low level EMG activation during imagery (Bonnet, et al., 1997; Guillot, et al., 2007;

Wehner, et al., 1984). Indeed, further study of our data with respect to ensemble EMG averages of the left and right arms for the imagery control trials in which a partial response was recorded, did show a similar but reduced unilateral activation pattern in the imaged hand, as compared to physical movement trials (Figure 5, top panel, light black line). This activation pattern was not found for the observation control trials (Figure 5, top panel, grey line) nor for the startle trials (Figure 5, bottom panel), although any effects in startle trials may have been masked by the startle reflex activation, seen bilaterally from approximately 50-100ms following the SAS.

(INSERT FIGURE 5 ABOUT HERE)

If we consider these partial responses to be reflective of subliminal motor commands “leaking out” to the motor neuron pool, it is not surprising that startle trials resulted in a greater percentage of responses, as the SAS is thought to involuntarily and rapidly increase cortical activation (Carlsen, et al., 2012; Maslovat, Hodges, Chua, & Franks, 2011), which would cause a greater number of neurons to reach firing threshold. While our data seems to provide support for low level activation during imagery and observation, we should note that this does not preclude the additional presence of inhibitory commands as it is possible that these mechanisms both occur in parallel to prevent unwanted overt movements (Jeannerod, 2001).

A number of results from the current experiment suggest quantifiable differences between the imagery and observation conditions. During imagery we recorded a higher percentage of partial responses (Figure 3), increased activation in the startle indicator (Figure 4), and more consistent unimanual EMG activation in control trials (Figure 5). Cumulatively, these results suggest a greater degree of response preparation was achieved during imagery as compared to observation, perhaps through a higher level of subthreshold activation. In support of this explanation, previous research has shown increased corticospinal excitability (Williams, Pearce, Loporto, Morris, & Holmes, 2012) and neural representations in motor areas of the brain (Macuga & Frey, 2012) in imagery as compared to observation.

During the imagery condition, simulation was encouraged, and thus it may not be surprising that EMG activation specific to the imagined movement was recorded. As half the participants performed imagery prior to the observation condition, it is possible that engagement in imagery primed participants to employ similar intentional simulation while observing the confederate. However, participants that performed observation prior to any imagery instructions also showed partial responses (Figure 3, bottom panel), suggesting that observation may automatically engage the observer in preparatory activities that can be measured via low-level muscle activation and are amplified by the presence of a startling stimulus. Given recent research showing differences in corticospinal excitability between passive observation (without instructions), active observation, and imagery (Roosink & Zijdewind, 2010), further research may be warranted to continue to examine differences in the preparation process between different forms of non-physical practice.

As a final point, given that the startle reflex itself has been shown to cause activation in the arm muscles (Brown, et al., 1991), it is important for us to underscore the fact that the partial responses we observed were due to preparatory activity and not a result of the startle reflex. First, we observed partial responses on control trials which would not involve the startle reflex. Second, if participants were not consistently preparing a response, we would have expected habituation in the startle reflex activation as compared to the physical movement trials. A lack of movement preparation has been shown to result in rapid habituation of the startle indicators (Brown, et al., 1991; Valls-Sole, Valldeoriola, Tolosa, & Nobbe, 1997), whereas the engagement in movement preparation has been shown to result in a lack of habituation (Carlsen, Chua, Inglis, Sanderson, & Franks, 2003; Maslovat, Carlsen, et al., 2012). In the current experiment, a similar magnitude of startle reflex activation was found between all three conditions (Figure 4), and a startle indicator was observed on a high percentage of trials for both imagery (83%) and observation (73%). Third, we examined participants' reflexive response by exposure to the SAS without any specific movement preparation instructions, and excluded any participants that showed a unimanual response to the startling stimulus before experimental testing.

In summary, the presence of unilateral responses when startled during imagery and observation provides support that movements are prepared in a similar manner to physical response trials, even though no overt movement is required. As most responses were partial in nature, we believe that imagery and observation involve subthreshold activation, which can "leak out" to the motor neuron pool when exposed to a stimulus that rapidly increases cortical activation. We also found a greater incidence of responses during imagery as compared to observation, which we attribute to increased cortical and subcortical activation.

References

Anquetil, T., & Jeannerod, M. (2007). Simulated actions in the first and in the third person perspectives share common representations. *Brain Research, 1130,* 125-129.

Baldissera, F., Cavallari, P., Craighero, L., & Fadiga, L. (2001). Modulation of spinal excitability during observation of hand actions in humans. *European Journal of Neuroscience, 13*, 190-194.

Bonnet, M., Decety, J., Jeannerod, M., & Requin, J. (1997). Mental simulation of an action modulates the excitability of spinal reflex pathways in man. *Brain Research. Cognitive Brain Research, 5*, 221-228.

Brass, M., Derrfuss, J., & von Cramon, D. Y. (2005). The inhibition of imitative and overlearned responses: a functional double dissociation. *Neuropsychologia, 43,* 89-98.

Brass, M., Zysset, S., & von Cramon, D. Y. (2001). The inhibition of imitative response tendencies. *Neuroimage, 14*, 1416-1423.

Brown, P., Rothwell, J. C., Thompson, P. D., Britton, T. C., Day, B. L., & Marsden, C. D. (1991). New observations on the normal auditory startle reflex in man. *Brain, 114 (4),* 1891-1902.

Carlsen, A. N., Chua, R., Dakin, C. J., Sanderson, D. J., Inglis, J. T., & Franks, I. M. (2008). Startle reveals an absence of advance motor programming in a Go/No-go task. *Neuroscience Letters, 434,* 61-65.

Carlsen, A. N., Chua, R., Inglis, J. T., Sanderson, D. J., & Franks, I. M. (2003). Startle response is dishabituated during a reaction time task. *Experimental Brain Research, 152*, 510-518.

Carlsen, A. N., Chua, R., Inglis, J. T., Sanderson, D. J., & Franks, I. M. (2004). Can prepared responses be stored subcortically? *Experimental Brain Research, 159,* 301-309.

Carlsen, A. N., Chua, R., Inglis, J. T., Sanderson, D. J., & Franks, I. M. (2008). Motor preparation in an anticipation-timing task. *Experimental Brain Research, 190,* 453-461.

Carlsen, A. N., Dakin, C. J., Chua, R., & Franks, I. M. (2007). Startle produces early response latencies that are distinct from stimulus intensity effects. *Experimental Brain Research, 176*, 199-205.

Carlsen, A. N., & Mackinnon, C. D. (2010). Motor preparation is modulated by the resolution of the response timing information. *Brain Research, 1322,* 38-49.

Carlsen, A. N., Maslovat, D., & Franks, I. M. (2012). Preparation for voluntary movement in healthy and clincial populations: Evidence from startle. *Clinical Neurophysiology, 123*, 21-33.

Carlsen, A. N., Maslovat, D., Lam, M. Y., Chua, R., & Franks, I. M. (2011). Considerations for the use of a startling acoustic stimulus in studies of motor preparation in humans. *Neuroscience and Biobehavioral Reviews, 35,* 366-376.

Carrillo-de-la-Peña, M. T., Galdo-Álvarez, S., & Lastra-Barreira, C. (2008). Equivalent is not equal: Primary motor cortex (MI) activation during motor imagery and execution of sequential movements. *Brain Research, 1226*, 134-143.

Clark, S., Tremblay, F., & Ste-Marie, D. (2003). Differential modulation of corticospinal excitability during observation, mental imagery and imitation of hand actions. *Neuropsychologia, 42*, 105-112.

Demougeot, L., Normand, H., Denise, P., & Papaxanthis, C. (2009). Discrete and effortful imagined movements do not specifically activate the autonomic nervous system. *PLoS ONE, 4*, e6769.

Fadiga, L., Craighero, L., & Olivier, E. (2005). Human motor cortex excitability during the perception of others' action. *Current Opinion in Neurobiology, 15*, 213-218.

Guillot, A., & Collet, C. (2005). Contribution from neurophysiological and psychological methods to the study of motor imagery. *Brain Research. Brain Research Reviews, 50*, 387-397.

Guillot, A., Lebon, F., Rouffet, D., Champely, S., Doyon, J., & Collet, C. (2007). Muscular responses during motor imagery as a function of muscle contraction types. *International Journal of Psychophysiology, 66*, 18-27.

Hall, C. R., & Martin, K. A. (1997). Measuring movement imagery abilities: a revision of the Movement Imagery Questionnaire. *Journal of Mental Imagery, 21*, 143-154.

Hohlefeld, F. U., Nikulin, V. V., & Curio, G. (2011). Visual stimuli evoke rapid activation (120 ms) of sensorimotor cortex for overt but not for covert movements. *Brain Research, 1368*, 185-195.

Holmes, P., & Calmels, C. (2008). A neuroscientific review of imagery and observation use in sport. *J Mot Behav, 40*, 433-445.

Iacoboni, M. (2005). Neural mechanisms of imitation. *Current Opinion in Neurobiology, 15*, 632-637.

Jeannerod, M. (2001). Neural Simulation of Action: A Unifying Mechanism for Motor Cognition. *Neuroimage, 14*, S103-S109.

Jeannerod, M., & Frak, V. (1999). Mental imaging of motor activity in humans. *Current Opinion in Neurobiology, 9*, 735-739.

Kaufman, M. T., Churchland, M. M., Santhanam, G., Yu, B. M., Afshar, A., Ryu, S. I., & Shenoy, K. V. (2010). Roles of monkey premotor neuron classes in movement preparation and execution. *Journal of Neurophysiology, 104*, 799-810.

Kranczioch, C., Mathews, S., Dean, P. J. A., & Sterr, A. (2009). On the equivalence of executed and imagined movements: Evidence from lateralized motor and nonmotor potentials. *Human Brain Mapping, 30*, 3275-3286.

Kumru, H., Soto, O., Casanova, J., & Valls-Sole, J. (2008). Motor cortex excitability changes during imagery of simple reaction time. *Experimental Brain Research, 189*, 373-378.

Kumru, H., Urra, X., Compta, Y., Castellote, J. M., Turbau, J., & Valls-Solé, J. (2006). Excitability of subcortical motor circuits in Go/noGo and forced choice reaction time tasks. *Neuroscience Letters, 406*, 66-70.

MacKinnon, C. D., Bissig, D., Chiusano, J., Miller, E., Rudnick, L., Jager, C., Zhang, Y., Mille, M. L., & Rogers, M. W. (2007). Preparation of Anticipatory Postural Adjustments Prior to Stepping. *Journal of Neurophysiology, 97*, 4368-4379.

Macuga, K. L., & Frey, S. H. (2012). Neural representations involved in observed, imagined, and imitated actions are dissociable and hierarchically organized. *Neuroimage, 59*, 2798-2807.

Maslovat, D., Carlsen, A. N., Chua, R., & Franks, I. M. (2009). Response preparation changes during practice of an asynchronous bimanual movement. *Experimental Brain Research, 195*, 383-392.

Maslovat, D., Carlsen, A. N., & Franks, I. M. (2012). Subcortical motor circuit excitability during simple and choice reaction time. *Behavioral Neuroscience, 126*, 499-503.

Maslovat, D., Carlsen, A. N., Ishimoto, R., Chua, R., & Franks, I. M. (2008). Response preparation changes following practice of an asymmetrical bimanual movement. *Experimental Brain Research, 190*, 239-249.

Maslovat, D., Chua, R., Spencer, H. C., Forgaard, C. J., Carlsen, A. N., & Franks, I. M. (in review). Evidence for a response preparation bottleneck during dual-task performance: Effect of a startling acoustic stimulus on teh psychological refractory period.

Maslovat, D., Hodges, N. J., Chua, R., & Franks, I. M. (2011). Motor preparation and the effects of practice: Evidence from startle. *Behavioral Neuroscience, 125*, 226-240.

Maslovat, D., Kennedy, P. M., Forgaard, C. J., Chua, R., & Franks, I. M. (2012). The effects of prepulse inhibition timing on the startle reflex and reaction time. *Neuroscience Letters, 513,* 243-247.

Munzert, J., Zentgraf, K., Stark, R., & Vaitl, D. (2008). Neural activation in cognitive motor processes: comparing motor imagery and observation of gymnastic movements. *Experimental Brain Research, 188,* 437-444.

Muthukumaraswamy, S. D., & Johnson, B. W. (2004). Changes in rolandic mu rhythm during observation of a precision grip. *Psychophysiology, 41,* 152-156.

Muthukumaraswamy, S. D., Johnson, B. W., & McNair, N. A. (2004). Mu rhythm modulation during observation of an object-directed grasp. *Cognitive Brain Research, 19,* 195-201.

Oude Nijhuis, L. B., Janssen, L., Bloem, B. R., van Dijk, J. G., Gielen, S. C., Borm, G. F., & Overeem, S. (2007). Choice reaction times for human head rotations are shortened by startling acoustic stimuli, irrespective of stimulus direction. *The Journal of Physiology, 584,* 97-109.

Prut, Y., & Fetz, E. E. (1999). Primate spinal interneurons show pre-movement instructed delay activity. *Nature, 401,* 590-594.

Queralt, A., Weerdesteyn, V., van Duijnhoven, H. J., Castellote, J. M., Valls-Sole, J., & Duysens, J. (2008). The effects of an auditory startle on obstacle avoidance during walking. *Journal of Physiology, 586,* 4453-4463.

Rizzolatti, G., & Craighero, L. (2004). The Mirror-Neuron System. *Annual Review of Neuroscience, 27,* 169-192.

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

Rodriguez, M., Llanos, C., Gonzalez, S., & Sabate, M. (2008). How similar are motor imagery and movement? *Behavioral Neuroscience, 122,* 910-916.

Roosink, M., & Zijdewind, I. (2010). Corticospinal excitability during observation and imagery of simple and complex hand tasks: Implications for motor rehabilitation. *Behavioural Brain Research, 213,* 35-41.

Schwoebel, J., Boronat, C. B., & Branch Coslett, H. (2002). The man who executed "imagined" movements: evidence for dissociable components of the body schema. *Brain and Cognition, 50,* 1-16.

Solodkin, A. (2004). Fine Modulation in Network Activation during Motor Execution and Motor Imagery. *Cerebral Cortex, 14,* 1246-1255.

Touzalin-Chretien, P., & Dufour, A. (2008). Motor Cortex Activation Induced by a Mirror: Evidence From Lateralized Readiness Potentials. *Journal of Neurophysiology, 100*, 19-23.

Valls-Solé, J., Kumru, H., & Kofler, M. (2008). Interaction between startle and voluntary reactions in humans. *Experimental Brain Research, 187,* 497-507.

Valls-Sole, J., Valldeoriola, F., Tolosa, E., & Nobbe, F. (1997). Habituation of the auditory startle reaction is reduced during preparation for execution of a motor task in normal human subjects. *Brain Research, 751*, 155-159.

Wehner, T., Vogt, S., & Stadler, M. (1984). Task-specific EMG-characteristics during mental training. *Psychological Research, 46*, 389-401.

Williams, J., Pearce, A. J., Loporto, M., Morris, T., & Holmes, P. S. (2012). The relationship between corticospinal excitability during motor imagery and motor imagery ability. *Behavioural Brain Research*, *226*, 369-375.

Author Note

Dana Maslovat, School of Kinesiology, University of British Columbia; Romeo Chua, School of Kinesiology, University of British Columbia; Nicola J. Hodges, School of Kinesiology, University of British Columbia;

Acknowledgements for this study go to a Social Sciences & Humanities Research Council of Canada grant awarded to Nicola J. Hodges. We would also like to acknowledge the assistance of Chris Forgaard for his role as the confederate and Paul Nagelkerke for his technical support.

Correspondence concerning this article should be addressed to Nicola J. Hodges, School of Kinesiology, University of British Columbia, War Memorial Gymnasium 210-6081 University Boulevard, Vancouver, British Columbia CANADA V6T 1Z1. Email: : nicola.hodges@ubc.ca.

Figure Captions

Figure 1. Experimental set-up including location of participant, confederate, loudspeaker computer monitor and EMG electrodes.

Figure 2. Sample partial unimanual response for a startle trial during the imagery condition. Note the significant increase in force production and agonist (ECR – extensor carpi radialis) EMG activation on the right side only consistent with a prepared response, and the bilateral activation in the startle indicator (SCM – sternocleidomastoid) confirming a startle reflex was elicited.

Figure 3. Percent incidence, by participant, of partial unilateral responses during control and startle trials for imagery (top panel) and observation (bottom panel). Subject numbers with an asterisk (*) denote those that performed imagery trials first.

Figure 4. Ensemble averages showing rectified raw EMG by condition for startle indicator activation of the sternocleidomastoid (SCM; collapsed left and right). Note that imagery showed a significantly higher activation level as compared to observation, with neither imagery nor observation significantly different to physical movement trials.

Figure 5. Ensemble averages showing raw EMG for left arm (negative values) and right arm (positive values) for control trials (top panel) and startle trials (bottom panel) during physical movement trials (thick black line) and those imagery (thin black line) and observation (grey line) trials in which a partial response was observed. Note the small muscle activation burst in the right arm for imagery control trials (grey circle) at a similar time course to muscle activation during physical trials, with a lack of activation in the left arm, suggesting the activation is related to a prepared, unilateral response.

Figure 1

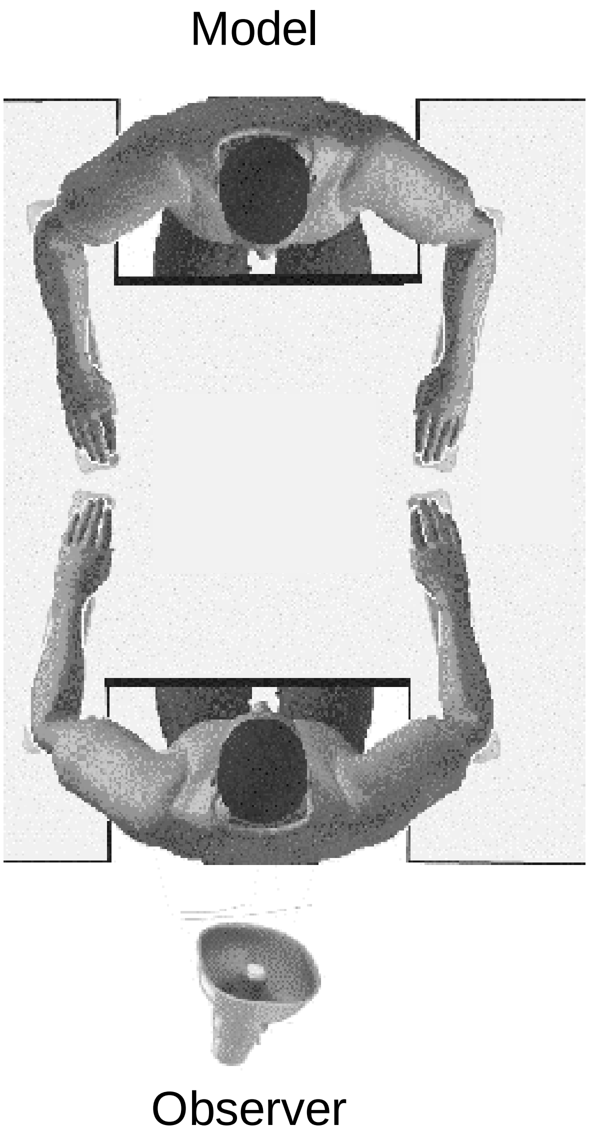


Figure 2

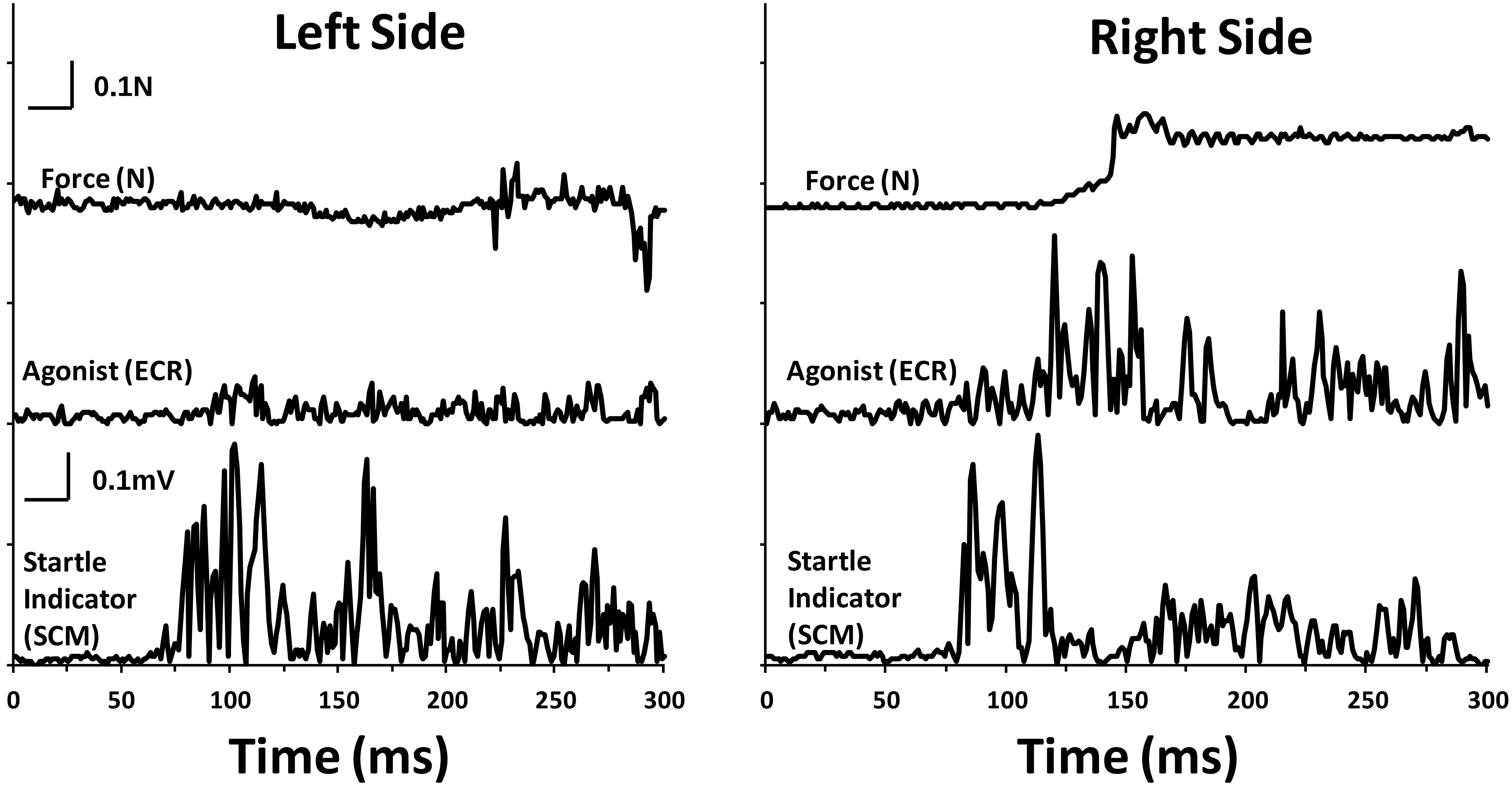

Left Side
0.1N
Force (N)
Agonist (ECR)
0.1mV
Startle
Indicator
(SCM)
0
50
100
150
200
250
300
Time (ms)
Right Side
Force (N)
Agonist (ECR)
Startle
Indicator
(SCM)
0
50
100
150
200
250
300
Time (ms)

Figure 3

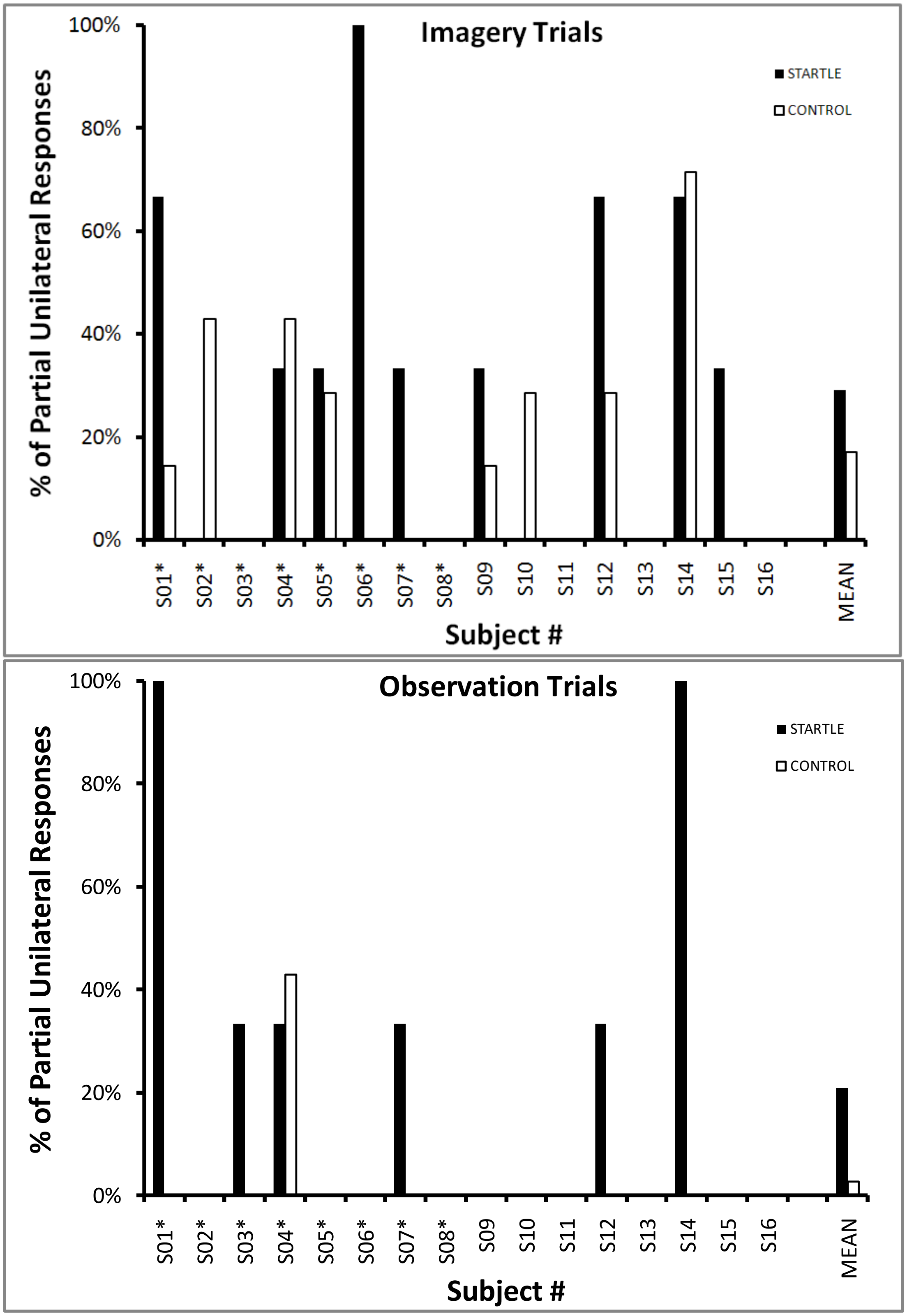

Imagery Trials
■ STARTLE
☐ CONTROL
% of Partial Unilateral Responses
100%
80%
60%
40%
20%
0%
S01*
S02*
S03*
S04*
S05*
S06*
S07*
S08*
S09
S10
S11
S12
S13
S14
S15
S16
MEAN
Subject #
Observation Trials
■ STARTLE
☐ CONTROL
% of Partial Unilateral Responses
100%
80%
60%
40%
20%
0%
S01*
S02*
S03*
S04*
S05*
S06*
S07*
S08*
S09
S10
S11
S12
S13
S14
S15
S16
MEAN
Subject #

Figure 4

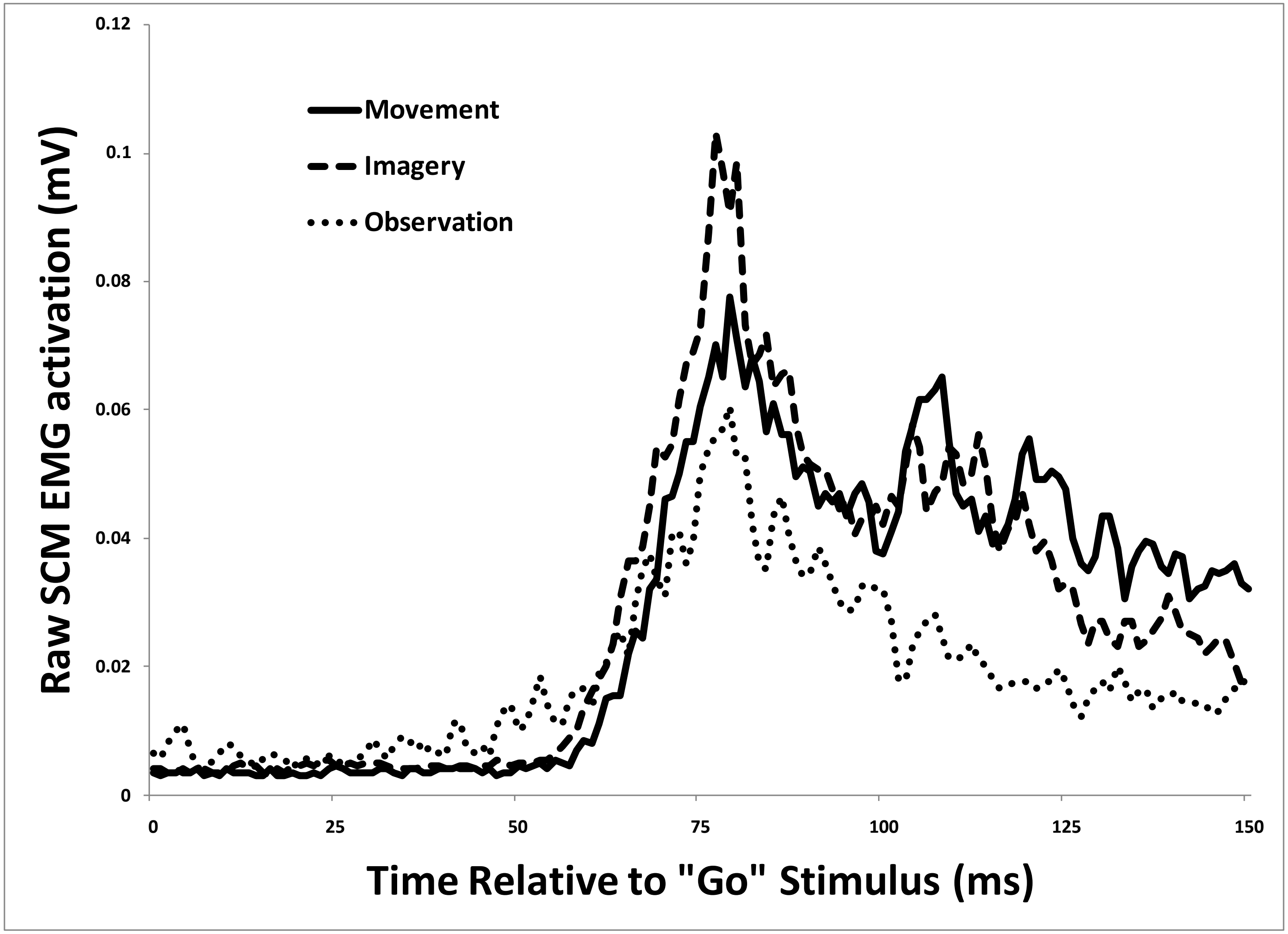
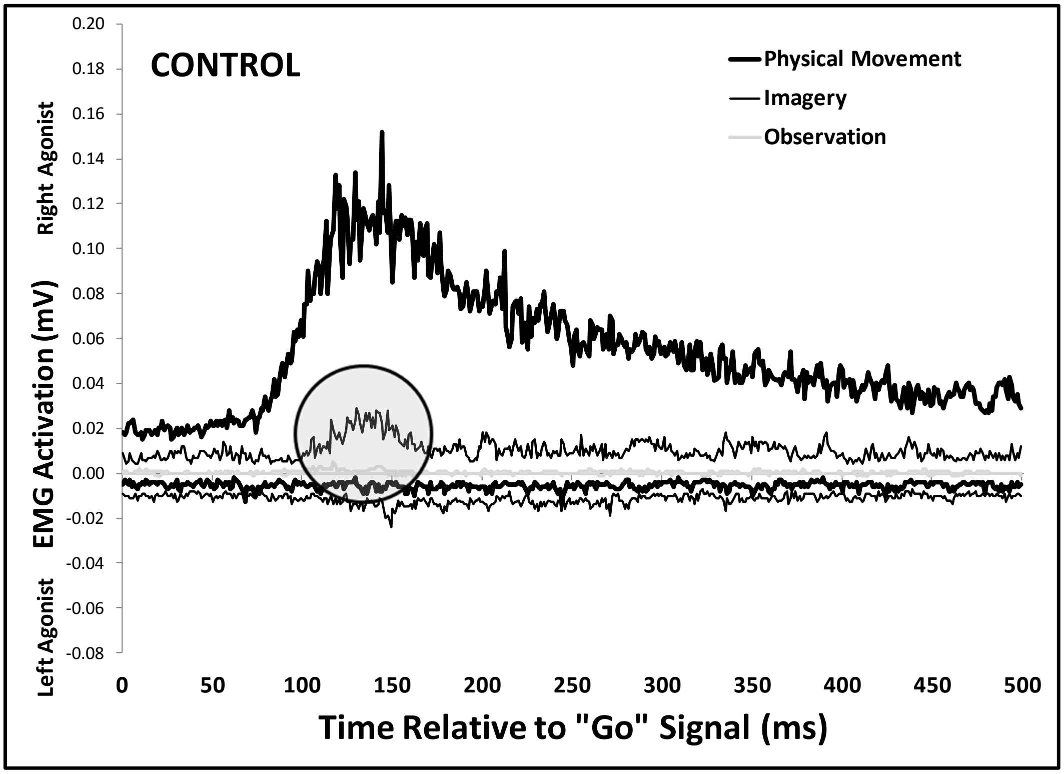
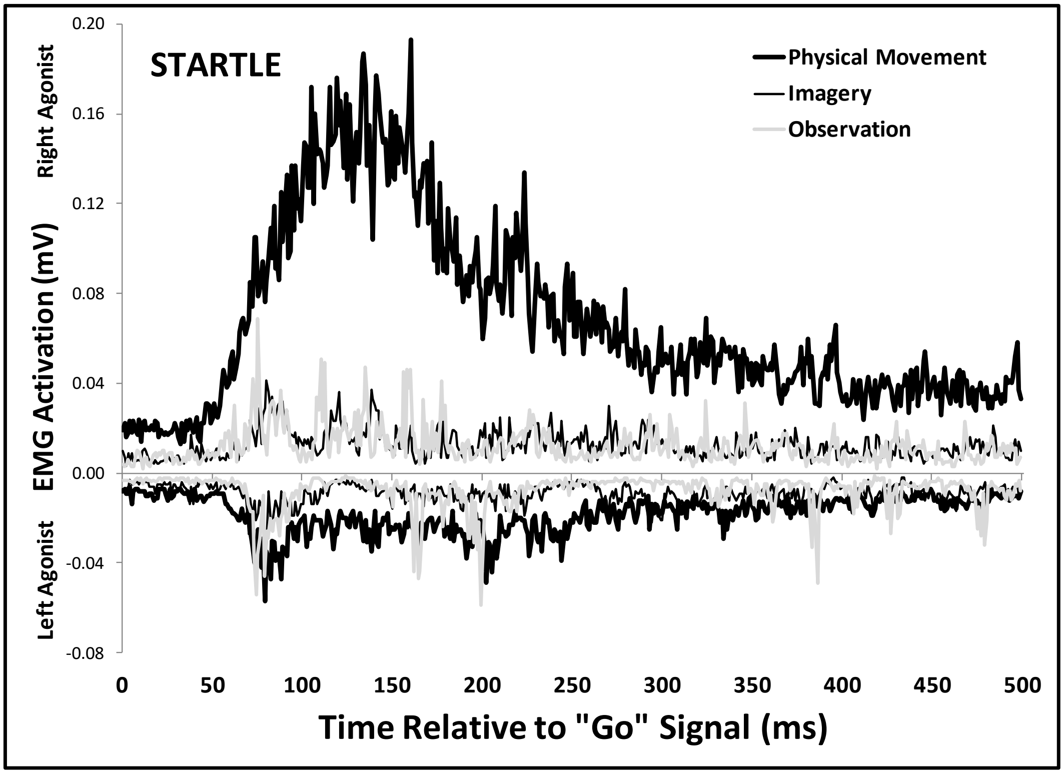

Raw SCM EMG activation (mV)
0.12
0.1
0.08
0.06
0.04
0.02
0
Movement
Imagery
Observation
0
25
50
75
100
125
150
Time Relative to "Go" Stimulus (ms)

Figure 5


CONTROL
Physical Movement
Imagery
Observation
Right Agonist
Left Agonist
EMG Activation (mV)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
0
50
100
150
200
250
300
350
400
450
500
Time Relative to "Go" Signal (ms)


STARTLE
Physical Movement
Imagery
Observation
Right Agonist
Left Agonist
EMG Activation (mV)
0.20
0.16
0.12
0.08
0.04
0.00
-0.04
-0.08
0
50
100
150
200
250
300
350
400
450
500
Time Relative to "Go" Signal (ms)