Timing of Motor Preparation for Indirectly Cued vs. Directly Cued Movements During a Visuomotor Mental Rotation Task

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General Introduction

Generally, humans reach to objects in their environment in a direct manner, such that the response they have to prepare and execute is acted upon the object of interest. For instance, when reaching to grab a cup or keys sitting on a table, the visual stimulus directly overlaps with the movement goal, directly cuing the location of the target. But what happens to responses, in particular motor preparation, if the visual stimulus indirectly cues the required movement such that the actual movement is made towards a different location than that indicated visually (e.g., when moving a cursor on a screen using a computer mouse)? To date, research on response preparation underlying motor tasks has primarily focused on responses to directly cued targets with less attention being given to the processes that underlie indirectly cued motor responses.

The purpose of this research was to investigate the processes underlying motor preparation when one is required to make an indirectly cued movement. Specifically, I was interested in determining the time course of motor preparation when one is required to make an indirectly cued movement. To cue targets indirectly we used a visuomotor mental rotation (VMR) task. A startling acoustic stimulus (SAS) was used to explore the time course of motor preparation and how it is influenced by the angle of transformation (i.e. how closely the visual cue matches the indirect movement target).

Chapter I: Literature Review

In the literature review I will begin with an introduction to reaction time (RT) and how it is typically used in the laboratory to study motor preparation. I will then discuss what different RT tasks can tell us about motor preparation (i.e., response programming) when they are analyzed from a (1) behavioural perspective, (2) neurophysiological perspective and (3) when combined with a startle stimulus. In discussing response programming, I will highlight current findings regarding the time course of motor preparation. Finally, I will discuss the visuomotor mental rotation task and outline how it can be used to examine motor preparation underlying direct and indirectly cued movements.

On average, 100 meter sprinters react to an auditory go-signal in less than 200 ms; the time interval measured from when the horn sounds signaling the start of the race to when the sprinters apply force to the starting blocks (Pain & Hibbs, 2007). From an athlete’s perspective, a shorter reaction time gives one an advantage over his or her opponents in getting to the finish line first. From a motor control perspective, reaction time (RT), and differences in RT between individuals within a task and across tasks, allows one to explore the nature of the processes underlying movement initiation.

It has been proposed that RT is influenced by the amount of time it takes one to complete processes associated with the following three stages prior to movement initiation: stimulus identification, response selection and response programming (Donders, 1969/1868). Stimulus identification involves the detection and identification of the stimulus of interest. In addition to identifying the stimulus, one needs to decide on the appropriate response and the response (or motor program) needs to be prepared. According to original definitions, the motor program can be defined as "a set of muscle commands that are structured before a movement sequence begins" (Keele, 1968, p.387). Today, the motor program is thought of as generalized, containing an abstract code about the order of events, the relative timing of the events, and the relative force with which the events are to be produced (Schmidt & Lee, 2011).

Collectively, these processes, by which one transforms information related to the stimulus into the corresponding response, are referred to as the information processing model. In support of the proposal that different stages in the information processing model take time, Donders (1969) found differences in RT when he manipulated task constraints. In Donders' experiment, participants completed a number of different RT tasks and changes in RT were then examined. Specifically, participants completed various simple RT tasks, choice RT tasks, and go/no-go tasks.

During one set of the simple RT tasks, participants were told to verbally respond with the sound "ki" when they heard a "ki" sound; knowing that "ki" was the only stimulus and response available to them. Given that there was only one stimulus and response and the individual knew what response was required prior to the go-signal, this task is referred to as a simple RT task. Donders (1969) found that participants had the quickest RTs in this task.

A choice RT task included multiple auditory stimuli ("ku," "ki," "ka" and "ke") and participants were told to verbally repeat the sound they heard. Participants did not know what sound they would hear on any given trial and therefore did not know what response they would have to make. Donders (1969) found that RTs were slower in this task compared to the simple RT task and, based on these results, suggested that neural processes related to decision making take time. Specifically in this choice RT task, one must wait for information from the go-signal to know which response to prepare and thus all three stages of information processing model (stimulus identification, response selection, and response programming) are expected to occur after the go-signal.

A go/no-go task involved the same auditory stimuli as the choice RT task, but in this task participants were told to respond verbally with the sound “ki” only on trials in which they heard the sound “ki.” If they did not hear the sound “ki,” they were to make no response. This type of task is referred to as a go/no-go task because the participant is instructed to respond with a particular response only when a specific stimulus is presented and not respond if another stimulus is presented. Donders (1969) found slower RTs in this task compared to the simple RT task, but faster RTs than the choice RT task. Similar to a simple RT task, during a go/no-go task the participant knows exactly what movement needs to be performed prior to the imperative go-signal, and thus, according to Donders, can prepare the required response ahead of time, prior to the go-signal. However, unlike a simple RT task, participants must identify the stimulus and decide whether or not to respond, leading Donders to suggest that the processes associated with stimulus identification account for the increase in RT compared to simple RT tasks.

The differences in RT observed by Donders (1969) in his different RT tasks clearly demonstrate that the processes underlying movement initiation are influenced by task constraints. By subtracting RTs between the different tasks, Donders (1969) suggested the amount of time it takes to perform various neural processes (or certain processing stages). For example, by subtracting RT in the simple RT task from the go/no-go task, he determined the time required for processes related to stimulus identification (in particular stimulus discrimination) and by subtracting the RT time in the go/no-go task from the choice RT task, he determined the length of time required for response selection processes. Subsequent research, which will be discussed later in this proposal, has since identified a number of flaws with Donders’ subtractive method for identifying the time course of different neural processes, and indicated that processes associated with the different stages of the information processing model can occur in a more parallel manner (Cisek & Kalaska, 2010). Despite these flaws, Donders' methodology of examining RTs to investigate information processing and his suggestion that different stages of processing are included in certain RT tasks has served as a foundation for current research examining the processes underlying movement preparation.

1. Motor Preparation: A Behavioural Perspective

Donders' research outlined above provided insight into the speed of various mental (or neural) processes and he suggested that under certain conditions, movements could be prepared in advance, prior to the go-signal. However, Donders' work did not examine processes associated with response programming or determine the temporal demands associated with response programming directly. In this section of the literature review, I will discuss processes related to response programming in more detail, highlighting a number of behavioural paradigms that have been used to examine the processes underlying response programming and the associated temporal demands. Initial behavioural research examining response programming manipulated the complexity of the response required and the conditions under which the movement was to be performed (e.g. movement precuing and movement blocking paradigms).

1.1 Movement Complexity

In a classic experiment by Henry and Rogers (1960), they compared RT between three different rapid movements that varied in complexity. The simplest (or least complex) movement required participants to release a telegraph key as quickly as possible after an auditory go-signal (movement A). The second movement required participants to perform movement A and then move as rapidly as possible to grasp a hanging tennis ball (movement B). The most complex movement required participants to perform movement A, then reach forward and strike the hanging tennis ball, reverse their hand direction and push a button, and finally reach forward and grasp another hanging tennis ball (movement C). Henry and Rogers found that RT for movement A was the fastest (165ms), followed by movement B (199ms), and finally movement C (212ms), demonstrating that RT increased with movement complexity (i.e. the number of elements included in the required action). Similar to Henry and Rogers's experimental protocol, Klapp and colleagues (1974) investigated differences in RT between a short button press "dit" and a long button press "dah." In an initial experiment which used a choice RT paradigm, the short button press was found to have a shorter reaction time compared to the long button press, suggesting that the less complex short button press movement required less time to program. However, in contrast to these initial experiments and the results of Henry and Rogers, Klapp and colleagues found that differences in RT between movements of varying complexity were eliminated when participants performed the same "dit" or "dah" button presses in a simple RT paradigm. Klapp and colleagues suggested that a simple RT paradigm was able to eliminate the differences in RT due to the participant's ability to program the response in advance, and store the resultant program until needed.

To explore the different results in simple RT tasks when movement complexity was manipulated in greater detail, Klapp (1995) had participants perform another RT task. In this task, participants performed one of four different response patterns; a short (S) "dit," a long (L) "dah," or a combination of the short and long elements (e.g. SLLS or LSSL sequences). Unique to this experiment was the fact that participants were tested once a day for eight days. In the first experiment, using a simple RT paradigm, it was found that on day one single element RT responses were faster than the four element RT responses. Interestingly as the days progressed, the four element responses began to have faster and faster RTs, until they were as fast as the RTs observed when participants were making single element movements. According to Klapp, this change in RT following practice was due to the four element response being recoded into a single “chunk,” and RT is determined by the number of movement chunks that need to be prepared. Early on, the four element responses were coded as four chunks, thus the RT was longer compared to one element responses. However, following practice, both one and four element responses were represented as single chunks and hence had similar RTs. This proposal suggests that Henry and Rogers (1960) saw increases in RT between their different movements as movement complexity increased because the movements were composed of a different number of chunks, with the most complex movement having the most movement chunks.

In summary, these studies indicate that simple RTs are faster than choice RTs due to the fact that participants are able to program the movement in advance. Moreover, the results suggested that the RT differences in a simple RT task between different movements is due to the amount of chunks the response is/can be coded in (i.e. the complexity of the movement), and, with practice, responses can be recoded into smaller chunks.

1.2 Movement Precuing

Another paradigm that has been used to investigate response programming is a precuing paradigm. The original precuing paradigm was developed by Rosenbaum in 1980, and in his task participants were provided with information regarding a component of the upcoming movement to be completed prior to the go-signal. In particular, participants were provided with precue information regarding which arm to use, what direction to move and/or how far to move. Participants were instructed to make movements to buttons on a response panel, in which there was four rows of response buttons, with two buttons (a left and a right) within each row for a total of 8 possible response buttons. Half of the response buttons were above the home position (a row of 2 far targets and a row of two near targets) and the other buttons were below the home position (a row of 2 far targets and a row of two near targets). A trial began with participants touching the home position (in the center of the 8 response buttons) with their right and left index fingers. Precue information was then provided on some trials prior to the go-signal. Precues were displayed at the top of the response panel for 3000 ms and consisted of typed capital letters that conveyed information as follows; arm information: R or L for right arm or left arm respectively, direction information: F or B to move forwards or backwards respectively from the home position and extent information: N or D for near or distant (far) targets respectively. On each trial, no precues, one, two, or all three precues were presented. When any of the three possible precues were not provided the letter X would be shown instead to ensure that three letters were consistently displayed on each trial. The go-signal was presented 500 ms after the precues were extinguished. Results indicated that participants were fastest when all movement parameters were specified (i.e. it was a simple RT task), and slowest when no movement parameters were specified (i.e. an 8 choice RT task). Surprisingly, Rosenbaum found that when the same number of response choices were available (2 choices), there was a difference in RT depending on the precues available. In particular, RT was fastest when arm and direction information were provided, second fastest when arm and extent information were provided, and slowest when direction and extent information were provided.

The above results suggest several things about motor processes. First, Rosenbaum suggested that increases in RT may be due to an increased amount of specifications or auxiliary motor decisions associated with the non precued characteristic. Specifically, longer RTs are reflective of the time required to program the non-precued movement component, a proposal also put forth by Larish and Frekany (1985). For example, Rosenbaum's results suggest that it takes longer to specify arm than movement direction and longer to specify movement direction than movement extent when preparing a movement. Second, Rosenbaum's results provided support that pre-programming can indeed occur. Lastly, the results suggested that motor programming does not necessarily occur in a hierarchical (top-down ordered) manner, as no matter what characteristic of the movement (i.e. arm/direction/extent) was precued participants were able to use the information available to pre-program that distinctive feature of the movement in advance and hence shorten their RTs. In accordance with Rosenbaum's findings, others have demonstrated similar RT advantages when components of a movement are precued (e.g., Bock & Arnold, 1992; Goodman & Kelso, 1980; Larish & Frekany, 1985; Lepine, Glencross, & Requin, 1989; Zelaznik & Hahn, 1985). Several of these studies will be discussed later on in the literature review when we discuss response programming underlying direct vs. indirect movements.

1.3 Movement Blocking

A final behavioural paradigm that I will discuss that has been used to investigate response programming is a blocking paradigm (e.g., Wadman, Denier Van der Gon, Geuze, & Mol, 1979). In Wadman's paradigm, participants performed fast goal directed arm extension movements. Participants were allowed to initiate these movements whenever they wanted, as long as they performed them as fast as possible. On some trials, participants' arms were blocked when they attempted to move. By recording muscle activity of the agonist (triceps) and antagonist (biceps brachii) using electromyography (EMG), Wadman observed a typical triphasic EMG pattern of muscle activation on control (non-blocked) trials; where the triphasic pattern consisted of an agonist burst used to initiate the movement, followed by the onset of the antagonist and then finally a second burst of activity in the agonist after antagonist offset (Agarwal & Gottlieb, 1972; Hallett, Shahani, & Young, 1975). Surprisingly, EMG results from the blocked movements were almost identical to those of the unblocked movements for at least the first 100 ms following "movement onset" and consisted of the same triphasic muscle activation pattern even when no actual movement occurred. These results suggest that the timing of different muscle actions, as evident by the EMG muscle activation pattern, had been pre-programmed and executed without influence from sensory feedback (i.e. proprioceptive information).

Taken together, these behavioural studies demonstrate that if one is provided with information regarding the movement to be performed, response programming can occur prior to the go-signal. Moreover, the entire movement can be pre-programmed in advance. Finally, from the results discussed earlier it is evident that the complexity of a movement influences the time it takes to prepare a response. In the next two sections, I will discuss research in which many of the same behavioural paradigms outlined above are used to examine response programming. In the following section (Response Programming: A Neurophysiological Perspective), these paradigms are used while neural activity is recorded or modified. Finally, in the third section of the literature review (Response Programming: The StartReact Effect), these behavioural paradigms are used when a loud acoustic signal accompanies the go-signal.

2. Motor Preparation: A Neurophysiological Perspective

In addition to the behavioural evidence discussed above, neurophysiological evidence can also provide insight into the processes underlying response programming. Specifically, as outlined below, researchers have used electroencephalography (EEG) and transcranial magnetic stimulation (TMS) to demonstrate advanced motor preparation prior to movement initiation and investigate the locus of this response preparation. Given the current research question of interest, and the fact that I will not be using neurophysiological tools in the present study, I will only briefly outline how EEG and TMS can be used to investigate neural correlates underlying motor preparation, highlighting a few key studies that have used these techniques.

2.1 Electroencephalography

In the EEG literature, it has been demonstrated that there is a negative-going shift in electrical potential [the so-called Bereitschaftspotential (BP) or readiness potential (RP)] that starts approximately 2 seconds prior to voluntary movement initiation (Kornhuber & Deecke, 1965; as cited in Shibasaki & Hallett, 2006). This activation is maximal at the midline centro-parietal areas, and is symmetrically and widely distributed (Shibasaki & Hallett, 2006). As movement onset approaches, the RP becomes lateralized such that the activity recorded from electrodes placed over left and right motor areas (i.e. at sites C3’ and C4’ respectively) differs in magnitude. Specifically, by determining the difference in neural activation at sites C3’ from C4’ it has been demonstrated that there is more cortical activation in the hemisphere contralateral to the responding hand. This asymmetry in electrical potentials [i.e. the lateralized readiness potential (LRP)] can be used to provide an index of the level of motor preparation prior to movement onset (Leuthold, Sommer, & Ulrich, 1996).

To investigate the neural correlates underlying motor preparation, Leuthold and colleagues (1996) used a precuing paradigm while recording neural activity using EEG. Participants sat facing a screen which had four gray open boxes arranged in the shape of a square, which corresponded to specific responses. In particular, the two boxes on the right indicated right finger responses and the two boxes on the left indicated left finger responses. The top boxes corresponded to finger extension and the bottom boxes corresponded to finger flexion. There were four categories of trials in which different precue information was provided (which were represented by shading the boxes 1000ms prior to the go-signal). In one type of trial (the simple RT task) only one square was shaded. In a second set of trials, finger precue information was provided by shading in the boxes on the left or right. In the third set of trials, information regarding movement direction (extension or flexion) was provided by shading either the top or bottom boxes. In the fourth type of trials no information regarding the upcoming response was provided and all four boxes were shaded. Results indicated that motor preparation was initiated, as indicated by the onset of the LRP, well in advance of motor execution during the first and second set of trials (see also Ulrich, Moore, & Osman, 1993). There was no evidence of advanced motor preparation on trials in which only movement direction was precued (i.e. the finger to be used was not precued) or no information regarding the upcoming movement was provided (Leuthold, et al., 1996).

Motor preparation has also been shown to occur well in advance of motor execution during a go/no-go mental rotation task (Band & Miller, 1997). In the go/no-go task of Band and Miller (1997), participants were instructed that certain letters and numbers were assigned to particular responses. For example, participants were to respond with the left finger to a “2, G, P, or R” and with the right finger to a “7, F, J, or Q.” In addition, the letter or digit’s form (i.e. normal vs. mirror image) determined whether the response was to be made (press the left or right button) or withheld (no-go). At the start of the trial, participants would need to mentally rotate various letters and digits to an upright position, and then determine the required response. EEG results showed that response preparation began before mental rotation was complete, as demonstrated by LRP onset on trials in which the participant both responded and did not respond. These results suggest that motor preparation of the response began as soon as the corresponding letter or digit was identified, as opposed to waiting until mental rotation was complete to program the required response (Band & Miller, 1997).

2.2 Transcranial Magnetic Stimulation

TMS can also be used as a tool to examine the neural correlates underlying motor preparation. Specifically, in order to examine motor pathway excitability, a magnetic pulse is delivered over a specific motor cortical area (e.g. the hand area of the motor cortex), which activates the underlying neurons. If the magnitude of the pulse is great enough, the firing of these neurons will result in activation of the targeted muscle. By examining the resultant EMG trace, the resultant muscle activation or motor evoked potential (MEP) can then be observed and quantified. It has been suggested that an increase in MEP is reflective of an increase in excitability within the motor pathway (Day et al., 1989; Pascual-Leone et al., 1992; Starr, Caramia, Zarola, & Rossini, 1988). Research by Leocani and colleagues (2000) investigated corticospinal excitability during RT tasks with the use of TMS. Participants were to perform either a right or left thumb extension in a simple, choice, or go/no-go task while TMS was applied to the extensor pollicis brevis (EPB) region of the motor cortex bilaterally at various times after the auditory go-signal. The results showed that for all paradigms the MEP amplitude recorded from the soon to be active EPB increased in the 80-120ms before voluntary EMG onset, while inhibition was evident in the resting side (i.e. the EPB that was not activated). These results are similar to previous TMS results, demonstrating an increase in MEP amplitude prior to EMG onset (e.g., Chen et al., 1998; Hoshiyama et al., 1997; Hoshiyama et al., 1996), as well as suppression of activity in the non-responding hand (e.g., Duque et al., 2005; Liepert, Dettmers, Terborg, & Weiller, 2001; Sohn, Jung, Kaelin-Lang, & Hallett, 2003). The rise in MEP amplitude prior to voluntary movement initiation observed by Leocani and colleagues (2000) was suggested to be associated with an increase in excitability within the corticospinal pathway caused by increasing motor preparation.

With the understanding of the modulation of corticospinal excitability during a RT task, Michelet and colleagues (2010) wanted to examine the idea that during a choice RT task (in which one has to make a decision regarding the response required), multiple options are initially activated in the cortex in parallel and then gradually eliminated in a competition for overt execution [i.e. affordance-competition hypothesis (Cisek, 2006, 2007)]. To test this question, Michelet and colleagues, applied TMS over the first dorsal interosseus (FDI, a finger flexor) region of the primary motor cortex at five different time points following cue onset in an Eriksen flanker task. In the control condition, a single arrow (← or →) appeared on the screen, and subjects were asked to flex if it pointed to the left or extend if it pointed to the right. In the congruent condition, four addition flanker arrows appeared beside the central arrow, pointing in the same direction (←←←←← or → →→→→). In the incongruent condition, the flanker arrows pointed in the opposite direction and the subject was instructed to respond only on the basis of the central arrow (flexion for →→←→→ and extension for ←←→←←). Results from the control and congruent conditions showed a gradual increase in MEP amplitude in the agonist muscle as the response was being prepared (i.e. over the course of RT), reaching a constant maximum level just before movement initiation, suggesting an increase in corticospinal excitability. Conversely, when the same muscle acted as an antagonist, MEP size decreased, suggesting a decrease in corticospinal excitability. Interestingly, results from the incongruent condition showed a brief increase in MEP's in the muscle corresponding to an initial default response to the flanker arrows, which was later replaced by a MEP corresponding to the correct central arrow response. The authors suggested that the dynamic modulation of MEP's (corticospinal excitability) as seen in the incongruent condition, provides evidence that multiple response options are initially specified in parallel and then gradually eliminated in a competition for overt execution (i.e. affordance-competition hypothesis).

Taken together both behavioural and neurophysiological studies have enhanced our understanding of response programming. Behavioural studies have demonstrated that if one is provided with information regarding the movement in advance, motor response programming can occur prior to the go-signal. In addition it has been clearly demonstrated that the complexity of the movement influences the time it takes to prepare the response. Neurophysiological studies provide additional support that response programming can occur well in advance of motor execution and multiple responses can be prepared in parallel, in addition to providing evidence that this preparation can occur in the motor areas of the cortex and corticospinal pathways.

While the behavioural and neurophysiological experimental paradigms discussed can be used as tools to investigate motor preparation, they provide limited information regarding exactly how much preparation has occurred or what has been prepared at a particular point in time prior to movement onset. Fortunately, researchers have found another tool, “startle,” to address some of these questions surrounding motor preparation.

3. Motor Preparation: The StartReact Effect

Recently, response programming has been investigated using a loud acoustic stimulus (i.e. a SAS), as this technique allows researchers to peer into motor preparatory processes by means of examining “voluntary movement production” during startle reactions (StartReact effect) to see what components of a movement are programmed and when. In this next section, I will discuss results from the startle literature in detail, as I will be employing a SAS in the current research project. I will first describe the general startle response, followed by an explanation of the StartReact effect. I will then discuss current literature in which a SAS was used to probe response pre-programming (i.e. determine what components of a movement can be pre-programmed. This will be followed by a discussion of the time course of response pre-programming. Finally, a review of the proposed neurophysiological mechanism underlying the StartReact effect will be discussed.

A typical response to a SAS is a fast reflexive response. It is characterized by a general symmetrical flexion of the body, with the most prominent responses observed in the face, neck, and shoulders, then tapering caudally (Landis, Hunt, & Strauss, 1939; Wilkins, Hallett, & Wess, 1986). Onset latencies of EMG activity during a reflexive response occur in the orbicularis oculi (OOC) 30-40 ms following the SAS. This is then followed by muscle activation in the masseter and sternocleidomastoid (SCM) at 55-85 ms, biceps brachii at 85-100 ms, hamstrings and quadriceps at 100-125 ms, and tibialis anterior at 130-140 ms (Wilkins, et al., 1986). The muscle activation patterns that have been shown to be most consistent and resistant to startle habituation (after being exposed to a SAS repeatedly) are contraction of the OOC and the SCM (Brown et al., 1991). However, while OOC activation is consistent and resistant to habituation, it has been proposed to be due to a separate blink reflex because of the extremely short latency at which it is activated following a SAS, (Brown, et al., 1991). Thus activation in the SCM is proposed to be the most reliable indicator that someone has been startled (Carlsen, Chua, Inglis, Sanderson, & Franks, 2003). It is important to note that the startle response described above is a reflexive response, meaning that the associated response is involuntary.

Initial research by Valls-Solé et al. (1995) sought to determine what would happen if a SAS, that typically gave rise to the involuntary startle reflex described above, was paired with a visual go-signal in a simple RT task. Participants were instructed to raise their hand and hit a switch as fast as possible in response to the go-signal. Valls-Solé found that when the SAS was delivered at the same time as the visual go-signal, RT was shorter. In fact, premotor RT, which is the time measured from stimulus onset to recorded EMG agonist muscle activation, was 80 ms; 72 ms faster than trials in which just the visual go-signal was displayed. Following this work, Valls-Solé et al. (1999) then investigated the effect of a SAS on the pattern of muscle activity observed during a simple wrist flexion movement to a target. The EMG data again demonstrated a remarkable shortening effect of the SAS on premotor RTs (171ms to 77ms), and it also revealed something important about the movement itself. In particular, in the SAS trials, a similar pattern of muscle activation was observed as that seen in the control (non-SAS) trials. Specifically, a similar triphasic muscle activation pattern emerged, beginning with activation of the agonist, then antagonist, followed again by activation of the agonist. In fact, the only differences between the two sets of trials was that SCM activation was observed in the SAS trials and the EMG pattern was shifted forward in time in the SAS trials compared to the control (non-SAS) trials due to the earlier release of the movement.

Based on these results Valls-Solé and colleagues concluded that the movement released by the SAS was not simply a startle reflex, rather, the intended voluntary response was being performed at a very short latency. Moreover, it was suggested that the initiation of these movements could not involve the cortex given the onset latencies of the responses. Typically, a premotor RT of 140 ms is seen in response to an auditory go-signal (Brebner & Welford, 1980). On these SAS trials, responses were elicited with a mean premotor RT of 77 ms, with premotor activity occasionally occurring at latencies less than 65 ms (Valls-Solé et al., 1999). Given that the minimum time needed to transduce an auditory stimulus and for corresponding activation to arrive in the auditory cortex is suggested to be 35 ms (Erwin & Buchwald, 1986), and the efferent conduction time to the forearm is approximately 20 ms, this would leave only 10 ms for cortico-cortical transmission in the SAS trials (Valls-Solé et al., 1999). Thus, it was suggested that the SAS triggered a pre-programmed movement (motor program) stored subcortically in the brainstem and spinal centers.

In contrast to Valls-Solé's pre-programming and storage explanation, it could be argued that faster RTs in the SAS trials arose due to (1) the increased intensity of the go-signal and hence faster perceptual processing of the stimulus or (2) increased excitability in motor pathways. However, research by Carlsen and colleagues following Valls-Solé's work has clearly demonstrated that faster reaction times observed in SAS trials cannot be explained by faster perceptual processing (Carlsen, Dakin, Chua, & Franks, 2007) or increased excitability in the motor pathways (Carlsen, R. Chua, Inglis, Sanderson, & Franks, 2004). In a study by Carlsen at al. (2007), they manipulated the intensity of the go-signal from 84 dB to 124 dB and examined the effect of stimulus intensity on RT. They found that participants were startled (i.e., showed SCM activity) on a greater number of trials as stimulus intensity increased. However, regardless of the stimulus intensity level, if a participant was startled, RT was very fast (less than 100 ms). Thus it can be concluded that the stimulus intensity of the go-signal was not responsible for the shortened RTs observed by Valls-Solé and colleagues (1999). As long as participants are startled, they show evidence of a StartReact effect, regardless of stimulus intensity level.

To rule out the possibility that increased motor pathway excitability lead to faster RTs on SAS trials compared to control non-SAS trials, Carlsen at al. (2004) examined RTs to SAS trials presented in simple and choice RT tasks. As outlined above, in the choice RT trials, it is not possible for participants to pre-program their responses prior to the go-signal. Thus, if the SAS acts to trigger a movement stored subcortically in the brainstem and spinal centres, as suggested by Valls-Solé and colleagues (1999), there should not be a significant reduction in RT on SAS trials compared to control (non-SAS trials) in the choice RT task. In contrast, if changes in the level of excitability of the motor pathways are responsible for the observed changes in RT on SAS trials versus non-SAS trials, RT should be significantly reduced regardless of whether the SAS is introduced during the simple or choice RT tasks. In the simple RT task, participants were to perform a 20° wrist extension movement with the right hand. In the choice RT task, 2 or 4 response alternatives were presented and participants had to perform a 20° flexion or extension movement with either the left or right hand in response to the go-signal. The SAS was delivered in conjunction with the go-signal in 12% of the right hand extension trials, whether simple RT, 2-choice RT, or 4-choice RT trials. Similar to previous findings, results showed that on SAS trials in the simple RT task, premotor RT was significantly shorter (mean premotor RT = 86 ms) than on the non-SAS trials (mean premotor RT = 142 ms). In contrast to the simple RT results, the SAS did not influence RT in the 2- or 4-choice RT tasks, with similar premotor RTs observed in the non-SAS and SAS trials (e.g. 2 choice RT: non-SAS mean premotor RT = 204 ms, SAS mean premotor RT = 203 ms; 4 choice RT: non-SAS mean premotor RT = 236 ms, SAS mean premotor RT = 234 ms). These results indicate that a SAS only shortens the time it takes to initiate a response when it is possible for the response to be prepared ahead of time, hence supporting the suggestion that the SAS triggers a pre-programmed movement and does not simply facilitate responses due to increased perceptual or motor activation (Carlsen et al., 2004).

In summary, the presentation of a SAS can trigger a pre-programmed movement, resulting in a quick release of the movement (Carlsen et al., 2004; Carlsen et al., 2007; Valls-Solé et al., 1999). With this information regarding the use of a SAS and the StartReact effect, a

SAS can be used as a tool to investigate the components of a movement that can be pre-programmed and when pre-programming occurs.

3.1 What is Pre-Programmed?

As previously discussed, the presentation of a SAS can lead to the early release of a goal directed movement in a simple RT task, but not in a choice RT task (Carlsen et al., 2004). Thus, it has been suggested that when a response is known in advance, that response can be pre-programmed such that it is prepared in entirety and ready for execution. In contrast, when the response is not known in advance (i.e. in a choice RT task), pre-programming cannot occur (Carlsen et al., 2004). With this background, researchers have used a SAS to probe the components of a movement that are pre-programmed and examine the circumstances under which movements are pre-programmed.

In a recent experiment by Carlsen and colleagues (Carlsen et al., 2008) they included a SAS in a go/no-go task. Participants knew in advance what movement (key press) they had to perform, but were unaware of whether they were to initiate (go), or withhold (no-go) the response. If the imperative go-signal was blue, they were to make a response. If the imperative stimulus was red, they were to make no response. According to previous research, in a simple RT task the response is programmed prior to the go-signal (Carlsen et al., 2004; Valls-Solé et al., 1999). Furthermore, Donders (1969) suggested that in a go/no-go task the response is also prepared prior to the go-signal. Thus, given these previous findings, the authors hypothesized that if the response is indeed pre-programmed, it would be released early by the SAS on both go and no-go trials. However, the data revealed that when a SAS was presented in addition to the go or no-go stimulus, there was no StartReact effect, such that there was no early response initiation of the movement when startled on either type of trial (go or no-go trial). Instead, results indicated no differences in RT between SAS and non-SAS go-trials, even when participants were startled. These results indicate that response pre-programming had not occurred prior to the go-signal. Thus, in contrast to Donders' proposal, Carlsen et al. (2008) concluded that response pre-programming does not occur during a go/no-go task. Instead, participants treat this type of task like a choice RT task and wait until the imperative stimulus arrives to prepare the required response. These results further suggest that humans employ a strategy of accuracy (correct response) over speed (fast response) during go/no-go and choice RT tasks, indicating that one's ability to execute the accurate response may dictate if a movement is pre-programmed.

Further work by Carlsen and colleagues (2009) has begun to examine what movement components are pre-programmed when only part of an upcoming movement is known in advance of the imperative go-signal. Using a precuing paradigm similar to that developed by Rosenbaum (1980), the authors paired a SAS with the imperative go-signal to determine what, if any components of the response was pre-programmed based on the precued information provided. Participants sat facing a screen which had four boxes displayed in a horizontal manner; each box corresponded to a specific target and movement with either the left of right hand. The two boxes on the left represented the two possible movements for the left hand (outside box = wrist extension, inside box = wrist flexion), and the two boxes on the right represented the two possible movements for the right hand (outside box = wrist extension, inside box = wrist flexion). Precue information was provided by the boxes, which represented potential targets (and hence movements), lighting up in four different patterns: (1) hand precue information was provided (e.g. both right boxes were illuminated), (2) direction precue information was provided (e.g. both inside boxes were illuminated), (3) ambiguous precue information was provided (e.g.

the left inside box and right outside box were provided), (4) the exact target was illuminated (e.g. right outside box, simple RT task). It is important to note that participants were instructed to prepare all the components of the movement possible based on the precue. In contrast to Rosenbaum's (1980) suggestion that whatever part of the movement was known in advance would be pre-programmed resulting in shorter RTs, results revealed that multiple full responses were pre-programmed and released by the SAS during the direction and ambiguous precue conditions(Carlsen et al., 2009). For example, when a precue provided information regarding movement direction (i.e. the boxes indicating right and left wrist extension were highlighted), the SAS in conjunction with the visual go-signal indicating a right hand extension movement was to be performed, resulted in the early release of both right and left hand extension movements. These results indicated that instead of pre-programming only the part of the response that was specified by the precue, participants used the precue to pre-program both possible responses in their entirety. Contrary to results achieved when direction or ambiguous precues were presented, when the precues indicated the hand to respond with but not direction (boxes indicating right flexion and extension were illuminated), a SAS did not lead to a clear extension or flexion movement. Instead, participants committed a great number of errors (63%), with the majority involving movement initiation towards flexion with a subsequent quick correction into extension. These errors suggest that the SAS elicited a natural startle reflex as opposed to a pre-programmed movement, signifying that participants did not or could not prepare multiple responses when the responses were associated with the same hand. In summary, this research provides evidence that one is only able to pre-program multiple responses when they are associated with multiple limbs, demonstrating that precues improve RT by aiding motor programming processes (Carlsen et al., 2009).

By using startle, it is evident that what is pre-programmed is dependent on a number of factors. Such as, (1) the certainty that a specific response will be executed, (2) the opportunity for errors to influence whether a movement will be pre-programmed. When one knows the exact movement one has to perform, he or she can program the full response prior to the go-signal. Interestingly one is also able to plan parts of a movement (e.g. direction information) in advance when those parts are certain to occur. Finally it appears that two whole movements can be pre-programmed simultaneously, as long as the two movements involve different limbs.

3.2 When Are Responses Pre-Programmed?

3.21 Temporal Uncertainty

As discussed above, using a SAS in a simple RT task or precuing paradigm can be useful when attempting to determine what components of the movement are pre-programmed in a RT task. In addition to examining what is pre-programmed, a SAS can also be used to examine the time course of pre-programming. Cressman and colleagues (2006) began to investigate this question using startle by looking at the effect of temporal uncertainty of the auditory go-signal on RT in a simple RT task. In order to manipulate the temporal uncertainty of the go-signal, they adjusted the foreperiod by changing the time between the warning (i.e. get-ready) signal and the actual go-signal. When the time between the warning and go-signal varies over trials, it is referred to as a variable foreperiod experimental design. Previous research investigating response preparation has found that minimal RT is achieved when the go-signal is presented when it is most expected (i.e. go-signal presented at the average foreperiod) (Drazin, 1961; Niemi & Näätänen, 1981). In the experiment by Cressman and colleagues (2006), participants were required to perform a 20° wrist extension movement in response to an auditory tone that was presented between 2500 to 5500 ms after a warning stimulus. On certain trials, the control auditory stimulus was unexpectedly replaced by the SAS. Similar to previous findings, results showed that when participants were startled, the intended movement was initiated with a significantly faster RT that the control (non-SAS) trials (mean RT on control trials: 144 ms (SD 43) vs. mean RT on SAS trials: 73 ms (SD 11), regardless of foreperiod duration. Moreover, consistent with previous RT findings (e.g., Drazin, 1961; Niemi & Näätänen, 1981), RT varied as a function of foreperiod duration in the control RT trials. Specifically premotor RT was fastest when the go-signal was presented during the centre of the range of foreperiods, suggesting that participants attempted to synchronize response readiness with the average estimated time at which the go-signal was presented. However, in contrast to the control trials, RT was similar across all SAS trials when participants were startled, regardless of foreperiod duration. These results suggest that in a variable foreperiod task, the movement is fully prepared by the time the earliest go-signal is presented and remains prepared for at least three more seconds without decay (Cressman et al., 2006).

Further research examining the effect of temporal predictability of the go-signal on motor preparation had participants perform a right wrist extension in which the go-signal was presented within a variable foreperiod (2 to 3 seconds after the get-ready signal) or when the go-signal was always presented 3 seconds after (i.e. a fixed foreperiod design) (Carlsen & Mackinnon, 2010). Participants were not aware of the foreperiod duration or whether the foreperiod varied between trials, therefore both conditions were considered to have low temporal resolution. To determine when pre-programming took place the SAS was administered at three time points (-1500 ms, -500 ms, -150 ms) prior to the go-signal. Results showed that when the SAS was delivered 1500 ms prior to the go-signal, 57.5% of the variable foreperiod and 68.1% of the fixed foreperiod trials resulted in an early StartReact effect. Interestingly when the SAS was delivered 500 ms prior to the go-signal the percent of trials that resulted in an early StartReact effect rose to more than 87.5% and 92.5% respectively. It was suggested that since the proportion of trials in which the SAS elicited the motor command increased from 60% to over 90% as the anticipated go-signal neared, it appeared that the response was likely “programmed” and thus ready for initiation sometime between 2 seconds and 1 second prior to the go-signal (Carlsen & Mackinnon, 2010). Similar results have also been observed in a task in which participants were required to perform a stepping movement in response to the go-signal (MacKinnon et al., 2007). In this task, it appeared that the movement was programmed progressively, such that parts of the multidimensional movement were programmed before others. This was demonstrated by small amplitude anticipatory postural adjustments being released 1400 ms prior to the go-signal by a SAS, which were then combined with the full stepping movement when the startle was presented 100 ms prior to the go-signal.

In summary, during a simple RT task when the temporal predictability of the go-signal is low (uncertain), we prepare the response so that it is fully pre-programmed prior to the earliest possible go-signal. Moreover, a response appears to be programmed in full at least 500 ms prior to the earliest possible go-signal for single joint movements and 100 ms prior to the earliest possible go-signal for multi joint movements. These paradigms also demonstrate the ability of SAS to probe the temporal dynamics of motor preparation.

3.22 Temporal Certainty

Researchers have also investigated the effect that temporal certainty and high temporal predictability of the go-signal have on response pre-programming. In a recent task by Carlsen and Mackinnon (2010), participants performed a right wrist extension in response to a go-signal, when they had high predictability of when the go-signal would be presented. Specifically, in one paradigm, the clock-face countdown condition, an analog clock face without any numbers was displayed. Participants were told to initiate their response at the precise time that the clock hand, reached the 9 o'clock position (indicated by a horizontal red arrow). The clock hand began at the 12 o'clock position and took 3 seconds on each trial to reach the 9 o'clock position. In a second paradigm, the countdown condition, four squares appeared sequentially from left to right on the display at 1000ms intervals (total time from the onset of the first square to the fourth square was 3 seconds). Participants were instructed to initiate their response at the precise moment the last square lit up. Both of these paradigms are considered anticipation-timing tasks due to the high temporal resolution/predictability of the time at which the response was to be initiated (the anticipated go-signal), as opposed to RT tasks in which one is reacting to the onset of a stimulus (e.g., visual or auditory stimulus). Once again, to determine when pre-programming took place the SAS was delivered at three possible time points (-1500 ms, -500 ms, -150 ms) prior to the go-signal. Contrary to the results discussed above when the temporal uncertainty was high, when the SAS was delivered 1500 ms and 500 ms prior to the go-signal, 0% and 18% of the trials resulted in an early StartReact effect in the clock-face countdown and countdown paradigms respectively. Interestingly when then SAS was delivered 150 ms prior to the go-signal the percent of trials that resulted in an early StartReact effect was 98% for both types of countdown trials. Therefore, these results suggest that unlike a variable foreperiod simple RT task, when the temporal predictability of the response is high, humans employ an efficiency strategy, such that they do not begin to prepare the movement until approximately 200 ms prior to when a response needs to be initiated (Carlsen & Mackinnon, 2010).

These temporal results are in agreement with previous findings using a stop-signal anticipation-timing task (Slater-Hammel, 1960). Specifically, Slater-Hammel demonstrated that responses could be suppressed when the “stop” (no-go) signal was presented a minimum of 150 ms prior to the anticipated go-signal, but when the “stop” (no-go) signal was presented less than 150 ms prior to the anticipated go-signal, the response could not be suppressed. Taken together, results from anticipation tasks show that response preparation is deferred until just before the movement is to be executed, allowing just enough time for the response to be programmed and then executed in anticipation of the go-signal (Carlsen & Mackinnon, 2010; Coxon, Stinear, & Byblow, 2006).

In addition to temporal predictability influencing the time at which a response is prepared, it has also been demonstrated that spatial cues can influence motor preparation. For example, motor preparation is delayed when the spatial predictability of movement initiation is high. To investigate the influence of spatial predictability on response preparation, Carlsen and colleagues (2003) had participants perform a continuous active elbow extension movement (starting at 80° of extension and ending at 180°) through a target (located 55° from the starting position) with the goal of opening their fingers from a closed position (as if releasing a Frisbee) when the target was achieved. The elbow extension movement was performed while attached to a custom made manipulandum which ensured the axis of rotation at the elbow, with the movement occurring in the horizontal plane. A SAS was delivered at three points (5°, 25°, and 45°) into the arm movement prior to the target goal. In accordance with the high temporal resolution response results, the SAS only resulted in an early opening of the fingers when the arm was near the target (i.e. when the SAS was presented at 45°). Based on these results it was suggested that the response was not programmed until just before it was required, using kinesthetic feedback from proprioceptive receptors in the joint to detect when the fingers should be released (Carlsen, Hunt et al., 2003).

Based on the results discussed above, it appears that when a response could be required at any time (i.e. an unpredictable response time, variable foreperiod RT task) responses are pre-programmed early (Carlsen & Mackinnon, 2010; MacKinnon, et al., 2007). In contrast, when it is known when responses are to be performed (i.e. a predictable response time, anticipation-timing task), responses are programmed later (Carlsen, Hunt, et al., 2003; Carlsen & Mackinnon, 2010; Coxon et al., 2006). It has been suggested that these responses are programmed later in order to conserve energy, such that one only begins to prepare the response so that it will be fully prepared approximately 150 ms prior to response execution (Carlsen & Mackinnon, 2010).

As a whole, the literature reviewed on paradigms employing a SAS, indicates that a SAS can be used as a tool to investigate two important aspects of motor preparation: (1) what components of a movement have been pre-programmed and (2) when a motor response has been programmed. In order to probe the timing of motor preparation, the SAS can be presented at different times prior to the normal (or anticipated) go-signal.

3.3 Proposed Startle Mechanism

Having reviewed the pertinent startle literature, I will briefly outline the proposed mechanism thought to be responsible for the StartReact effect. It should be noted that the current study will not attempt to investigate the proposed startle mechanism. In the initial experiment by Valls-Solé (1995) it was demonstrated that when a SAS was delivered at the same time as a visual go-signal, premotor RT was sped up so that it was only 80 ms, 72 ms faster than trials in which just the visual go-signal was displayed. In attempt to explain these results Valls-Solé suggested that the startle stimulus enhanced the excitability of neural structures used for driving the voluntary command to execution. These neural structures included everything from the brainstem to motor neurons, with enhanced excitability by way of the reticulospinal pathway.

Following this first proposal, the early release of a pre-programmed movement was then suggested to be due to the release of the movement from subcortical structures [based on RTs which were proposed to be too fast to include cortical involvement and identical EMG movement patterns observed on SAS and control trials (A.N. Carlsen, et al., 2004; Rothwell, 2006; Valls-Solé, et al., 1999)]. In order for the movement to be released early it was thought that a pre-programmed motor program was likely stored in subcortical areas (i.e. the reticular formation), that were common to both the voluntary response and startle response pathways, and then involuntarily triggered by the SAS (Carlsen et al., 2004; Rothwell, 2006; Valls-Solé et al., 1999). It is important to note that this original proposed startle mechanism implies that there are two representations of the motor program, one in the cortex and a second stored subcortically.

More recently, Carlsen and colleagues (Carlsen, Maslovat, & Franks, 2012) have suggested an alternative mechanism for the StartReact effect. After reviewing the startle literature they suggest that “a SAS acts to facilitate RT by quickly and directly increasing the activation of the initiation mechanism via a subcortically mediated ascending pathway such that the cortically stored response (in the form of a cell assembly) is triggered without the usual cortical processing” (p.26). This proposed mechanism does two things, first it eliminates the need for a motor program to be stored subcortically, and second, it implies that motor response preparation and initiation are two distinct processes. Carlsen and colleagues state that so long as sufficient response preparation has occurred, the SAS is able to increase response initiation activation levels to an extent that will reach the required threshold and trigger the movement. Although it is important to understand and be aware of the possible mechanisms behind the quick release of a pre-programmed movement, the purpose of the current study is not to examine or contrast these alternative mechanisms. Rather we will apply previous knowledge regarding the SAS and the StartReact effect to further probe when motor preparation occurs, specifically for indirect movements.

4. Motor Preparation: Direct vs. Indirectly Cued Movements

In the research employing a SAS, response preparation has typically been examined under conditions in which participants are required to make a response to a target that is directly cued (i.e. respond by moving to or in the direction of a target). In the current proposal, I am interested in determining what happens to response preparation when one has to make response to an indirectly cued target (i.e. move to a position that is dependent on the position of a visual cue). Previous paradigms used to examine indirectly cued movements include, pro-pointing vs. anti-pointing, symbolic precues vs. direct precues and a mental rotation task.

4.1 Pro- vs. Anti-pointing

Recent research examining response preparation underlying direct and indirectly cued movements has demonstrated that RTs are longer when one has to make a response that is indirectly cued. Specifically, Heath et al. (2009) found slower RTs when participants were to complete an anti-point movement (i.e. make a movement to a position opposite or 180° from the seen target) compared to pro-pointing movements (i.e. pointing directly to the target). It was proposed that the slower RTs were a result of the increased processing demands associated with countermanding a (directly cued) stimulus driven response. In addition to RT differences, research examining anti-pointing movements has found reduced spatial and temporal resolution compared to pro-pointing (Maraj & Heath, 2010). These results have led Maraj and Heath (2010) to propose that anti-pointing is controlled offline with input from perceptual processes, such that the preparation of an indirectly cued motor response is completed prior to movement onset. Thus, the increase in RT observed during indirectly cued movements (or reaches to "indirect targets") is proposed to be due to additional mental processes that are required to occur prior to response initiation.

Neurophysiological evidence indicating that anti-pointing is a more (cognitively) demanding task than pro-pointing has been shown by Connolly and colleagues (2000) with the use of functional magnetic resonance imaging (fMRI). FMRI indirectly measures neural activation by detecting changes in blood flow to different parts of the brain. High levels of oxygen consumption indicate a high degree of activation, while low levels of oxygen consumption suggest a low degree of activation. Connolly et al. (2000) found that the production of anti-pointing movements recruited additional frontoparietal areas in comparison to pro-pointing. Specifically, in the parietal cortex the posterior superior parietal area (pSPR), medial superior parietal area (mSPR), and middle inferior parietal area (mIPR) were activated in addition to shared activated areas during pro- and anti-pointing. Additional activation areas in the frontal cortex during anti-pointing included the pre-frontal eye field (FEF), the pre-supplemental motor areas (SMA), and the anterior cingulate (anCing). The activation of these additional brain areas during anti-pointing suggests that supplementary cognitive control is needed to process additional operations important to cognitively mediated movements (Connolly et al., 2000).

Differences in response preparation processes' underlying direct vs. indirectly cued movements to visual targets has been suggested to be due to the motor output being mediated differently by the two visual pathways (Milner & Goodale, 2008). According to Goodale and Milner (1992), visual processing is mediated by two cortical pathways; a dorsal stream, projecting from the primary visual cortex to the posterior parietal cortex and a ventral stream, projecting from the primary visual cortex to the infero-temporal cortex. According to Goodale and Milner, the dorsal stream mediates the visual control of skilled actions, such as reaching and grasping, directed at objects in the world. In contrast, the ventral stream is proposed to transform visual inputs into perceptual representations that embody the enduring characteristics of objects and their spatial relations (Goodale & Milner, 1992; Milner & Goodale, 1993, 2008). It is important to note that in Milner and Goodale's model, and in other models of visuomotor control (Glover & Dixon, 2002; Glover & Dixon, 2001), processes in the perceptual (ventral) stream can influence action. For example, the ventral stream can play a role in selecting the correct action in response to a stimulus, thus influencing the subsequent implementation of that action by the dorsal stream. Moreover, when a delay is inserted between presentation of the target stimulus and when the go-signal is presented, individuals with optic ataxia (damage to the dorsal stream) show a significant improvement in their pointing accuracy (Dijkerman, Milner, & Carey, 1999; Himmelbach & Karnath, 2005; Milner, Dijkerman, McIntosh, Rossetti, & Pisella, 2003). This improvement is argued to occur because the patient can now use a memory of the stimulus location, based on visual perceptual processing from the relatively intact ventral stream. In support of this explanation for improved pointing accuracy being guided by processes occurring in the ventral stream, experimental data from an individual with visual form agnosia (i.e. damage to the ventral stream), demonstrated the opposite pattern of results in the same experimental paradigm (Milner, Paulignan, Dijkerman, Michel, & Jeannerod, 1999). Specifically, the individual with visual form agnosia was very inaccurate in pointing when a delay was introduced between stimulus presentation and the go-signal, compared to trials when no delay was present. This detriment in performance was argued to have occurred due to the required ventral stream processing in the delay task, which was not functioning in the visual agnostic patient.

From this body of literature it can be concluded that response preparation when pointing directly to targets (pro-pointing) is different than response preparation when making indirectly cued movements (anti-pointing). The source of this difference is suggested to lie in the two streams having a different impact on motor output. For example, the dorsal stream seems to be heavily implicated in actions completed to objects directly, while the ventral stream is proposed to mediate indirect movements. However, this is not to say that the dorsal or ventral streams do not have a role in both types of action. Recent evidence suggests that there are many connections between the two streams and processes in the dorsal stream can be implicated in movements that require more and less cognitive input (Pisella et al., 2009).

4.2 Symbolic vs. Direct precues

In addition to direct and indirectly cued responses being examined using a pro-pointing vs. anti-pointing paradigm, direct and indirectly cued movements have been investigated by manipulating precue presentation (i.e. symbolic (indirect) or direct precues). As mentioned previously, original research by Rosenbaum (1980) using precues to examine motor preparation determined that the time to program the arm component of a movement was longer than the time needed to specify movement direction, which in turn was longer than the time required to specify movement extent. In Rosenbaum’s study, the precues were provided by typed capital letters that conveyed information about the upcoming response (e.g. (R) right arm, (F) Forward target, (N) near target); meaning that before the participant made a motor response they first had to cognitively transform the precued information into the corresponding movement or movement components.

In 1980, Goodman and Kelso suggested that the manner in which the precues were presented in Rosenbaum’s original experiment was not “natural.” For example, in normal everyday interactions, information about a target location is coded more directly, not coded indirectly by letters. Thus, Goodman and Kelso (1980) developed a direct precuing paradigm which involved a direct one-to-one correspondence between the precue and the movement goal. Specifically, in contrast to Rosenbaum's precue of (R) to convey that the movement goal would be on the right and participants should use their right hand to respond, Goodman and Kelso lit up all four targets on the right side of the workspace. With this highly compatible direct precuing technique they found that RT decreased with increasing precue information. Moreover, and more importantly, unlike Rosenbaum (1980), they found no differences in RT when movement arm, direction or extent was precued. Goodman and Kelso (1980) therefore suggested that the differences in RTs seen in Rosenbaum's (1980) experiment were due to the cognitive transformation of these precues into the appropriate movement, not differences in motor preparation (see also Larish & Frekany, 1985; Zelaznik & Hahn, 1985). Taken together the RT precue results reveal that different preparation processes arise when preparing a response to a stimulus/target that has been cued directly compared to stimulus/target that has been indirectly cued, which requires cognitive processing to transform the stimulus into the appropriate motor target (Goodman & Kelso, 1980; Larish & Frekany, 1985).

Another precuing experimental paradigm examining processes underlying direct versus indirectly cued movements has required participants to perform movements with both limbs (i.e. bimanual movements). Typically, participants are required to complete either the same movement with both limbs or different movements with both limbs. Evidence suggests that when the two limbs are moving the movements become coupled, both spatially and temporally (Franz, Zelaznik, & Mccabe, 1991; Sherwood, 1994; Swinnen, Walter, & Shapiro, 1988; Swinnen, Beirinckx, Meugens, & Walter, 1991; Swinnen, Young, Walter, & Serrien, 1991;

Walter & Swinnen, 1990). Spatial-coupling refers to the phenomenon that bimanual movements, in which the two limbs are supposed to complete different spatial trajectories at the same time, typically end up being completed such that movements performed by the two hands have similar spatial characteristics. For example, if one were to use one hand to draw a circle and the other to draw a line, instead of seeing two distinct shapes, one draws a shape that resembles a combination of a line and a circle (Franz, Eliassen, Ivry, & Gazzaniga, 1996; Franz et al., 1991). This spatial coupling suggests that there is a great deal of cross talk between the two effectors. It has been proposed that this cross talk occurs during motor programming, specifically when specifying spatial movement parameters prior to movement execution (Heuer, 1993).

In addition to spatial coupling, temporal-coupling has also been demonstrated between the limbs. Temporal-coupling refers to the phenomenon that bimanual movements, in which the two limbs are supposed to complete different spatial trajectories at the same time, typically end up being completed such that movements performed by the two hands have similar RTs, movement times, and total response times (Kelso, Southard, & Goodman, 1979; Sherwood, 1994; Spijkers, Heuer, Kleinsorge, & van der Loo, 1997; Swinnen, Young et al., 1991; Weigelt, 2007). For example, Kelso and colleagues (1979) found that when participants reached to a target 6 cm laterally from their midline with their right hand and reached to a target 24 cm laterally from their midline with their left hand in response to an auditory go-signal, they altered the velocity and acceleration of the short right hand reach in order to coordinate with the longer left hand reach. In an experiment by Spijkers and colleagues (1997), the participants' task was to reach to targets of either long or short amplitude when target amplitude for each hand was indicated by the German word "long" or "short." As expected, the RTs between the two hands did not differ. Moreover, when the two movements were different, mean RTs were 83 ms longer then when the two movements had the same amplitude. However, Diedrichsen et al. (2001) proposed that this temporal-coupling effect observed by Spijkers et al. (1997) was due to the processes involved in identifying the symbolic cues and translating these cues into specific actions. Using the same bimanual movement paradigm as Spijkers et al. (1997) but replacing the symbolic precues “L” (Long) and “S” (Short) with direct target precues (like Goodman & Kelso, 1980), Diedrichsen et al. (2001) found that the slower RT temporal-coupling effect was eliminated, such that even when the two movements were different in amplitude there was no significant difference in RT compared to when they were the same. Therefore the authors concluded that the increased RT for symbolically cued asymmetric bimanual movements was partly due to increased difficulty with stimulus identification, but more importantly a cost associated with transforming the symbolic stimuli into the proper response codes (Diedrichsen et al., 2001).

In summary, from the literature regarding direct versus indirectly cued movements we can conclude that motor preparation in response to directly cued targets differs from preparation processes underlying indirectly cued movements. The source of this difference is suggested to lie in the separate streams having a differential impact on motor output. For example, directly cued responses may be mediated by information processes arising in the dorsal stream, while indirectly cued responses require additional perceptual processing in the ventral stream. This perceptual/cognitive processing is required to transform the indirect stimulus into the appropriate motor target, leading to increased RT.

4.3 Mental Rotation

As discussed above, one way to present a target indirectly is to have participants perform an anti-point movement (e.g. point to a target that is 180° from the seen target). In the proposed research, I will use a variation of this paradigm to cue our indirect responses (i.e. the visuomotor mental rotation task).

A 2-dimensional spatial transformation involves shifting an image in coordinate space by adding specific values to the x and y coordinates. An example of a paradigm that examines spatial transformations is the classic mental rotation task pioneered by Shepard and Metzler (1971). In their paradigm, participants were shown two asymmetric objects consisting of ten solid cubes attached face-to-face to form a rigid arm like structure with exactly three right-angled "elbows." The objects were rotated relative to each other and were either congruent or mirror images. For each trial the asymmetry (angular difference) between the two objects was created by either a rigid rotation of one of the two identical pictures in its own picture plane or by a rigid rotation of the three-dimensional object in depth. For each pair the participant was asked to pull a right-hand lever as soon as he or she determined that the two drawings portrayed objects that were congruent with respect to three-dimensional shape and to pull a left-hand lever if the two drawings depicted objects of different three-dimensional shapes (mirror images). Results indicated that RT for a correct judgment was a linear function of the angular difference between the two objects, suggesting that the object was mentally rotated until superimposed on the reference object (Shepard & Metzler, 1971).

The same finding of greater RT with increasing rotation angles was obtained by Georgopoulos and Massey (1987) when they used a variant of the classic mental rotation task called the visuomotor mental rotation (VMR) task. Their VMR task required participants to point from a central home position to a position that deviated clockwise (CW) or counter clockwise (CCW) from a visual target located on the perimeter of a circle by a predetermined angle of rotation (angles used: 5˚, 10˚, 15˚, 35˚, 70˚, 105˚, 140˚). Based on their findings of a linear increase in RT with increasing angles of rotation, Georgopoulos and Massey proposed that responses are prepared according to the mental rotation model (MRM). The MRM suggests that for a given angle of rotation, one mentally rotates the movement vector from its start position (angled through the start position of the reach to the visual cue), through increasing degrees until the corresponding angle of rotation is obtained.

Further support for the MRM was obtained from single-cell recordings in monkeys during the response preparation phase of a VMR task (Georgopoulos, Lurito, Petrides, Schwartz, & Massey, 1989). In this study, the weighted vector sum of neural activity (i.e. the population vector, in which each neuron is represented by a vector, with direction corresponding to its preferred direction of movement and amplitude representing its preference for movement in a given direction) in the motor cortex initially reflected the location of the visual target. However, over time the population vector rotated to reflect the direction of the (mentally rotated) movement target. It is important to note that such a transient increase in the recruitment of neurons with intermediate (i.e. between the stimulus and movement) preferred directions during the middle of the RT interval was observed, providing neural evidence for mental rotation and its nature as an analog (continuous) process(Georgopoulos et al., 1989).

In contrast to Georgopoulos and colleagues (1989; 1987) explanation of the processes underlying the VMR task, Cisek and Scott (1999) proposed an alternative interpretation, the ‘response substitution’ hypothesis (RSH), to explain the findings of greater RT with increasing rotation angles. The RSH states that the VMR elicits two distinct neural responses, one corresponding to the visual target and the other to the rotated movement target. Through a process of response substitution, the activity representing the stimulus must first be inhibited, and then replaced with that of the rotated movement target until it increases to the threshold required for movement initiation.

In accordance with Cisek and Scott’s (1999) response substitution’ hypothesis, Neely and Heath (2010) have recently shown that RT does not increase in a linear fashion over all rotation angles. Specifically, Neely and Heath (2010) adapted Georgopoulos and Massey’s (1987) paradigm and included rotation angles of 90˚ and 180˚. In contrast to Georgopoulos and Massey’s MRM proposal, results showed that participants were more efficient (decreased RT) and effective (increased accuracy) at response programming and movement execution when the required transformation involved perceptually familiar angles (90˚ and 180˚) compared to the remaining angles of 30°, 60 °, 120°, 150° and 210°. Thus, RTs did not increase in a linear fashion with increasing rotation angles. Instead, the data was best described by a fifth-order polynomial (Neely & Heath, 2010). These results conflict with the MRM, providing further support for the response substitution theory, with the time required to substitute responses (i.e. RT) being influenced by the angular disparity between response alternatives, as well as perceptual familiarity of the required transformation angle.

Although there is conflicting views regarding how responses are prepared in a VMR task, results indicate that motor cortical areas are involved. Specifically, it is possible that motor cortical areas are involved in the cognitive transformations underlying movement preparation (Georgopoulos et al., 1989). Moreover, regardless of which proposal correctly explains results achieved in a VRM task, the VRM task can be used to examine response preparation underlying indirect movements.

5. Objectives and Hypotheses

The purpose of the present research experiment was to investigate the time course of motor preparation in humans when they are required to make a response to an indirectly cued target during an anticipation-timing task, in which there is high temporal certainty regarding when the response is to be initiated. To cue targets indirectly we used a VMR task. A startle stimulus was used to explore the time course of motor preparation and how it is influenced by the angle of transformation required (i.e. how closely the visual cue matches the indirect movement goal and the perceptual familiarity of the required angle of rotation). It was hypothesized that while participants would program the response in advance of the go-signal for both types of movements, motor preparation would occur at different times prior to the go-signal as a function of the type of movement required. Specifically it was hypothesized that indirectly cued movements would take longer to prepare and hence motor preparation would begin earlier in order to have the response ready for execution coincident with the anticipated go-signal. In contrast, similar to previous results of Carlsen and Mackinnon (2010), we did not expect motor preparation to occur until approximately 200ms prior to the go-signal for directly cued movements.

In summary, by examining the underlying motor processes occurring during a VMR task I will gain new insight into how the human brain prepares responses to indirectly cued movements.

Chapter II: Research Article

Motor preparation is delayed for both directly and indirectly cued movements during an anticipation-timing task

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Abstract Previous investigations comparing direct versus indirectly cued movements have consistently shown that indirectly cued movements take longer to prepare (Neely and Heath, 2010) and involve the recruitment of additional brain areas (Connolly et al., 2000). This increase in processing time has been associated with the additional cognitive transformations required of the task (Neely and Heath, 2010). In the present study we investigated whether differences between direct versus indirectly cued movements are also reflected in the time course of motor preparation. Participants performed a targeting task, moving directly to the location of a visual cue (i.e., directly cued movement) or to a location that differed by 60˚, 90˚, or 120˚ with respect to the visual cue provided (i.e., indirectly cued movements). Participants were instructed to initiate their movements concurrently with an anticipated go-signal. To examine the time course of motor preparation, a startling acoustic stimulus (SAS, 124dB) was randomly presented 150 ms, 500 ms, or 1000 ms prior to the go-signal. Results from the startle trials revealed that the time course of motor preparation was similar regardless of the angle of rotation required and hence whether it was a direct or indirectly cued trial. Specifically, motor preparation was delayed until less than 500 ms prior to movement initiation for both direct and indirectly cued movements. These findings indicate that similar motor preparation strategies are engaged for both types of cued movements, suggesting that the time to prepare a motor response may be similar regardless of whether a cognitive transformation is required.

Keywords Visuomotor mental rotation · Response preparation · Startle · Motor preparation · Anticipation-timing

Introduction

Many movements in everyday life involve an established relationship between a target goal and the movement required to reach the given target. For example, a visual stimulus may be the movement goal, directly cuing the location of the target (e.g., when reaching for a cup of coffee). In other instances, the visual stimulus indirectly corresponds to the required movement such that the actual movement is made towards a different location than that indicated visually (e.g., when controlling a cursor on a screen by moving a computer mouse). Direct versus indirectly cued movements have been shown to differ with regards to the cognitive demands associated with them. Specifically, indirectly cued movements are proposed to incur greater cognitive demands (as seen by the additional recruitment of frontoparietal areas (Connolly et al., 2000)), resulting in longer response times (Heath et al., 2009a; Maraj and Heath, 2010; Rosenbaum, 1980).

To study the sensorimotor processes underlying movement initiation to direct versus indirectly cued movements in the laboratory, researchers have used a visuomotor mental rotation (VMR) task (Georgopoulos and Massey, 1987; Neely and Heath, 2010). Typically, the VMR task requires participants to point from a central home position to a location that deviates from a visual cue by a predetermined / instructed angle of rotation. Initial research using this VMR task has demonstrated that reaction time (RT) increases linearly as the angle of rotation is increased from 5º to 140º (Georgopoulos and Massey, 1987). To account for this increase in RT, Georgopoulos & Massey (1987) proposed a mental rotation model (MRM), suggesting that during indirectly cued movements, participants mentally rotate a movement vector from its starting position (i.e., a movement directed to the visual cue) through increasing angular degrees until the movement goal is obtained. In support of the MRM, single-cell recordings in monkeys during the response preparation phase of a VMR task revealed that the weighted vector sum of neural activity (i.e., the population vector) in the motor cortex initially reflected the location of the visual cue; however, over time the population vector rotated to reflect the direction of the mentally rotated movement goal (Georgopoulos et al., 1989; see Georgopoulos and Pellizzer, 1995 for a review).

In contrast to the MRM, Cisek and Scott (1999) have proposed an alternative explanation, the response substitution hypothesis (RSH), to explain the increases in RT with increasing angles of rotation observed during a VMR task. According to the RSH, the onset of a visual cue in a VMR task elicits two distinct neural responses, one to the visual cue and the other to the (rotated) movement goal. During indirectly cued movements, the initial motor activity related to the visual location of the stimulus (i.e., a directly cued response) must then be inhibited, and replaced with that of the rotated movement goal in order for the movement to be initiated as required. Although the two neural models (i.e., MRM and RSH) differ in their explanation for how indirectly cued movements are carried out within the VMR task, they both highlight the notion that there is initial activation related to the location of the visual cue. Given that indirectly cued movements are not initiated to the location of the visual cue, the rotation or substitution required to achieve the correct movement goal then takes longer to prepare and requires additional processing compared to directly cued trials.

While the studies discussed above indicate that additional processes are required to complete indirect versus directly cued movements, it is unclear if the observed increases in RT associated with indirectly cued movement arise due to 1) more time being required to complete non-motoric processes (e.g., visual/cognitive transformations) or 2) increased time associated with motor-related processes (e.g., preparing the desired response and inhibiting an unwanted action). In the current study we investigated whether differences in RT between direct versus indirectly cued movements arise due to differences in the time required to prepare (i.e., activate) motor-related neural structures between the two movement types. In other words, we asked if it takes longer for participants to activate motor-related neural structures in an indirectly cued movement in comparison to a directly cued movement.

In order to determine whether a buildup of response related activity in motor structures requires different amounts of time for direct versus indirectly cued movements, we instructed participants to initiate their movements concurrent with an anticipated go-signal, and then used a "startle" technique to probe motor preparation at various intervals preceding the go-signal (see Carlsen et al., 2012 for a review). The startle technique involves the presentation of a loud (>120 dB), startling acoustic stimulus (SAS) during the motor preparatory phase. Previous research has shown that when a person is startled, the typical reaction consists of a fast, reflexive response, characterized by a general symmetrical flexion of the body including activation of the sternocleidomastoid (SCM) muscle (Brown et al., 1991; Landis et al., 1939; Wilkins et al., 1986). However, if a person is startled when preparing to react to a go-signal in a directly cued simple RT task, the SAS not only gives rise to the typical startle response, but also involuntarily triggers the release of the planned voluntary movement. Specifically, a startle can result in a response that is produced at a significantly shorter latency (<80 ms from SAS onset) compared to trials in which the SAS is not presented (Carlsen et al., 2003, 2004a, 2004b, 2007, 2009a; Castellote et al., 2007; Cressman et al., 2006; MacKinnon et al., 2007; Maslovat et al., 2008, 2009; Valls-Solé et al., 1995, 1999, 2005). Importantly, this early response initiation suggests that if an action is sufficiently "prepared" the SAS can act to directly trigger it, thus allowing one to examine motor preparation. This early initiation of a planned movement in conjunction with an observed startle reflex response (e.g., SCM activity) is termed the StartReact effect (Carlsen et al., 2012).

The startle technique not only provides insight into the components of a movement that are prepared (Carlsen et al., 2009b), but can also be used to probe the time course of motor preparation (Carlsen and Mackinnon, 2010; Cressman et al., 2006; MacKinnon et al., 2007). For example, when a SAS was presented in a task in which the temporal predictability of response onset was high (e.g., in an anticipation-timing task), the SAS only triggered the prepared movement when participants were startled just prior (i.e., 150 ms) to an anticipated go-signal (Carlsen and Mackinnon, 2010).Thus a SAS can provide insight into when there is substantive activation in motor-related neural structures. For directly cued movements, even though the response was known well in advance of the temporally predictable go-signal, motor-related preparation was delayed until just prior to response initiation (Carlsen et al., 2008; Carlsen and Mackinnon, 2010).

The purpose of the present experiment was to determine if indirectly cued movements require additional motor-related activation time compared to directly cued movements. To address this question, this experiment investigated whether there were differences in the proportion of responses triggered by a SAS presented at various times prior to an anticipated go-signal. If so, this would provide evidence that at least part of the RT difference observed between these two types of tasks arises due to motor-related factors. Participants completed a VMR task (e.g., Georgopoulos and Massey, 1987; Neely and Heath, 2010) involving a forearm rotation to a target in which there was high temporal predictability regarding when the response was to be initiated. A SAS was pseudo-randomly presented at one of three time points (1000 ms, 500 ms, 150 ms) prior to the anticipated go-signal to probe at what point prior to the go-signal motor preparatory activity began to increase (given that this was an anticipation-timing task, all responses were expected to be fully prepared to coincide with the go-signal). Electromyographic (EMG) and kinematic data were collected (see section 5 for further details regarding the experimental procedures). It was hypothesized that motor preparation for indirectly cued movements would indeed take longer (and thus would be initiated earlier), and that this would be reflected by an increased incidence of early movement release in response to the SAS at the earliest startle presentation times (i.e., 500 and/or 1000 ms prior to the anticipated go-signal). In contrast, we did not expect motor preparation for directly cued movements to occur until approximately 200 ms prior to the go-signal (similar to previous results of Carlsen and Mackinnon, 2010). Therefore an increased incidence of early movement release in response to the SAS would only be observed at the latest startle presentation time (150 ms prior to the anticipated go-signal) for directly cued movements.

Experimental Procedures

Participants

Fourteen right-handed participants (5 males, 9 females; age 22.6 ± 2.6 years) with normal or corrected to normal vision, and with no history of neurological, sensory, or motor disorders participated in the study. Testing of each participant took place in a single testing session, and took approximately 1.5 hrs to complete. All participants provided informed consent, and the study was conducted in accordance with ethical guidelines approved by the University of Ottawa’s Research Ethics Board. Data from four participants were not included in the analyses described below due to the lack of a reliable startle response (see sections 5.5 & 5.7 for details). Therefore, the final analyses were based on data from ten participants (3 males, 7 females; age 22.1±1.66).

Experimental Set-up

Participants sat facing a computer monitor with a circular aperture placed over the monitor in order to eliminate orientation cues related to the cardinal axes (see Coppola et al., 1998). The participant’s right forearm was semi-prone and placed in a custom made brace, such that their wrist and hand hung over the edge of the brace (see Fig. 1A). The forearm was then secured to the brace using Velcro straps placed around the proximal and distal ends of the radius and ulna, allowing for forearm pronation and supination. The elbow was flexed in a comfortable position at approximately 70˚ to allow for an optimal balance between agonist and antagonist maximal voluntary electrical activity (O'Sullivan and Gallwey, 2002), and the shoulder abducted approximately 15˚. Participants gripped a handle (120 mm long x 25 mm in diameter) with their right hand which could be rotated in the coronal plane via pronation/supination of the forearm (see Fig. 1A). Participants were told that the axis of rotation of the handle corresponded to the center of the circle displayed on the monitor in front of them, and therefore the handle could be used like a pointer. Participants were instructed that they were to use the handle to make targeted supination movements to targets presented on the perimeter of the visual display (for more details on the visual display and trials see sections 5.3 & 5.4 respectively). For example, when the handle was parallel to the floor with the forearm pronated, it pointed at the 9 o’clock position, and when the forearm was supinated such that the handle was perpendicular to the floor, it pointed at the 12 o’clock position. Once participants understood how they were to move to targets using the handle, the right arm was secured in position and covered by a black cloth, preventing vision of the distal arm and hand.

Stimuli Displayed

The visual stimuli displayed to participants are shown in Fig. 1B and were generated using a custom written LabVIEW program. On the computer monitor a black circle was displayed (diameter = 185 mm, surrounded by a 20 mm wide circular border). In the center of the circle an adapted clock-face (diameter = 90 mm) was presented. There were no numbers presented on the clock-face but there was a 45 mm long black clock hand (initially positioned at the 12 o'clock position). During each trial, this arrow hand rotated at a speed of 0.25 Hz, such that it made one full revolution in 4000 ms. The movement of the clock hand is referred to as the "clock-face countdown." A 15 mm green indicator arrow was displayed just outside the clock-face, which pointed directly at the 9 o'clock position for the duration of the trial (shown in Fig. 1B and 2 as a grey arrow).

At the start of each trial, the hand (and handle) had to be oriented parallel to the floor such that the forearm was pronated with the palm down. The starting position was indicated by a small green dot (diameter = 8 mm) appearing in the border of the circle on the far left (shown in Fig. 1B, Time 1 as a black dot). Once this position was maintained for 2000 ms, the dot disappeared and an "angle of rotation" appeared above the central clock-face (Fig. 1B, Time 2). Participants were instructed that they were to make a movement that corresponded to a location that deviated by the instructed "angle of rotation" in the clockwise direction from the upcoming visual cue. The visual cue (white circle with diameter = 10 mm) appeared inside the circular border of the display 2000 ms after the angle of rotation was presented and the clock-face countdown was started simultaneously (Fig. 1B, Time 3, shown as a black dot). The "angle of rotation" was presented prior to visual cue onset as we wanted to separate the processes associated with processing the angle of rotation from the processes associated with preparing the required response. This therefore made our task more similar to a previous VMR RT task (Neely and Heath, 2010), such that participants were well aware of the angle of rotation that was required on all upcoming trials. Participants were instructed to initiate the required ballistic supination movement coincident with when the black clock hand arrow reached the 9 o'clock position (i.e., pointed at the green indicator arrow) (Fig. 1B, Time 4). Given that the clock hand arrow moved at a constant speed, the go-signal always occurred 3000 ms after the visual cue appeared. Movements were to be made as quickly and as accurately as possible.

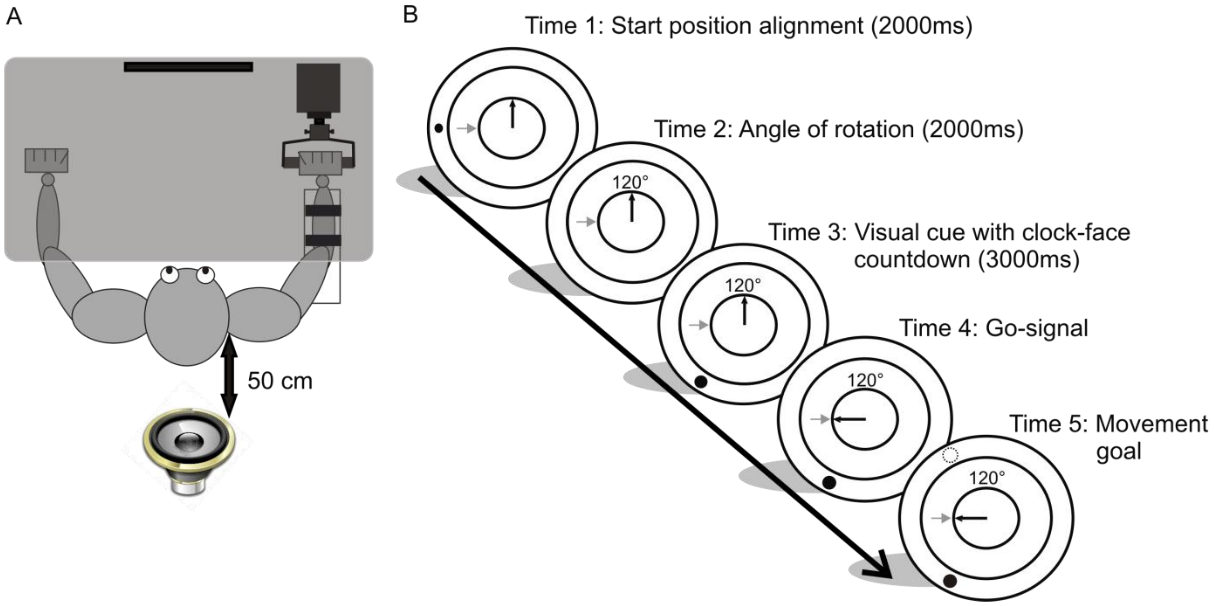

Figure 1. Overview of experimental set-up. **A.** Schematic illustration of participants' position. Participants sat facing the monitor with no vision of their right arm. The speaker that delivered the SAS was located 50 cm behind the participants' ears. Participants started each trial with their right hand pronated (0°), and the movement goals were located at 30°, 40°, and 50° of forearm supination. The movement was completed by the right hand while gripping a handle (120 mm long x 25 mm diameter). **B.** Temporal schematic of the visual stimuli displayed within a trial. In this trial the visual cue was to be mentally rotated 120°, such that the participant was to move to a movement goal of 50° of forearm supination (as shown by the dashed circle at Time 5).

Trials

A schematic representation of each of the four instructed rotation angles, including the locations of the visual cues and corresponding movement goals is shown in Fig. 2. In Fig. 2A., the instructed angle of rotation is 0°, thus these trials were directly cued trials in which the location of the visual cues (solid black circles) and movement goal targets (dotted white circles) overlapped. Additional instructed rotation angles included (B) 60°, (C) 90° and (D) 120°. As illustrated in Fig. 2, the movement goals remained consistent at 50° (position a), 40° (position b) and 30° (position c) of arm supination in the clockwise direction from the start position for all instructed angles of rotation. These movement goal targets corresponded to the matching visual cues (a, b, or c).

Participants began by completing a brief practice session prior to the actual testing trials. The practice trials were identical to those in the testing session with two exceptions: the number of trials and the availability of visual feedback. Participants completed 3 randomized trials for each angle of rotation in the practice session for a total of 12 trials. Feedback was provided after each trial and displayed on the computer monitor, such that response time with respect to the temporal goal (± error in ms: positive time indicated that participants responded too late, while a negative score indicated that participants responded too early) and movement accuracy with respect to the required target (± error in degrees: positive score indicated that participants overshot the target, while a negative score indicated that participants undershot the target) were provided.

Following the practice trials, participants performed 5 blocks of 60 trials for a total of 300 testing trials. Within each testing block, all instructed angles of rotation were presented. Feedback regarding their response time with respect to the go-signal was provided after each trial. In contrast to the practice trials, participants were not provided with any feedback regarding their movement accuracy, in order to prevent participants from memorizing the locations of the movement goals. Testing was completed in one day in order to ensure that trials could be averaged across blocks. In order to limit the effects of muscular fatigue, rest periods were given between blocks.

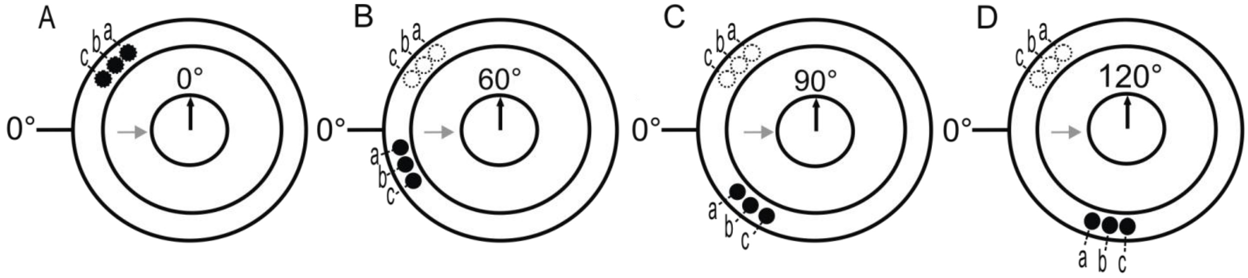

Figure 2. Schematic illustration representing the four instructed angles of rotation and their corresponding visual cues and movement goal locations (A: 0°, B: 60°, C: 90°, D: 120°). Solid black circles represent the three possible visual cue locations. Dotted white circles represent the three corresponding movement goals at 30° (position c), 40° (position b) and 50° (position a) of forearm supination from the starting position of the hand. Visual cue (a) corresponds with movement goal (a), visual cue (b) corresponds with movement goal (b), and visual cue (c) corresponds with movement goal (c). Note that the startling acoustic stimuli (SAS trials) were only delivered on trials in which the movement goal was 40° (i.e., movement goal [b]).

Startling acoustic stimulus (SAS)

Twelve trials within each of the 5 testing blocks were accompanied by a loud startling acoustic stimulus (SAS). This SAS (1000 Hz, 40 ms) was generated by a custom written LabVIEW (National Instruments Inc.) program which was amplified and presented via a loudspeaker (<1 ms rise time) placed 50 cm behind the head of the participant (see Fig. 1A), such that the intensity measured at the ear was 124 dB (A-weighted scale, impulse setting, Casella-254). Participants were instructed that this tone was irrelevant to the task. The SAS was presented pseudo-randomly such that the SAS never occurred on 2 consecutive trials and only occurred in trials in which the movement goal was 40°, therefore limiting the number of startle exposures experienced by participants.

In order to keep the proportion of SAS to control trials small (to limit exposure to the SAS), and to minimize the total amount of trials completed (to limit participant fatigue and keep participants engaged), the SAS was delivered at three time points, 1000 ms, 500 ms or 150 ms prior to the anticipated go-signal. These different time points were chosen based on previous work by Carlsen and MacKinnon (2010), which demonstrated that the extent of motor preparation for directly cued movements at 150 ms, 500 ms and 1000 ms prior to the go-signal was dependent on task demands, with motor preparation not starting until approximately 200 ms prior to the go-signal during an anticipation-timing task. A breakdown of testing trials, including trials in which a SAS was presented, is shown in Table 1. The SAS was presented on 60 trials in total (12 trials per block), for a total of 5 SAS trials per time point and instructed angle of rotation.

Table 1

Summary of trials. Breakdown of total trials by instructed angle of rotation and movement goal, completed by each participant over the entire testing session. N/A denotes that there was no startling acoustic stimulus (SAS) presented

			Trials/Angles of Rotation			
Movement Goal	Auditory Stimulus	Time of SAS Prior to go-signal	0°	60°	90°	120°
50° (a)	N/A	N/A	20 trials	20 trials	20 trials	20 trials
40° (b)	N/A	N/A	20 trials	20 trials	20 trials	20 trials
40° (b)	124dB	150 ms	5 trials	5 trials	5 trials	5 trials
40° (b)	124dB	500 ms	5 trials	5 trials	5 trials	5 trials
40° (b)	124dB	1000 ms	5 trials	5 trials	5 trials	5 trials
30° (c)	N/A	N/A	20 trials	20 trials	20 trials	20 trials

Recording Equipment

Surface electromyographic (EMG) data were collected from the muscle bellies of the following muscles: right biceps brachii (BB, agonist), right pronator teres (PT, antagonist), and left sternocleidomastoid (SCM, startle indicator), using bipolar pre-amplified (gain=10) surface electrodes (Delsys Bagnoli DE-2.1) connected via shielded cabling to an external amplifier system (Delsys Bagnoli-8). The EMG recording sites were prepared and cleansed in order to decrease electrical impedance. The electrodes were placed such that they were oriented parallel to the muscle fibers, and then attached to the skin using double sided adhesive strips. A grounding electrode was placed on the participant's left lateral epicondyle.

Forearm angular position data were collected using a potentiometer connected to the central axis of the manipulandum. On each trial, data collection was initiated by the computer 3000 ms prior to the go-signal on all trials. Unfiltered EMG and position data were digitally sampled at 1 kHz (National Instruments PCI-6030E) for 4000 ms using a customized program written in LabVIEW (National Instruments Inc.) and stored for offline analysis.

Data Analysis

Movement onset was defined as the first point in time at which angular displacement was greater than 0.2° from the home position location following the go-signal or SAS. Timing errors were calculated by determining the difference between the time of movement onset and the go-signal, such that a negative anticipation-timing error indicated movement onset occurred prior to the go-signal and a positive anticipation-timing error indicated movement onset occurred after the go-signal. Movement final position was defined as the first point at which angular velocity fell below 8°/s and then remained below 8°/s for at least 150 ms. Movement time was defined as the time from movement onset to the time that movement final position was achieved. Peak displacement and peak velocity were defined as the greatest displacement and velocity achieved during a movement respectively.

EMG data from all muscles measured were analyzed for timing of burst onsets and offsets as well as EMG amplitude. Signals were rectified and filtered using a 25Hz low pass elliptic filter, and displayed on a computer monitor using a custom written LabVIEW program. EMG burst onsets were defined as the point in time at which EMG activity first reached a value 2 standard deviations above baseline levels (i.e., mean EMG activity 100 ms prior to muscle activity). Similarly, EMG offset was defined as the point in time at which EMG activity first fell below a value 2 standard deviations above baseline levels (i.e., mean EMG activity 100 ms following the termination of movement), with the activity between EMG onset and EMG offset defined as a distinct burst. EMG markers were manually adjusted to compensate for any errors due to the strictness of the LabVIEW algorithm. EMG activity in the SCM, as well as movement related EMG activity from two distinct bursts in the biceps (agonist 1 and agonist 2) and one burst in the pronator teres (antagonist) were marked for each trial (e.g, triphasic EMG pattern, see Wadman et al., 1979). Peak EMG amplitudes were defined as the largest EMG amplitude recorded within an interval of 100 ms following EMG burst onset.

In order to determine if a participant responded early due to the SAS (i.e., StartReact), we first determined whether the participant showed startle response-related SCM activation in response to the SAS. If a participant showed a startle response, we then determined whether the movement was initiated early (defined as movement initiation within 250 ms of the SAS; see Carlsen and Mackinnon, 2010). For four participants, SCM activation was not observed in 60% or more of the trials in which the SAS was presented 150 ms prior to the go-signal, thus data from these participants were excluded from all analyses.

Statistical Analysis

Dependent variables were analyzed using Repeated Measures Analysis of Variance (RM ANOVA), to determine if differences existed between control and SAS trials, between different presentation times of the SAS (i.e., 1000, 500 or 150 ms prior to the anticipated go-signal), between different angles of rotation (i.e., 0°, 60°, 90° or 120°), and between the 5 testing blocks. Prior to statistical analysis, proportion values were subjected to an arcsine square root transformation (Osborne, 2010). All analyses of SAS trials were collapsed across testing blocks as each angle of rotation and SAS presentation time combination was presented only once per block, thus precluding an analysis across blocks. In cases when the assumption of sphericity was violated, Greenhouse-Geisser values are reported. Differences with a probability of less than

0.05 were considered significant and Tukey's Honestly Significant Difference (HSD) post-hoc tests were administered to determine the locus of any significant differences.

Results

Task performance: Control Trials

Final positions achieved during control trials were analyzed using a 3 movement goal (30°, 40°, vs. 50°) x 4 angle of rotation (0°, 60°, 90°, vs. 120°) x 5 testing block (1, 2, 3, 4, vs. 5) RM ANOVA. Analysis revealed significant main effects for movement goal ($F(2,18) = 7.667$, $p = 0.016$, $\eta^2p = 0.460$), angle of rotation ($F(3,27) = 4.715$, $p = 0.034$, $\eta^2p = 0.344$), and testing block ($F(4,36) = 8.361$, $p = 0.004$, $\eta^2p = 0.482$). Importantly, there were no two or three-way significant interactions ($p > 0.05$). Post-hoc analysis of movement goal indicated that the final position achieved when the target goal was 30° was significantly different than when the final target goal was 50° ($p < 0.05$). While the final positions on trials in which the target goal was 40° (M = 37.7°, SD = 11.7) did not differ from final positions achieved on trials in which the target goal was 30° (M = 36.0°, SD = 11.4) or 50° (M = 40.0°, SD = 12.2), in each block of trials there was a trend such that the smallest movement was completed when the movement goal was 30° and the largest movement was completed when the movement goal was 50°. Although a main effect was found on final position due to angle of rotation, post-hoc analysis failed to reveal any significant differences between angles of rotation; the greatest difference in final positions occurred between movements where the angle of rotation was 0° (M = 33.9°, SD = 9.8) and 120° (M = 43.0°, SD = 18.2). With respect to performance over testing blocks, post-hoc analysis revealed that final positions achieved in block 1 were significantly larger than the final positions achieved in block 5 ($p < 0.05$). Thus, it appears that while participants made smaller movements as testing went on (mean final positions: block 1 M = 44.4°, block 2 M = 39.6°, block 3 M =

37.9°, block 4 *M* = 34.6°, block 5 *M* = 32.9°), they still performed the task correctly over all blocks such that they were moving to different positions for the different movement goals across all angles of rotation.

Proportion of Startle Responses and StartReact Effects

The proportion of startle responses and StartReacts were analyzed using a 3 SAS presentation time (1000 ms, 500 ms, vs. 150 ms prior to go-signal) x 4 angle of rotation (0°, 60°, 90°, vs. 120°) RM ANOVA. The proportion of startle responses on trials in which the SAS was presented is shown in Fig. 3. Analyses revealed a significant main effect of SAS presentation time ($F(2,18) = 15.10$, $p < 0.001$, $\eta^2p = 0.627$), but no main effect of angle of rotation ($F(3,27) = 0.144$, $p = 0.81$, $\eta^2p = 0.016$), and no significant interaction ($F(6,54) = 0.709$, $p = 0.644$, $\eta^2p = 0.073$). Post-hoc analyses indicated that the proportion of startle responses when the SAS was presented 150 ms prior to the go-signal (*M* = 0.84, SD = 0.21) was significantly greater than when the SAS was presented both 500 ms (*M* = 0.55, SD = 0.32) and 1000 ms (*M* = 0.57, SD = 0.32) prior to the anticipated go-signal ($p < 0.01$).

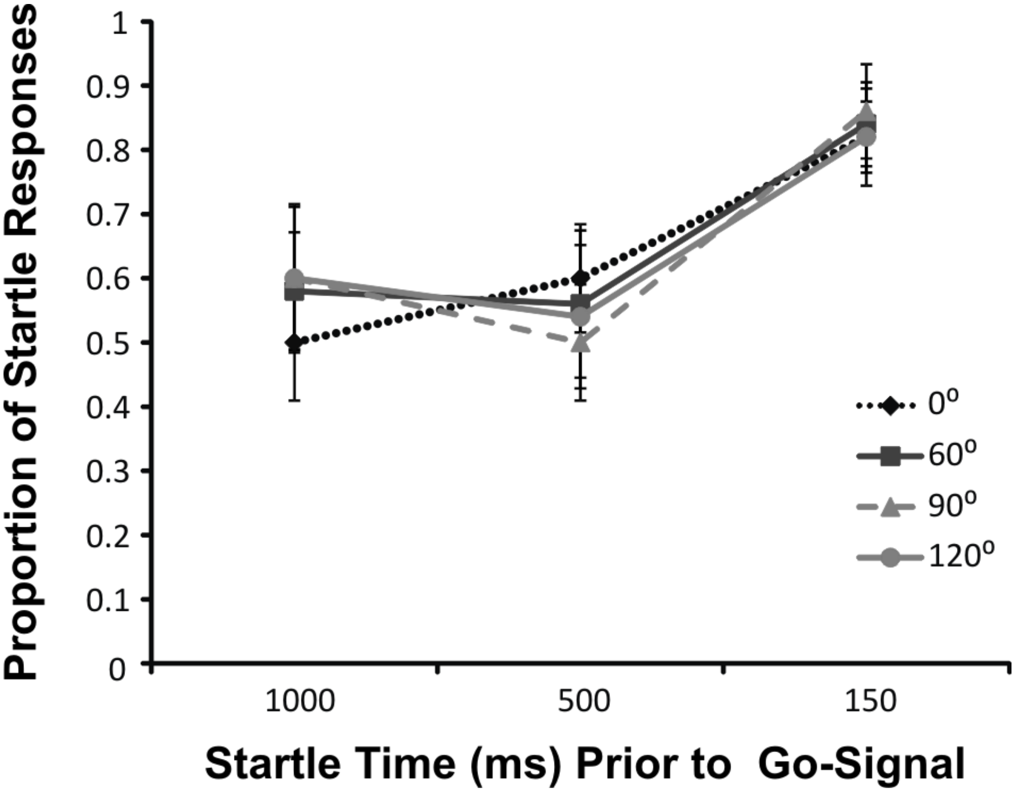

Figure 3. Mean proportion of startle responses elicited by the SAS as a function of the time of SAS presentation prior to the go-signal (1000, 500 or 150 ms prior to the go-signal) and the instructed angle of rotation (0º = black diamonds, 60º = black squares, 90° = grey triangles, 120° = grey circles). Error bars denote standard errors.

The proportion of early responses (StartReacts) due to the SAS on trials in which participants were startled is presented in Fig. 4. Similar to the analysis regarding proportion of startles responses, statistical analysis revealed a significant main effect of SAS presentation time ($F(2,18) = 74.567$, $p<0.001$, $\eta^2 p = 0.892$), but no main effect of angle of rotation ($F(3,27) = 0.381$, $p = 0.767$, $\eta^2 p = 0.041$) and no SAS presentation time x angle of rotation interaction ($F(6,24) = 0.709$, $p = 0.644$, $\eta^2 p = 0.073$). Post-hoc analysis indicated that the proportion of StartReacts when the SAS was presented at 150 ms prior to the go-signal ($M = 0.80$, SD = 0.22) was significantly greater (*p*<0.01) than the proportion of StartReacts when the SAS was presented either at 500 ms (*M* = 0.14, SD = 0.28) or 1000 ms (*M* = 0.07, SD = 0.14) prior to the anticipated go-signal. In summary, 1) there were no differences between angles of rotation in either the proportion of startle responses or StartReacts, and 2) for all angles of rotation the proportion of startle responses and StartReacts were significantly greater when the SAS was presented at 150 ms prior to the go-signal compared to 500 ms or 1000 ms prior to the anticipated go-signal.

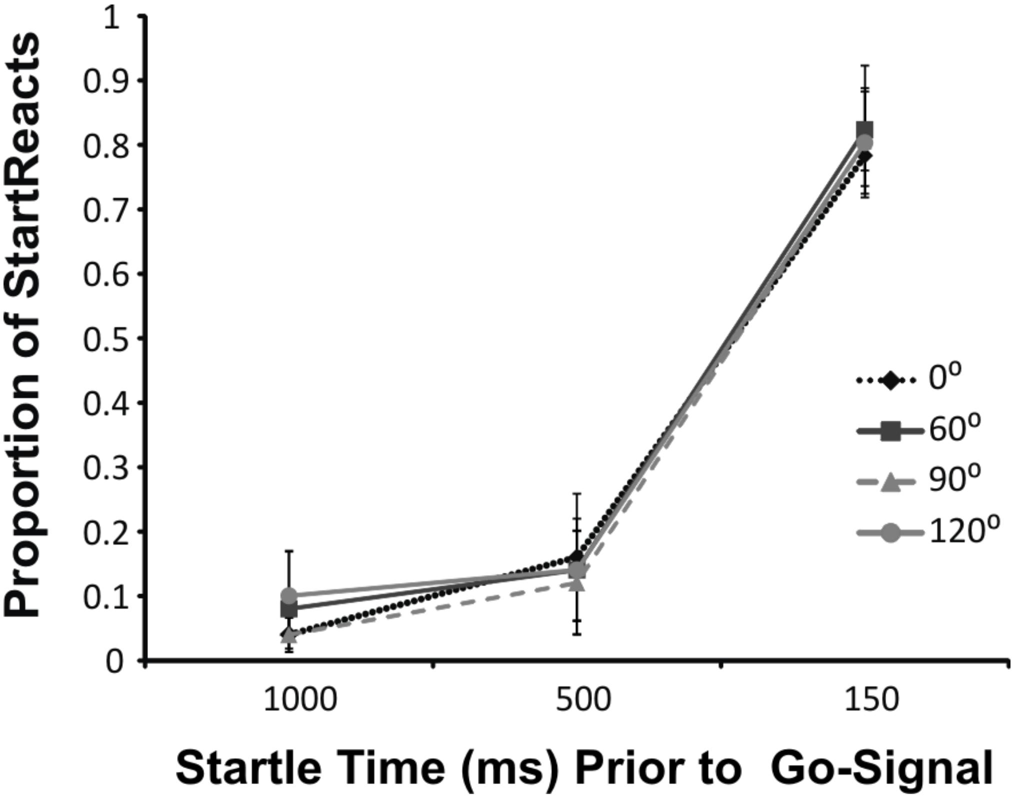

Figure 4. Mean proportion of early responses (i.e., StartReacts) elicited by the SAS as a function of the time of SAS presentation prior to the go signal (1000, 500 or 150 ms prior to the go-signal) and the instructed angle of rotation (0º = black diamonds, 60º = black squares, 90° = grey triangles, 120° = grey circles). Error bars denote standard errors.

Effect of Startle on EMG and Kinematic Measures

Given the limited number of trials which demonstrated a StartReact effect when the SAS was presented at 1000 ms and 500 ms prior to the anticipated go-signal, EMG and kinematic measures of StartReact trials were analyzed using a 2 stimulus (Control vs. SAS presented 150 ms prior to go-signal) x 4 angle of rotation (0°, 60°, 90°, vs. 120°) RM ANOVA. The timing of agonist 1 EMG onset and displacement onset were analyzed to determine if presenting a SAS 150 ms prior to the anticipated go-signal led to an earlier production of the movement compared to control trials. As seen in Fig. 5, analysis of agonist 1 onset revealed a significant main effect for stimulus ($F(1,9) = 10.575$, $p = 0.010$, $\eta^2p = 0.540$), such that agonist 1 onset with respect to the go-signal occurred earlier during the SAS trials ($M = -77.9$ ms, SD = 22.9) compared to control trials ($M = -45.9$ ms, SD = 35.4). There was no significant main effect for angle of rotation ($F(3,27) = 0.895$, $p = 0.456$, $\eta^2p = 0.090$), and no significant interaction ($F(3,27) = 0.963$, $p = 0.425$, $\eta^2p = 0.097$). Analysis of displacement onset revealed a similar trend. Specifically, a significant main effect for stimulus ($F(1,9) = 19.269$, $p = 0.002$, $\eta^2p = 0.682$), such that displacement onset occurred earlier during the SAS trials ($M = -35.6$ ms, SD = 23.6) compared to control trials ($M = 4.6$ ms, SD = 35.9). There was no significant main effect for angle of rotation ($F(3,27) = 0.494$, $p = 0.69$, $\eta^2p = 0.052$), and no significant interaction ($F(3,27) = 0.727$, $p = 0.545$, $\eta^2p = 0.075$). Together, these results demonstrate that regardless of angle of rotation, movement related EMG onset and displacement onset occurred significantly earlier in StartReact trials compared to control trials, providing evidence that the SAS did trigger the early release of the prepared movement when it was presented 150 ms prior to the go-signal.

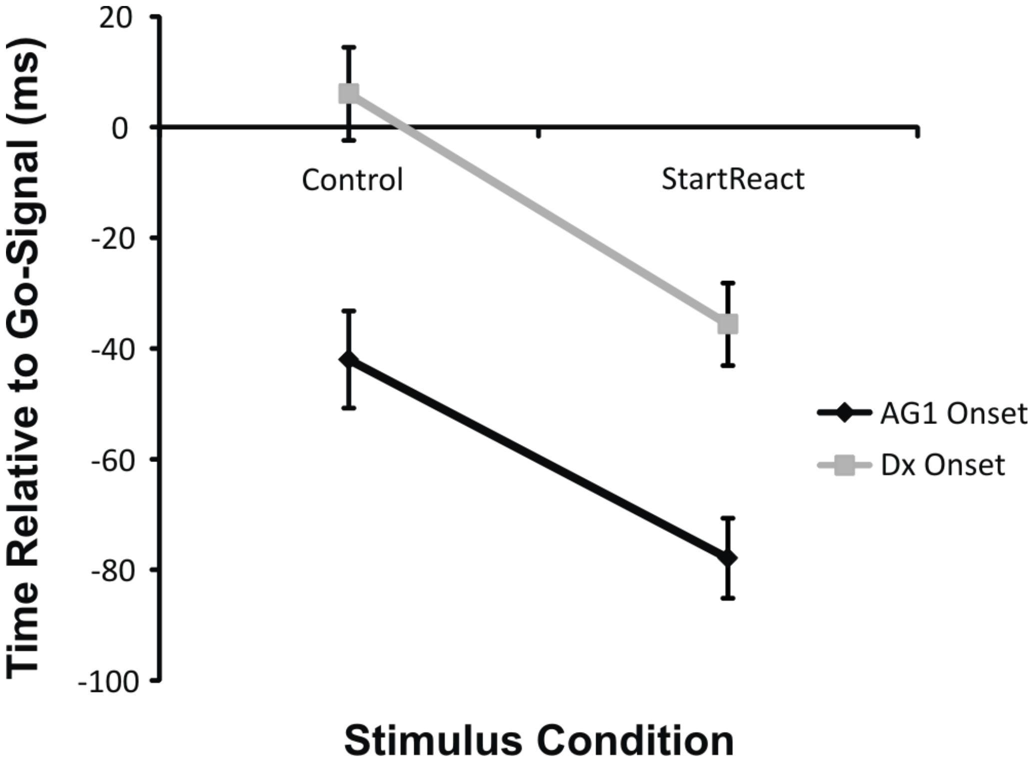

Figure 5. Mean agonist 1(AG1) onset times (black diamonds) and displacement (Dx) onset times (grey boxes) relative to the anticipated go-signal (0 ms) for both control and StartReact trials when the SAS was presented 150 ms prior to the go-signal. Error bars denote standard errors.

To determine if the movements triggered by the SAS were similar to those completed during comparable control trials (i.e. trials in which the movement goal was 40°), as seen in Fig. 6., the triphasic muscle burst patterns associated with all angles of mental rotation were analyzed using a 2 stimulus (Control vs. SAS 150 ms prior to go-signal) x 4 angle of rotation (0°, 60°, 90°, vs. 120°) RM ANOVA. An analysis of agonist 1 duration revealed no significant main effect for stimulus ($F(1,9) = 3.943$, $p = 0.078$, $\eta^2p = 0.308$), or angle of rotation ($F(3,27) = 0.118$, $p = 0.949$, $\eta^2p = 0.013$). As well, there was no significant interaction, $F(3,27) = 0.049$, $p = 0.985$, $\eta^2p = 0.005$. Thus, the duration of the first agonist burst was unaffected by the SAS, indicating that the early part of the EMG pattern associated with StartReact movements was not different than control movements. In contrast to agonist 1 duration, the analysis of agonist 2 duration revealed a significant main effect for stimulus ($F(1,9) = 8.505$, $p = 0.017$, $\eta^2p = 0.486$), but no significant main effect for angle of rotation ($F(3,27) = 0.676$, $p = 0.574$, $\eta^2p = 0.07$) and no significant interaction ($F(3,27) = 0.069$, $p = 0.976$, $\eta^2p = 0.008$). Similar to agonist 2 duration, the analysis of antagonist duration revealed a significant main effect for stimulus ($F(1,9) = 9.46$, $p = 0.013$, $\eta^2p = 0.512$), but no significant main effect for angle ($F(3,27) = 1.103$, $p = 0.344$, $\eta^2p = 0.109$) and no significant interaction ($F(3,27) = 0.622$, $p = 0.607$, $\eta^2p = 0.065$).

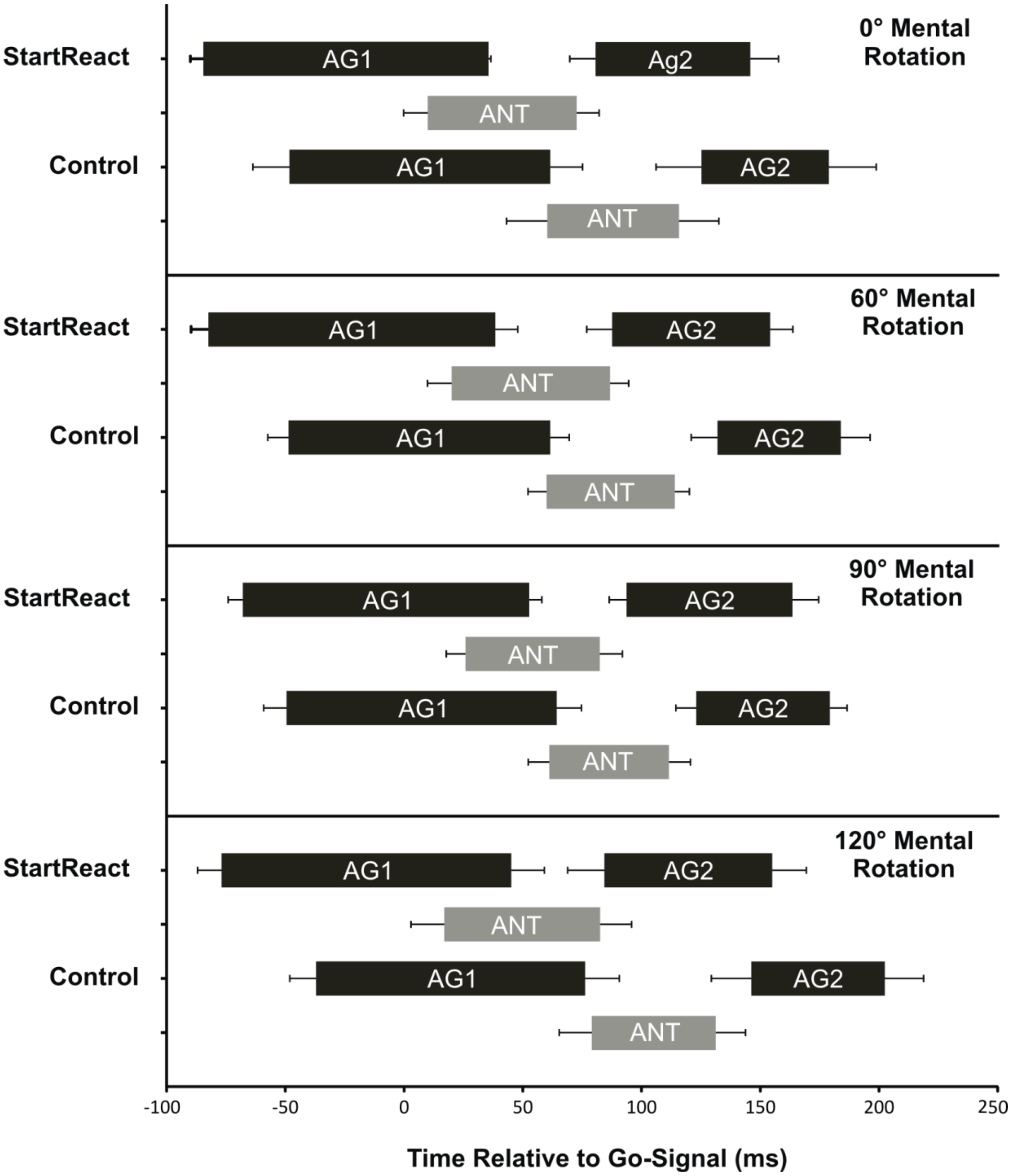

Figure 6. Plots of the triphasic EMG pattern associated with the 40° movement. Boxes represent mean EMG bursts with mean onsets and offsets with respect to the anticipated go-signal (0 ms). The mean EMG bursts for control and StartReact trials of all four angles of mental rotation are presented (0°, 60°, 90°, 120°). Agonist 1 (AG1) from right biceps brachii, antagonist (ANT) from right pronator teres, and agonist 2 (AG2) from right biceps brachii. Error bars denote standard error.

Since EMG burst duration for agonist 2 and antagonist differed between StartReact and control trials, movement kinematics may have been influenced by the SAS. Analysis of final positions revealed a significant main effect for both stimulus ($F(1,9) = 9.354$, $p = 0.014$, $\eta^2p = 0.51$), and angle of rotation ($F(3,27) = 3.654$, $p = 0.025$, $\eta^2p = 0.289$). There was no significant interaction ($F(3,27) = 0.966$, $p = 0.423$, $\eta^2p = 0.097$). Mean final position for StartReact trials was 39.9° (11.5), which was significantly greater than the mean final position for control trials ($M = 33.2°$, SD = 11.2). With respect to angle of rotation, post-hoc analysis indicated that the final position achieved for a directly cued movement (angle of rotation = 0°) was significantly smaller than the final position achieved for the indirectly cued 120° movement (0°: $M = 32.5°$ SD = 8.3; 120°: $M = 41.8°$ SD = 18.0; $p < 0.05$). Similar to final position results, the analysis of peak displacement revealed a significant main effect for stimulus ($F(1,9) = 17.597$, $p = 0.002$, $\eta^2p = 0.661$), but no main effect for angle of rotation ($F(3,27) = 2.863$, $p = 0.055$, $\eta^2p = 0.241$) and no significant interaction ($F(3,27) = 0.403$, $p = 0.752$, $\eta^2p = 0.043$), whereby peak displacement was greater during StartReact trials than control trials (StartReact: $M = 52.6°$ SD = 17.1; Control: $M = 41.0°$ SD = 14.7; $p < 0.05$). In accordance with the peak displacement results, analysis of peak velocity revealed a significant main effect for stimulus ($F(1,9) = 19.336$, $p = 0.002$, $\eta^2p = 0.682$), no main effect for angle of rotation ($F(3,27) = 1.773$, $p = 0.173$, $\eta^2p = 0.165$), and no significant interaction ($F(3,27) = 0.26$, $p = 0.853$, $\eta^2p = 0.028$). Specifically peak velocity was greater during StartReact trials than control trials (StartReact: $M = 950.0°/s$ SD = 324.2; Control: $M = 699.4°/s$ SD = 279.7; $p < 0.05$). In summary, final position, peak displacement, and peak velocity were greater during StartReact trials compared to control trials.

Given the differences in EMG activity and final positions achieved in the StartReact trials versus control trials, it was reasoned that there might be a difference in MT between StartReact and control trials. However, analysis of MT revealed no significant main effects for either stimulus ($F(1,9) = 1.525$, $p = 0.248$, $\eta^2p = 0.145$), or angle of rotation ($F(3,27) = 0.412$, $p = 0.604$, $\eta^2p = 0.044$), and there was no significant interaction ($F(3,27) = 0.44$, $p = 0.726$, $\eta^2p = 0.047$), indicating that movement times were similar between StartReact ($M = 185.1$ ms, SD = 35.5) and control trials ($M = 179.6$ ms, SD = 33.6), and that movement times to the same movement goal (i.e., 40°) were similar between angles of rotation.

Discussion

It has been proposed that movements made to directly cued targets require little cognitive demand and are simple, stimulus-driven responses (Fitts and Seeger, 1953; Kornblum et al., 1990). On the other hand, movements made to indirectly cued targets have been shown to incur longer reaction times (i.e. time before the response is initiated) in comparison to directly cued movements (Connolly et al., 2000; Heath et al., 2009a, 2009b; Neely and Heath, 2010; Rosenbaum, 1980), suggesting that additional processes are required. At present it is unclear if the RT differences between direct and indirectly cued movements are due to differences in the timing of motor-related preparation or non-motoric cognitive processes. To determine if the time course of motor preparation differs between direct and indirectly cued movements we used a SAS to probe the timing of motor-related activation in an anticipation timing task. If additional time is required for motor activation in the indirectly cued trials, we would expect to find a greater proportion of early movements triggered in response to the SAS (StartReacts) at earlier SAS presentation times prior to the go-signal in comparison to directly cued trials. The current results revealed no difference in proportion of early responses between cuing conditions, suggesting that motor preparation was delayed for both direct *and* indirectly cued movements until just prior to the anticipated go-signal. Thus, these results indicate that there was little difference in the timing of motor-related preparatory activation regardless of the degree of mental rotation required.

A startling acoustic stimulus (SAS) in an anticipation timing task

The StartReact effect

Previous experiments have demonstrated that when participants know the required response in advance of a go-signal in a simple RT task, a loud 124 dB SAS is able to elicit a classic startle response (i.e., indicated by SCM activation) and also trigger the prepared motor response at a latency too short to involve normal cortical initiation processes (Carlsen et al., 2003, 2004a, 2004b, 2007, 2009a; Castellote et al., 2007; Cressman et al., 2006; MacKinnon et al., 2007; Maslovat et al., 2008, 2009; Valls-Solé et al., 1995, 1999, 2005). However, studies examining the time course of motor preparation revealed that when the temporal predictability of response initiation was high (e.g., in an anticipation-timing task), participants waited to prepare a voluntary response, such that prepared responses were only elicited by the SAS when it was presented just prior (150-200 ms) to the go-signal (Carlsen and Mackinnon, 2010; Thickbroom et al., 2000). These results are in line with results from both the TMS and stop-signal anticipation-timing literature, which show peak response preparation occurring 150 ms prior to response onset (Coxon et al., 2006; Slater-Hammel, 1960). In the current study the early triggering of a voluntary motor response (i.e., a StartReact effect) was greatly influenced by the timing of SAS presentation. Specifically, when the SAS was presented at either 1000 ms or 500 ms prior to the anticipated go-signal, a StartReact rarely occurred (7% and 14% of trials respectively). In contrast, when the SAS was presented 150 ms prior to the anticipated go-signal, the percentage of StartReacts rose to 80%. However, there was no effect of mental rotation angle on the incidence of StartReacts: no difference was observed between directly cued movements (0° mental rotation) or indirectly cued movements. These results suggest that participants *delayed preparation* of the required movement until just prior to the anticipated go-signal for both direct and indirectly cued trials. Since very few responses were elicited by the SAS at 1000 ms and even 500 ms prior to the go-signal, this suggests that the motor response (irrespective of it being direct or indirectly cued) was rarely prepared.

This late response preparation was expected for the directly cued movements (i.e., 0° angle of rotation), as these results replicate those of Carlsen & Mackinnon (2010), who had participants perform a directly cued 20° angular wrist extension movement. In contrast to the directly cued movements, we hypothesized that motor preparation would begin earlier in the indirectly cued trials if the increased response time seen previously for these movements was due to processes related to activation in motor structures. Previous results have demonstrated that when preparation for direct and indirectly cued movements can begin at the same time (i.e. in response to an unexpected go-signal), RT is longer for indirectly cued movements (e.g., RT for a mental rotation of 60° = 615 ms, 90° = 575 ms, 120° = 725 ms) in comparison to directly cued movements (0° = 376 ms) (Neely and Heath, 2010). In the present experiment, participants were required to initiate their responses for both direct and indirectly cued movements exactly 3000 ms after the visual cue appeared. Our StartReact results indicate that even 500 ms prior to response initiation (2500 ms after the cue), the motor command for indirectly cued movements was rarely fully prepared. Given that in a RT task the greatest amount of time required to prepare an indirectly cued movement of 120° was less than 900 ms (Neely and Heath, 2010), the reason for not observing a StartReact 500 ms prior to the go-signal in our current study cannot be due to insufficient time to prepare the motor response following visual cue onset. Thus, since no differences were found in the proportion of StartReact responses between angles of mental rotation, our results suggest that the time required to prepare the motor commands is similar across direct and indirectly cued movements and takes a maximum of 350 ms. This value is calculated based on the finding that responses were rarely prepared 500 ms prior to the go-signal, and almost always fully prepared 150 ms prior to the go-signal. The results of the current study indicate that the increased RT observed for indirectly cued movements is a result of greater time being spent completing non-motoric cognitive and/or visuo-perceptual transformations prior to the preparation of the motor command in comparison to directly cued movements.

VMR task processes

The suggestion that cognitive transformations are the locus of increased RT in indirectly versus directly cued movements is consistent with the proposal that directly cued movements are processed through stimulus-driven visuomotor networks, and indirectly cued movements are processed (at least in part) through slow visuo-perceptual networks (Heath et al., 2009a, 2009b; Maraj and Heath, 2010). Further support for visuo-perceptual processing during indirectly cued movements has been revealed by VMR tasks that included the perceptually familiar rotation angles of 90˚ & 180˚, which have been shown to have a shorter RT compared to smaller angles of rotation (Neely and Heath, 2010). Faster response preparation times for these familiar angles is consistent with literature demonstrating perceptual expertise with right angles and straight lines (Mcfarland, 1968). In the present experiment, SAS trials did not reveal any differences in the timing of motor preparation between direct and indirectly cued movements, and no dissociation between perceptually familiar and unfamiliar angles of mental rotation. Although it has been suggested that an enhanced rate of response substitution may provide an advantage in movement planning for perceptually familiar angles of mental rotation (Neely and Heath, 2010), we contend that this advantage is related to performing cognitive transformations, not in the preparation of the motor command itself.

Cortical inhibition may provide an alternate explanation for the observation that the SAS only led to consistent early triggering of indirectly cued movements when it was presented just prior to the go-signal. It is possible that indirectly cued motor responses were prepared quite early (e.g., 500 ms or 1000 ms prior to the anticipated go-signal), but inhibitory motor cortical processes prevented the SAS from triggering these response. This explanation is supported by the response substitution hypothesis (RSH) or the affordance-competition hypothesis (Cisek, 2006; Cisek, 2007), which suggests that multiple response options are initially activated in the cortex in parallel and then gradually eliminated as they compete for overt execution. The RSH states that during an indirectly cued trial in the VMR task, two distinct neural responses are elicited: one corresponding to a response directed to the visual cue and the second to a target rotated with respect to the visual cue (Cisek and Scott, 1999). Through a process of response substitution, the motor activity related to the stimulus must first be inhibited, and then replaced with that of the rotated movement goal until the neural activity increases to the necessary threshold for movement initiation. While this hypothesis does provide an explanation for the activation seen in the motor cortex (Cisek and Scott, 1999), it is unclear what cortical processes underlie the cognitive transformation required for indirectly cued movements. To date, fMRI studies conducted to determine excitatory and inhibitory brain regions involved in a mental rotation task have led to inconsistent results. In general, results suggest that several cortical areas, including the superior and inferior parietal cortex, caudate nucleus, supplementary motor area (SMA), and the lateral premotor area are activated (Alivisatos and Petrides, 1997; Jordan et al., 2001; Richter et al., 2000). While it is difficult to draw firm conclusions regarding the time course of response preparation from previous fMRI results, it has been revealed that the SMA is one area that consistently shows greater activation during indirectly versus directly cued movements. The additional activation observed in the SMA during indirectly cued movement has tentatively been attributed to the attentional and coordinate transformation requirements in this complex task (Cohen et al., 1996; Connolly et al., 2000; Richter et al., 2000). The pre-SMA has also been suggested to play a critical part of the circuit that implements response inhibition, including inhibition during indirectly cued movements (Chao et al., 2009; Curtis and D'Esposito, 2003; Hampshire et al., 2010; Mostofsky and Simmonds, 2008; Sharp et al., 2010; Swick et al., 2011), with increased activation observed during stop-signal and other go/no-go tasks (Chao et al., 2009; Mostofsky and Simmonds, 2008). Therefore, results from these studies indicate that it is possible that cortical inhibition of the motor areas may have prevented the SAS from triggering the early release of an indirectly cued response. Continued work investigating the cortical areas responsible for response inhibition is needed, and further insight could be provided with techniques such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS).

The startle response

The proportion of trials in which a startle reaction (e.g. EMG activity in SCM) was observed provides a secondary piece of evidence that corroborates the notion that limited motor system activation was occurring 500 ms or more prior to the go-signal. Specifically, it has been shown that increased motor related preparation is associated with an increased incidence of observing a startle reaction in response to a SAS (Carlsen et al., 2011; Kumru et al., 2006; Maslovat et al., 2012; Valls-Solé et al., 1997). The current study showed that while the SAS elicited a startle response in over 50% of all trials when the SAS was presented 1000 ms and 500 ms prior to the go-signal, a startle response was elicited in significantly more trials (84%) when the SAS was presented 150 ms prior to the go-signal (Fig. 1). In high temporal resolution situations, similar to the task used in the current experiment, a decreased probability of observing a startle response when the SAS is presented more than 200 ms prior to the anticipated go-signal is thought to be reflective of an overall lack of motor preparation occurring well in advance of the anticipated go-signal (Carlsen et al., 2008; Carlsen and Mackinnon, 2010). Therefore, the proportion of startle responses can be seen as a secondary index of motor preparation (Carlsen et al., 2008; Maslovat et al., 2012). Our startle response data suggests that while some response preparation may have been occurring at least 1000 ms prior to response initiation, the motor preparatory level was significantly higher 150 ms prior to the go-signal.

Targeted response characteristics of forearm supination

Several previous studies using a SAS have had participants perform an extension movement of either the wrist or elbow, producing a characteristic triphasic muscle activation pattern (Carlsen et al., 2003, 2004a, 2004b , 2007, 2009b; Cressman et al., 2006; Maslovat et al., 2008, 2009; Valls-Solé et al., 1995, 1999, 2005). In the present study participants performed a forearm supination movement. Similar to these previous findings, we found that the duration of the first agonist burst was unaffected by the SAS. Importantly, this result provides evidence that at least the early part of the movement elicited by the SAS was unchanged from the prepared responses executed during control trials. However, the duration of the antagonist and second agonist EMG bursts were significantly longer during startle trials compared to control trials, which may reflect differences in the use of sensory feedback in this task (i.e., online control). Previous research has shown that corrections for suddenly presented changes in limb position can be initiated within 30-80 ms (Cole and Abbs, 1988; Dewhurst, 1967; Lee et al., 1983). In addition, research has shown that cortically controlled corrections in response to proprioceptive feedback can occur within 80-130 ms (Crago et al., 1976; Dewhurst, 1967). Given that the mean time from agonist 1 onset to antagonist and agonist 2 onsets during StartReact trials was 96.0 ms ± 21.8 and 163.3 ms ± 30.6 respectively, it is possible that sensory feedback influenced muscle activation patterns, leading to differences between control and StartReact trials in the present experiment. Specifically, the increase in EMG duration for the antagonist and agonist 2 during StartReact trials could be explained by the need to correct for the increased velocity experienced as a result of the SAS, which has been reported in prior research using a SAS (Carlsen et al., 2003, 2004b, 2011). Importantly, the EMG and kinematic facilitation due to the SAS was consistent over all angles of mental rotation, demonstrating that a similar prepared response was likely triggered by the SAS for all angles of rotation.

Conclusions

The present study investigated the time course of motor preparation when participants were required to make a movement to a direct or indirectly cued target during an anticipation-timing task. The use of a SAS revealed a similar incidence of early response triggering regardless of whether the target was directly cued (0˚) or whether a mental rotation was required (indirectly cued). These results indicate that motor-related preparation was delayed until less than 500 ms prior to movement initiation for both cueing conditions, and thus suggest that similar motor preparation strategies may be engaged for both types of cued movements. Furthermore, these results indicate that the time course of motor preparation may be similar regardless of whether or not a cognitive transformation is required. Thus, given that indirectly cued movements take longer to initiate compared to directly cued movements (as seen in typical RT tasks), we propose that this increased processing time is needed to cognitively transform the visual cue to the required response goal, not to prepare the subsequent motor response.

Acknowledgements

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References

Alivisatos, B., Petrides, M., 1997. Functional activation of the human brain during mental rotation. Neuropsychologia. 35**,** 111-118.

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**,** 1891-1902.

Carlsen, A.N., Hunt, M.A., Inglis, J.T., Sanderson, D.J., Chua, R., 2003. Altered triggering of a prepared movement by a startling stimulus. J. Neurophysiol. 89**,** 1857-1863.

Carlsen, A.N., Chua, R., Inglis, J.T., Sanderson, D.J., Franks, I.M., 2004a. Can prepared responses be stored subcortically? Exp. Brain Res. 159**,** 301-309.

Carlsen, A.N., Chua, R., Inglis, J.T., Sanderson, D.J., Franks, I.M., 2004b. Prepared movements are elicited early by startle. J. Motor Behav. 36**,** 253-264.

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

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

Carlsen, A.N., Chua, R., Inglis, J.T., Sanderson, D.J., Franks, I.M., 2009a. Differential effects of startle on reaction time for finger and arm movements. J. Neurophysiol. 101**,** 306-314.

Carlsen, A.N., Chua, R., Summers, J.J., Inglis, J.T., Sanderson, D.J., Franks, I.M., 2009b. Precues enable multiple response preprogramming: Evidence from startle. Psychophysiol. 46**,** 241-251.

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

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. Neurosci. Biobehav. Rev. 35**,** 366-76.

Carlsen, A.N., Maslovat, D., Franks, I.M., 2012. Preparation for voluntary movement in healthy and clinical populations: evidence from startle. Clin. Neurophysiol. 123**,** 21-33.

Castellote, J.M., Kumru, H., Queralt, A., Valls-Solé, J., 2007. A startle speeds up the execution of externally guided saccades. Exp. Brain Res. 177**,** 129-136.

Chao, H.H., Luo, X., Chang, J.L., Li, C.S., 2009. Activation of the pre-supplementary motor area but not inferior prefrontal cortex in association with short stop signal reaction time--an intra-subject analysis. BMC Neurosci. 10**,** 75.

Cisek, P., Scott, S.H., 1999. An alternative interpretation of population vector rotation in macaque motor cortex. Neurosci. Lett. 272**,** 1-4.

Cisek, P., 2006. Integrated neural processes for defining potential actions and deciding between them: A computational model. J. Neurosci. 26**,** 9761-9770.

Cisek, P., 2007. Cortical mechanisms of action selection: the affordance competition hypothesis. Philos T R Soc B. 362**,** 1585-1599.

Cohen, M.S., Kosslyn, S.M., Breiter, H.C., DiGirolamo, G.J., Thompson, W.L., Anderson, A.K., Bookheimer, S.Y., Rosen, B.R., Belliveau, J.W., 1996. Changes in cortical activity during mental rotation - A mapping study using functional MRI. Brain. 119**,** 89-100.

Cole, K.J., Abbs, J.H., 1988. Grip Force Adjustments Evoked by Load Force Perturbations of a Grasped Object. J. Neurophysiol. 60**,** 1513-1522.

Connolly, J.D., Goodale, M.A., DeSouza, J.F.X., Menon, R.S., Vilis, T., 2000. A comparison of frontoparietal fMRI activation during anti-saccades and anti-pointing. J. Neurophysiol. 84**,** 1645-1655.

Coppola, D.M., White, L.E., Fitzpatrick, D., Purves, D., 1998. Unequal representation of cardinal and oblique contours in ferret visual cortex. Proc. Natl. Acad. Sci. U. S. A. 95**,** 2621-2623.

Coxon, J.P., Stinear, C.M., Byblow, W.D., 2006. Intracortical inhibition during volitional inhibition of prepared action. J. Neurophysiol. 95**,** 3371-3383.

Crago, P.E., Houk, J.C., Hasan, Z., 1976. Regulatory actions of human stretch reflex. J. Neurophysiol. 39**,** 925-35.

Cressman, E.K., Carlsen, A.N., Chua, R., Franks, I.M., 2006. Temporal uncertainty does not affect response latencies of movements produced during startle reactions. Exp. Brain Res. 171**,** 278-282.

Curtis, C.E., D'Esposito, M., 2003. Success and failure suppressing reflexive behavior. J. Cogn. Neurosci. 15**,** 409-418.

Dewhurst, D.J., 1967. Neuromuscular control system. IEEE Trans. Biomed. Eng. 14**,** 167-71.

Fitts, P.M., Seeger, C.M., 1953. S-R compatibility: spatial characteristics of stimulus and response codes. J. Exp. Psychol. 46**,** 199-210.

Georgopoulos, A.P., Massey, J.T., 1987. Cognitive Spatial-Motor Processes .1. The Making of Movements at Various Angles from a Stimulus Direction. Exp. Brain Res. 65**,** 361-370.

Georgopoulos, A.P., Lurito, J.T., Petrides, M., Schwartz, A.B., Massey, J.T., 1989. Mental Rotation of the Neuronal Population Vector. Science. 243**,** 234-236.

Georgopoulos, A.P., Pellizzer, G., 1995. The Mental and the Neural - Psychological and Neural Studies of Mental Rotation and Memory Scanning. Neuropsychologia. 33**,** 1531-1547.

Hampshire, A., Chamberlain, S.R., Monti, M.M., Duncan, J., Owen, A.M., 2010. The role of the right inferior frontal gyrus: inhibition and attentional control. Neuroimage. 50**,** 1313-9.

Heath, M., Maraj, A., Gradkowski, A., Binsted, G., 2009a. Anti-pointing is mediated by a perceptual bias of target location in left and right visual space. Exp. Brain Res. 192**,** 275-286.

Heath, M., Maraj, A., Maddigan, M., Binsted, G., 2009b. The Antipointing Task: Vector Inversion Is Supported by a Perceptual Estimate of Visual Space. J. Motor Behav. 41**,** 383-392.

Jordan, K., Heinze, H.J., Lutz, K., Kanowski, M., Jancke, L., 2001. Cortical activations during the mental rotation of different visual objects. Neuroimage. 13**,** 143-152.

Kornblum, S., Hasbroucq, T., Osman, A., 1990. Dimensional overlap: cognitive basis for stimulus-response compatibility--a model and taxonomy. Psychol. Rev. 97**,** 253-70.

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. Neurosci. Lett. 406**,** 66-70.

Landis, C., Hunt, W.A., Strauss, H., 1939. The startle pattern. Vol., Farrar & Rinehart, New York.

Lee, R.G., Murphy, J.T., Tatton, W.G., 1983. Long-latency myotatic reflexes in man: mechanisms, functional significance, and changes in patients with Parkinson's disease or hemiplegia. Adv. Neurol. 39**,** 489-508.

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. J. Neurophysiol. 97**,** 4368-79.

Maraj, A., Heath, M., 2010. Antipointing: perception-based visual information renders an offline mode of control. Experimental brain research. Experimentelle Hirnforschung. Experimentation cerebrale. 202**,** 55-64.

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

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

Maslovat, D., Carlsen, A.N., Franks, I.M., 2012. Subcortical motor circuit excitability during simple and choice reaction time. Behav. Neurosci. 126**,** 499-503.

Mcfarland, J.H., 1968. Parts of Perceived Visual Forms - New Evidence. Percept. Psychophys. 3**,** 118-120.

Mostofsky, S.H., Simmonds, D.J., 2008. Response inhibition and response selection: two sides of the same coin. J. Cogn. Neurosci. 20**,** 751-61.

Neely, K.A., Heath, M., 2010. Visuomotor mental rotation: Reaction time is determined by the complexity of the sensorimotor transformations mediating the response. Brain Res. 1366**,** 129-140.

O'Sullivan, L.W., Gallwey, T.J., 2002. Upper-limb surface electro-myography at maximum supination and pronation torques: the effect of elbow and forearm angle. J. Electromyogr. Kinesiol. 12**,** 275-285.

Osborne, J.W., 2010. Improving your data transformations: Applying the Box-Cox transformation. Practical Assessment, Research & Evaluation. 15**,** 7.

Richter, W., Somorjai, R., Summers, R., Jarmasz, M., Menon, R.S., Gati, J.S., Georgopoulos, A.P., Tegeler, C., Ugurbil, K., Kim, S.G., 2000. Motor area activity during mental rotation studied by time-resolved single-trial fMRI. J. Cogn. Neurosci. 12**,** 310-320.

Rosenbaum, D.A., 1980. Human movement initiation: specification of arm, direction, and extent. Journal of Experimental Psychology-General. 109**,** 444-474.

Sharp, D.J., Bonnelle, V., De Boissezon, X., Beckmann, C.F., James, S.G., Patel, M.C., Mehta, M.A., 2010. Distinct frontal systems for response inhibition, attentional capture, and error processing. Proc. Natl. Acad. Sci. U. S. A. 107**,** 6106-11.

Slater-Hammel, A.T., 1960. Reliability, accuracy and refractoriness of a transit reaction. Res. Q. 31**,** 217-228.

Swick, D., Ashley, V., Turken, U., 2011. Are the neural correlates of stopping and not going identical? Quantitative meta-analysis of two response inhibition tasks. Neuroimage. 56**,** 1655-65.

Thickbroom, G.W., Byrnes, M.L., Sacco, P., Ghosh, S., Morris, I.T., Mastaglia, F.L., 2000. The role of the supplementary motor area in externally timed movement: the influence of predictability of movement timing. Brain Res. 874**,** 233-241.

Valls-Solé, J., Solé, A., Valldeoriola, F., Muñoz, E., Gonzalez, L.E., Tolosa, E.S., 1995. Reaction time and acoustic startle in normal human subjects. Neurosci. Lett. 195**,** 97-100.

Valls-Solé, 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 Res. 751**,** 155-159.

Valls-Solé, J., Rothwell, J.C., Goulart, F., Cossu, G., Muñoz, E., 1999. Patterned ballistic movements triggered by a startle in healthy humans. J. Physiol. (Lond.). 516**,** 931-938.

Valls-Solé, J., Kofler, M., Kumru, H., Castellote, J.M., Sanegré, M., 2005. Startle-induced reaction time shortening is not modified by prepulse inhibition. Exp. Brain Res. 165**,** 541-548.

Wadman, W.J., Denier Van der Gon, J.J., Geuze, R.H., Mol, C.R., 1979. Control of fast goal-directed arm movements. J Hum Movement Stud. 5**,** 3-17.

Wilkins, D.E., Hallett, M., Wess, M.M., 1986. Audiogenic Startle Reflex of Man and Its Relationship to Startle Syndromes - a Review. Brain. 109**,** 561-573.

Chapter III: General Discussion & Conclusion

Indirectly cued movements have been shown to require greater cognitive demands and thus more time is required to prepare and initiate these responses in comparison to directly cued movements (Connolly et al., 2000; Goodman & Kelso, 1980; Heath, Maraj, Gradkowski, et al., 2009; Heath, Maraj, Maddigan, & Binsted, 2009; Neely & Heath, 2010; Rosenbaum, 1980). Although it has been shown that direct and indirectly cued movements are prepared differently at the neural level, it was not known if the timing of advance response preparation differs between direct versus indirectly cued movements. To answer this question we used a SAS to probe the timing of response preparation, contrasting the proportion of early movements triggered by a startle under direct versus indirect cue conditions. Our results revealed that motor preparation was delayed for both direct and indirectly cued movements until just prior to the anticipated response initiation time, suggesting that there was little difference (if any) in the timing of motor-related preparation regardless of the degree of mental rotation required. These findings lead us to believe that the increased time needed to initiate a response to an indirectly cued target is a result of the time spent completing the cognitive transformation, prior to the actual preparation of the motor command. This explanation provides further insight to the results from Maraj and Heath (2010), which found that anti-pointing (indirectly cued movement) in comparison to pro-pointing (directly cued movement) was controlled by an offline mode of control. Specifically, we assert that the processes associated with the cognitive transformation must first be completed before the motor response can be fully prepared and initiated without online feedback.

The findings potentially reveal an important aspect of the processes underlying response preparation, such that the limiting factor in terms of time needed to initiate a response is determining where the response is to be initiated. Directly cued movements can provide metrical visual information regarding the location of movement target, therefore, stimulus-driven processing regarding where the response is to be made is very quick allowing for a fast motor output. In contrast, indirectly cued movements do not allow for this stimulus-driven processing because the visual information does not indicate the location of the response. Instead, the visual information must be integrated with the instructed transformation to create a cognitive representation of the response location. Our results suggest that once this transformation is complete and the cognitive representation of the response location is determined, the time needed to prepare the motor command is no different than that of a directly cued movement.

Although our results do provide evidence to suggest that motor preparation time is similar for both direct and indirectly cued movements, it must be noted that there are limitations to the study. First, it may be hard to generalize these results across all age groups given that our sample consisted of a university aged population. Research by Sterr and Dean (2008) has found differences in response preparation between young and old participants, revealing deficits in information processing related to the anticipation and preparation of an upcoming response for the elderly. Interestingly, our experimental paradigm may be able to determine whether deficits reside in the cognitive control of determining the response or preparing the motor command. Specifically, by startling at smaller time increments (e.g., 1000 ms, 800 ms, 600 ms, 400 ms, 200 ms, 150 ms) prior to the anticipated go-signal, we could determine whether older participants begin preparing the motor commands sooner in order to compensate for potential deficits in motor preparation.

A second limitation is that each time an early movement was observed (i.e., StartReact), participants were preparing their motor response in anticipation of the go-signal, such that the movement would have been accurately timed. Although it is an assumption that must be made, it can be substantiated based on the highly accurate response timing during control trials (*M* = 4.6 ms).

A third limitation is due to the resolution at which we were able to probe response preparation with startle. Given that we wanted to keep the proportion of startle to control trials small (to limit exposure to the SAS), as well as keep the total amount of trials to the minimum required (to limit participant fatigue and keep participants engaged), we restricted the total number of trials that we startled participants by presenting the SAS at three time points prior to the anticipated go-signal. Our results revealed that the motor command was rarely fully prepared 1000 ms and 500 ms prior to the go-signal, but often fully prepared 150 ms prior to the go-signal. Therefore, we can only conclude that the motor response was prepared sometime within less than 500 ms prior to the anticipated go-signal. To address this limitation, our laboratory is currently conducting a similar experiment, with the major difference being that participants are now initiating their response to a variable foreperiod go-signal. Motor preparation has been shown to occur well in advance of the earliest possible go-signal (Carlsen & Mackinnon, 2010), therefore the results of the current study many reveal if any differences in the time course of motor preparation exist between angles of rotation and direct versus indirect cued movements.

A fourth limitation is that our experimental design cannot speak to the alternate explanation that for indirectly cued movements the responses were prepared quite early but due to inhibitory motor cortical processes (e.g., inhibition from the SMA (Curtis & D'Esposito, 2003; Mostofsky & Simmonds, 2008; Sharp et al., 2010)), the SAS was prevented from triggering the prepared response. Due to the large delay between visual cue onset and the presentation of a SAS we are unable to determine if any early motor preparation had occurred. It is important that future research be conducted investigating the cortical areas responsible for response inhibition, and its potential role during indirectly cued movements.

In summary, the results from this experiment showed that motor preparation was delayed for both direct and indirectly cued movement until just prior to the anticipated response initiation time, suggesting that there was little difference in the timing of motor preparation regardless of the degree of mental rotation. These findings add to the body of literature regarding the differences in response preparation for indirectly cued movements, but further research is still needed to determine the specific response preparation processes underlying indirectly cued movements.

References

Agarwal, G. S., & Gottlieb, G. L. (1972). Muscle Silent Period and Reciprocal Inhibition in Man. *Journal of Neurology Neurosurgery and Psychiatry, 35*(1), 72-&.

Band, G. P. H., & Miller, J. (1997). Mental rotation interferes with response preparation. *Journal of Experimental Psychology-Human Perception and Performance, 23*(2), 319-338.

Bock, O., & Arnold, K. (1992). Motor Control Prior to Movement Onset - Preparatory Mechanisms for Pointing at Visual Targets. *Experimental Brain Research, 90*(1), 209-216.

Brebner, J. M. T., & Welford, A. T. (1980). Introduction: an historical background sketch. In A. T. Welford (Ed.), *Reaction times* (pp. 1-23). London: Academic Press.

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*, 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*(1), 61-65. doi: 10.1016/j.neulet.2008.01.029

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*(4), 510-518. doi: 10.1007/s00221-003-1575-5

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*(3), 301-309. doi: 10.1007/s00221-004-1924-z

Carlsen, A. N., Chua, R., Inglis, J. T., Sanderson, D. J., & Franks, I. M. (2004). Prepared movements are elicited early by startle. *Journal of Motor Behavior, 36*(3), 253-264.

Carlsen, A. N., Chua, R., Summers, J. J., Inglis, J. T., Sanderson, D. J., & Franks, I. M. (2009). Precues enable multiple response preprogramming: Evidence from startle. *Psychophysiology, 46*(2), 241-251. doi: 10.1111/j.1469-8986.2008.00764.x

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*(2), 199-205. doi: 10.1007/s00221-006-0610-8

Carlsen, A. N., Hunt, M. A., Inglis, J. T., Sanderson, D. J., & Chua, R. (2003). Altered triggering of a prepared movement by a startling stimulus. *Journal of Neurophysiology, 89*(4), 1857-1863. doi: 10.1152/jn.00852.2002

Carlsen, A. N., & Mackinnon, C. D. (2010). Motor preparation is modulated by the resolution of the response timing information. *Brain Research, 1322*, 38-49. doi: S0006-8993(10)00230-1 [pii]

10.1016/j.brainres.2010.01.076

Carlsen, A. N., Maslovat, D., & Franks, I. M. (2012). Preparation for voluntary movement in healthy and clinical populations: evidence from startle. *Clinical Neurophysiology, 123*(1), 21-33. doi: S1388-2457(11)00696-1 [pii]

10.1016/j.clinph.2011.04.028

Chen, R., Tam, A., Butefisch, C., Corwell, B., Ziemann, U., Rothwell, J. C., & Cohen, L. G. (1998). Intracortical inhibition and facilitation in different representations of the human motor cortex. *Journal of Neurophysiology, 80*(6), 2870-2881.

Cisek, P. (2006). Integrated neural processes for defining potential actions and deciding between them: A computational model. *Journal of Neuroscience, 26*(38), 9761-9770. doi: Doi 10.1523/Jneurosci.5605-05.2006

Cisek, P. (2007). Cortical mechanisms of action selection: the affordance competition hypothesis. *Philosophical Transactions of the Royal Society B-Biological Sciences, 362*(1485), 1585-1599. doi: DOI 10.1098/rstb.2007.2054

Cisek, P., & Kalaska, J. F. (2010). Neural Mechanisms for Interacting with a World Full of Action Choices. *Annual Review of Neuroscience, Vol 33, 33*, 269-298. doi: DOI 10.1146/annurev.neuro.051508.135409

Cisek, P., & Scott, S. H. (1999). An alternative interpretation of population vector rotation in macaque motor cortex. *Neuroscience Letters, 272*(1), 1-4.

Connolly, J. D., Goodale, M. A., DeSouza, J. F. X., Menon, R. S., & Vilis, T. (2000). A comparison of frontoparietal fMRI activation during anti-saccades and anti-pointing. *Journal of Neurophysiology, 84*(3), 1645-1655.

Coxon, J. P., Stinear, C. M., & Byblow, W. D. (2006). Intracortical inhibition during volitional inhibition of prepared action. *Journal of Neurophysiology, 95*(6), 3371-3383. doi: 10.1152/jn.01334.2005

Cressman, E. K., Carlsen, A. N., Chua, R., & Franks, I. M. (2006). Temporal uncertainty does not affect response latencies of movements produced during startle reactions. *Experimental Brain Research, 171*(2), 278-282. doi: 10.1007/s00221-006-0459-x

Curtis, C. E., & D'Esposito, M. (2003). Success and failure suppressing reflexive behavior. *Journal of Cognitive Neuroscience, 15*(3), 409-418.

Day, B. L., Rothwell, J. C., Thompson, P. D., Denoordhout, A. M., Nakashima, K., Shannon, K., & Marsden, C. D. (1989). Delay in the Execution of Voluntary Movement by Electrical or Magnetic Brain-Stimulation in Intact Man - Evidence for the Storage of Motor Programs in the Brain. *Brain, 112*, 649-663.

Diedrichsen, J., Hazeltine, E., Kennerley, S., & Ivry, R. B. (2001). Moving to directly cued locations abolishes spatial interference during bimanual actions. *Psychological Science, 12*(6), 493-498.

Dijkerman, H. C., Milner, A. D., & Carey, D. P. (1999). Motion parallax enables depth processing for action in a visual form agnosic when binocular vision is unavailable. *Neuropsychologia, 37*(13), 1505-1510.

Donders, F. C. (1969). On Speed of Mental Processes. *Acta Psychologica, 30,* 412-431.

Drazin, D. H. (1961). Effects of foreperiod, foreperiod variability, and probability of stimulus occurrence on simple reaction-time. *Journal of Experimental Psychology, 62*(1), 43-50.

Duque, J., Mazzocchio, R., Dambrosia, J., Murase, N., Olivier, E., & Cohen, L. G. (2005). Kinematically specific interhemispheric inhibition operating in the process of generation of a voluntary movement. *Cerebral Cortex, 15*(5), 588-593. doi: DOI 10.1093/cercor/bhh160

Erwin, R. J., & Buchwald, J. S. (1986). Midlatency Auditory Evoked-Responses - Differential Recovery Cycle Characteristics. *Electroencephalography and Clinical Neurophysiology, 64*(5), 417-423.

Franz, E. A., Eliassen, J. C., Ivry, R. B., & Gazzaniga, M. S. (1996). Dissociation of spatial and temporal coupling in the bimanual movements of callosotomy patients. *Psychological Science, 7*(5), 306-310.

Franz, E. A., Zelaznik, H. N., & Mccabe, G. (1991). Spatial Topological Constraints in a Bimanual Task. *Acta Psychologica, 77*(2), 137-151.

Georgopoulos, A. P., Lurito, J. T., Petrides, M., Schwartz, A. B., & Massey, J. T. (1989). Mental Rotation of the Neuronal Population Vector. *Science, 243*(4888), 234-236.

Georgopoulos, A. P., & Massey, J. T. (1987). Cognitive Spatial-Motor Processes .1. The Making of Movements at Various Angles from a Stimulus Direction. *Experimental Brain Research, 65*(2), 361-370.

Glover, S., & Dixon, P. (2002). Dynamic effects of the Ebbinghaus illusion in grasping: Support for a planning/control model of action. *Perception and Psychophysics, 64*(2), 266-278.

Glover, S. R., & Dixon, P. (2001). Dynamic illusion effects in a reaching task: Evidence for separate visual representations in the planning and control of reaching. *Journal of Experimental Psychology-Human Perception and Performance, 27*(3), 560-572.

Goodale, M. A., & Milner, A. D. (1992). Separate Visual Pathways for Perception and Action. *Trends in Neurosciences, 15*(1), 20-25.

Goodman, D., & Kelso, J. A. S. (1980). Are Movements Prepared in Parts - Not under Compatible (Naturalized) Conditions. *Journal of Experimental Psychology-General, 109*(4), 475-495.

Hallett, M., Shahani, B. T., & Young, R. R. (1975). Emg Analysis of Stereotyped Voluntary Movements in Man. *Journal of Neurology Neurosurgery and Psychiatry, 38*(12), 1154-1162.

Heath, M., Maraj, A., Gradkowski, A., & Binsted, G. (2009). Anti-pointing is mediated by a perceptual bias of target location in left and right visual space. *Experimental Brain Research, 192*(2), 275-286. doi: DOI 10.1007/s00221-008-1612-5

Heath, M., Maraj, A., Maddigan, M., & Binsted, G. (2009). The Antipointing Task: Vector Inversion Is Supported by a Perceptual Estimate of Visual Space. *Journal of Motor Behavior, 41*(5), 383-392.

Henry, F. M., & Rogers, D. E. (1960). Increased Response Latency for Complicated Movements and a Memory Drum Theory of Neuromotor Reaction. *Research Quarterly, 31*(3), 448-458.

Heuer, H. (1993). Structural constraints on bimanual movements. *Psychological Research, 55*(2), 83-98.

Himmelbach, M., & Karnath, H. O. (2005). Dorsal and ventral stream interaction: Contributions from optic ataxia. *Journal of Cognitive Neuroscience, 17*(4), 632-640.

Hoshiyama, M., Kakigi, R., Koyama, S., Takeshima, Y., Watanabe, S., & Shimojo, M. (1997). Temporal changes of pyramidal tract activities after decision of movement: a study using transcranial magnetic stimulation of the motor cortex in humans. *Electromyography and Motor Control-Electroencephalography and Clinical Neurophysiology, 105*(4), 255-261.

Hoshiyama, M., Kitamura, Y., Koyama, S., Watanabe, S., Shimojo, M., & Kakigi, R. (1996). Reciprocal change of motor evoked potentials preceding voluntary movement in humans. *Muscle and Nerve, 19*(2), 125-131.

Keele, S. W. (1968). Movement control in skilled motor performance. *Psychological Bulletin, 70*(6P1), 387-403.

Kelso, J. A. S., Southard, D. L., & Goodman, D. (1979). Coordination of 2-Handed Movements. *Journal of Experimental Psychology-Human Perception and Performance, 5*(2), 229-238.

Klapp, S. T. (1995). Motor Response Programming during Simple and Choice-Reaction Time - the Role of Practice. *Journal of Experimental Psychology-Human Perception and Performance, 21*(5), 1015-1027.

Klapp, S. T., Wyatt, E. P., & Lingo, W. M. (1974). Response Programming in Simple and Choice Reactions. *Journal of Motor Behavior, 6*(4), 263-271.

Kornhuber, H. H., & Deecke, L. (1965). Hirnpotentialänderungen bei Willkürbewegungen und passiven Bewegungen des Menschen: Bereitschaftspotential und reafferente Potentiale. *Pflügers Archiv European Journal of Physiology, 284*(1), 1-17.

Landis, C., Hunt, W. A., & Strauss, H. (1939). *The startle pattern*. New York: Farrar & Rinehart.

Larish, D. D., & Frekany, G. A. (1985). Planning and Preparing Expected and Unexpected Movements - Reexamining the Relationships of Arm, Direction, and Extent of Movement. *Journal of Motor Behavior, 17*(2), 168-189.

Leocani, L., Cohen, L. G., Wassermann, E. M., Ikoma, K., & Hallett, M. (2000). Human corticospinal excitability evaluated with transcranial magnetic stimulation during different reaction time paradigms. *Brain, 123*, 1161-1173.

Lepine, D., Glencross, D., & Requin, J. (1989). Some Experimental-Evidence for and against a Parametric Conception of Movement Programming. *Journal of Experimental Psychology-Human Perception and Performance, 15*(2), 347-362.

Leuthold, H., Sommer, W., & Ulrich, R. (1996). Partial advance information and response preparation: Inferences from the lateralized readiness potential. *Journal of Experimental Psychology-General, 125*(3), 307-323.

Liepert, J., Dettmers, C., Terborg, C., & Weiller, C. (2001). Inhibition of ipsilateral motor cortex during phasic generation of low force. *Clinical Neurophysiology, 112*(1), 114-121.

MacKinnon, C. D., Bissig, D., Chiusano, J., Miller, E., Rudnick, L., Jager, C., . . . Rogers, M. W. (2007). Preparation of anticipatory postural adjustments prior to stepping. *Journal of Neurophysiology, 97*(6), 4368-4379. doi: 01136.2006 [pii]

10.1152/jn.01136.2006

Maraj, A., & Heath, M. (2010). Antipointing: perception-based visual information renders an offline mode of control. [Research Support, Non-U.S. Gov't]. *Experimental brain research. Experimentelle Hirnforschung. Experimentation cerebrale, 202*(1), 55-64. doi: 10.1007/s00221-009-2111-z

Milner, A. D., Dijkerman, H. C., McIntosh, R. D., Rossetti, Y., & Pisella, L. (2003). Delayed reaching and grasping in patients with optic ataxia. *Neural Control of Space Coding and Action Production, 142*, 223-240.

Milner, A. D., & Goodale, M. A. (1993). Visual Pathways to Perception and Action. *Progress in Brain Research, 95*, 317-337.

Milner, A. D., & Goodale, M. A. (2008). Two visual systems re-viewed. *Neuropsychologia, 46*(3), 774-785. doi: DOI 10.1016/j.neuropsychologia.2007.10.005

Milner, A. D., Paulignan, Y., Dijkerman, H. C., Michel, F., & Jeannerod, M. (1999). A paradoxical improvement of misreaching in optic ataxia: new evidence for two separate neural systems for visual localization. *Proceedings of the Royal Society of London Series B-Biological Sciences, 266*(1434), 2225-2229.

Mostofsky, S. H., & Simmonds, D. J. (2008). Response inhibition and response selection: two sides of the same coin. [Review]. *Journal of Cognitive Neuroscience, 20*(5), 751-761. doi: 10.1162/jocn.2008.20500

Neely, K. A., & Heath, M. (2010). Visuomotor mental rotation: Reaction time is determined by the complexity of the sensorimotor transformations mediating the response. *Brain Research, 1366*, 129-140. doi: DOI 10.1016/j.brainres.2010.09.096

Niemi, P., & Näätänen, R. (1981). Foreperiod and simple reaction-time. *Psychological Bulletin, 89*(1), 133-162.

Pain, M. T. G., & Hibbs, A. (2007). Sprint starts and the minimum auditory reaction time. *Journal of Sports Sciences, 25*(1), 79-86. doi: Doi 10.1080/02640410600718004

Pascual-Leone, A., Valls-Sole, J., Wassermann, E. M., Brasil-Neto, J., Cohen, L. G., & Hallett, M. (1992). Effects of focal transcranial magnetic stimulation on simple reaction time to acoustic, visual and somatosensory stimuli. [Research Support, Non-U.S. Gov't]. *Brain, 115*, 1045-1059.

Pisella, L., Sergio, L., Blangero, A., Torchin, H., Vighetto, A., & Rossetti, Y. (2009). Optic ataxia and the function of the dorsal stream: Contributions to perception and action. *Neuropsychologia, 47*(14), 3033-3044. doi: DOI 10.1016/j.neuropsychologia.2009.06.020

Rosenbaum, D. A. (1980). Human movement initiation: specification of arm, direction, and extent. *Journal of Experimental Psychology-General, 109*(4), 444-474.

Rothwell, J. C. (2006). The startle reflex, voluntary movement, and the reticulospinal tract. In G. Cruccu & M. Hallett (Eds.), *Brainstem Function and Dysfunction*. Amsterdam: Elsevier.

Schmidt, R. A., & Lee, T. D. (2011). *Motor control and learning : a behavioral emphasis* (5th ed.). Champaign, IL: Human Kinetics.

Sharp, D. J., Bonnelle, V., De Boissezon, X., Beckmann, C. F., James, S. G., Patel, M. C., & Mehta, M. A. (2010). Distinct frontal systems for response inhibition, attentional capture, and error processing. [Research Support, Non-U.S. Gov't]. *Proceedings of the National Academy of Sciences of the United States of America, 107*(13), 6106-6111. doi: 10.1073/pnas.1000175107

Shepard, R. N., & Metzler, J. (1971). Mental Rotation of 3-Dimensional Objects. *Science, 171*(3972), 701-&.

Sherwood, D. E. (1994). Hand Preference, Practice Order, and Spatial Assimilations in Rapid Bimanual Movement. *Journal of Motor Behavior, 26*(2), 123-134.

Shibasaki, H., & Hallett, M. (2006). What is the Bereltschaftspotential? *Clinical Neurophysiology, 117*(11), 2341-2356. doi: DOI 10.1016/j.clinph.2006.04.025

Slater-Hammel, A. T. (1960). Reliability, accuracy and refractoriness of a transit reaction. *Research Quarterly, 31*, 217-228.

Sohn, Y. H., Jung, H. Y., Kaelin-Lang, A., & Hallett, M. (2003). Excitability of the ipsilateral motor cortex during phasic voluntary hand movement. *Experimental Brain Research, 148*(2), 176-185. doi: DOI 10.1007/s00221-002-1292-5

Spijkers, W., Heuer, H., Kleinsorge, T., & van der Loo, H. (1997). Preparation of bimanual movements with same and different amplitudes: specification interference as revealed by reaction time. *Acta Psychologica, 96*(3), 207-227.

Starr, A., Caramia, M., Zarola, F., & Rossini, P. M. (1988). Enhancement of Motor Cortical Excitability in Humans by Non-Invasive Electrical-Stimulation Appears Prior to Voluntary Movement. *Electroencephalography and Clinical Neurophysiology, 70*(1), 26-32.

Sterr, A., & Dean, P. (2008). Neural correlates of movement preparation in healthy ageing. *European Journal of Neuroscience, 27*(1), 254-260. doi: DOI 10.1111/j.1460-9568.2007.05975.x

Swinnen, S., Walter, C. B., & Shapiro, D. C. (1988). The Coordination of Limb Movements with Different Kinematic Patterns. *Brain and Cognition, 8*(3), 326-347.

Swinnen, S. P., Beirinckx, M. B., Meugens, P. F., & Walter, C. B. (1991). Dissociating the Structural and Metrical Specifications of Bimanual Movement. *Journal of Motor Behavior, 23*(4), 263-279.

Swinnen, S. P., Young, D. E., Walter, C. B., & Serrien, D. J. (1991). Control of Asymmetrical Bimanual Movements. *Experimental Brain Research, 85*(1), 163-173.

Ulrich, R., Moore, C., & Osman, A. (1993). Lateralized Readiness Potential and Reaction-Times - the Effect of Partial-Response Cues. *Zeitschrift fur Experimentelle und Angewandte Psychologie, 40*(2), 310-325.

Valls-Solé, J., Rothwell, J. C., Goulart, F., Cossu, G., & Muñoz, E. (1999). Patterned ballistic movements triggered by a startle in healthy humans. *Journal of Physiology, 516*(3), 931-938.

Valls-Solé, J., Solé, A., Valldeoriola, F., Muñoz, E., Gonzalez, L. E., & Tolosa, E. S. (1995). Reaction time and acoustic startle in normal human subjects. *Neuroscience Letters, 195*(2), 97-100.

Wadman, W. J., Denier Van der Gon, J. J., Geuze, R. H., & Mol, C. R. (1979). Control of fast goal-directed arm movements. *Journal of Human Movement Studies, 5*, 3-17.

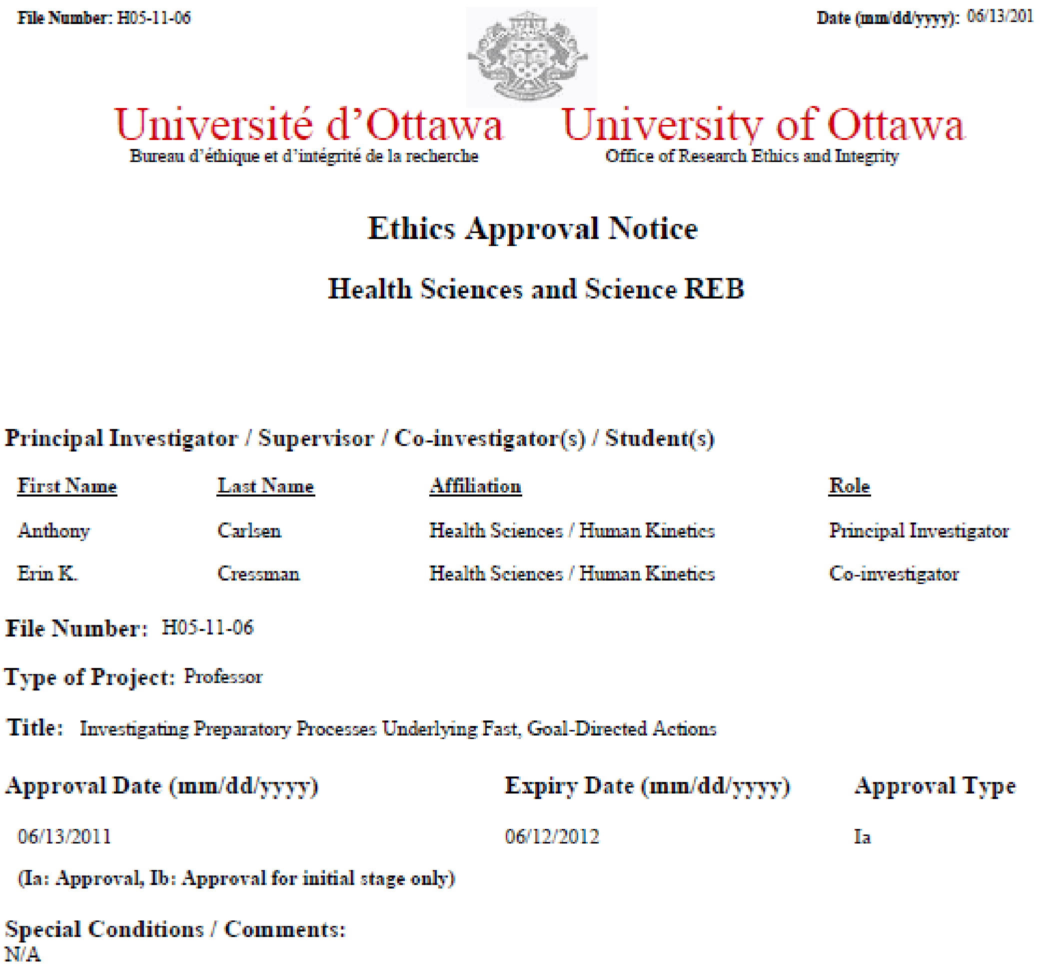
Walter, C. B., & Swinnen, S. P. (1990). Kinetic Attraction during Bimanual Coordination. *Journal of Motor Behavior, 22*(4), 451-473.

Weigelt, M. (2007). Re-examining structural constraints on the initiation of bimanual movements: The role of starting locations, movement amplitudes, and target locations. *Human Movement Science, 26*(2), 212-225. doi: DOI 10.1016/j.humov.2007.01.010

Wilkins, D. E., Hallett, M., & Wess, M. M. (1986). Audiogenic Startle Reflex of Man and Its Relationship to Startle Syndromes - a Review. *Brain, 109*, 561-573.

Zelaznik, H. N., & Hahn, R. (1985). Reaction-Time Methods in the Study of Motor Programming - the Precuing of Hand, Digit, and Duration. *Journal of Motor Behavior, 17*(2), 190-218.

Appendix



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Date (mm/dd/yyyy): 06/13/201

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Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Anthony	Carlsen	Health Sciences / Human Kinetics	Principal Investigator
Erin K.	Cressman	Health Sciences / Human Kinetics	Co-investigator

File Number: H05-11-06

Type of Project: Professor

Title: Investigating Preparatory Processes Underlying Fast, Goal-Directed Actions

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
06/13/2011	06/12/2012	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:
N/A