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An emerging target paradigm to evoke fast visuomotor responses on human upper limb muscles

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Dear Dr. Bajaj

April 8 2020

We are submitting a revision to our JoVE manuscript 61428R2, now entitled: “*An Emerging Target Paradigm to evoke fast visuomotor responses on human upper limb muscles*” for consideration at JoVE. We thank you for your editorial comments, and hope that all is in order. If not, please feel free to contact us at your convenience.

With best regards,

A handwritten signature in blue ink, appearing to read 'B. Corneil'.

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TITLE:

An Emerging Target Paradigm to Evoke Fast Visuomotor Responses on Human Upper Limb Muscles

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KEYWORDS:

stimulus-locked responses, reaction time, visually guided reaches, humans, electromyography, moving targets, sensorimotor transformation

SUMMARY:

Presented here is a behavioral paradigm that elicits robust fast visuomotor responses on human upper limb muscles during visually guided reaches.

ABSTRACT:

To reach towards a seen object, visual information has to be transformed into motor commands. Visual information such as the object's color, shape, and size are processed and integrated within numerous brain areas, then ultimately relayed to the motor periphery. In some instances, a reaction is needed as fast as possible. These fast visuomotor transformations, and their underlying neurological substrates, are poorly understood in humans as they have lacked a reliable biomarker. Stimulus-locked responses (SLRs) are short latency (<100 ms) bursts of electromyographic (EMG) activity representing the first wave of muscle recruitment influenced by visual stimulus presentation. SLRs provide a quantifiable output of rapid visuomotor transformations, but SLRs have not been consistently observed in all subjects in past studies. Here we describe a new, behavioral paradigm featuring the sudden emergence of a moving target below an obstacle that consistently evokes robust SLRs. Human participants generated visually guided reaches toward or away from the emerging target using a robotic manipulandum while surface electrodes recorded EMG activity from the pectoralis major muscle. In comparison to previous studies that investigated SLRs using static stimuli, the SLRs evoked with this emerging target paradigm were larger, evolved earlier, and were present in all participants. Reach reaction times (RTs) were also expedited in the emerging target paradigm. This paradigm affords

numerous opportunities for modification that could permit systematic study of the impact of various sensory, cognitive, and motor manipulations on fast visuomotor responses. Overall, our results demonstrate that an emerging target paradigm is capable of consistently and robustly evoking activity within a fast visuomotor system.

INTRODUCTION:

When we notice a message on our cellphone, we are prompted to perform a visually guided reach to pick up our phone and read the message. Visual features such as the shape and size of the phone are transformed into motor commands allowing us to successfully reach the goal. Such visuomotor transformations may be studied in laboratory conditions, which permit a high degree of control. However, there are scenarios where response time is important, e.g., catching the phone if it were to fall. Laboratory studies of fast visuomotor behaviors often rely on displaced target paradigms where on-going movements are modified in mid-flight following some change in target position (e.g., see ref.^{1,2}). While such online corrections can occur in <150 ms³, it is difficult to ascertain the exact timing of fast visuomotor output using kinematics alone due to the low-pass filtering characteristics of the arm, and because fast visuomotor output supersedes a movement already in mid-flight. Such complications lead to uncertainty about the substrates underlying fast visuomotor responses (see ref.⁴ for review). Some studies suggest that subcortical structures such as the superior colliculus, rather than fronto-parietal cortical areas, may initiate online corrections⁵.

This uncertainty regarding the underlying neural substrates may be due, at least in part, to the lack of a reliable biomarker for the output of the fast visuomotor system. Recently, we have described a measure of fast visuomotor responses that may be generated from static postures and recorded via electromyography (EMG). Stimulus-locked responses (SLRs) are time locked bursts of EMG activity that precede voluntary movement^{6,7}, evolving consistently ~100 ms after stimulus onset. As the name implies, SLRs are evoked by stimulus onset, persisting even if an eventual movement is withheld⁸ or moves in the opposite direction⁹. Furthermore, SLRs evoked by target displacement in a dynamic paradigm are associated with shorter latency online corrections¹⁰. Thus, SLRs provide an objective measure to systematically study the output of a fast visuomotor system involved in short latency RTs, as they may be generated from a static posture and parsed from other EMG signals unrelated to the initial phase of the fast visuomotor response.

The goal of the current study is to present a visually-guided reaching paradigm that robustly elicits SLRs. Previous studies investigating the SLR have reported less than 100% detection rates across participants, even when using more invasive intramuscular recordings^{6,8,9}. Low detection rates and a reliance on invasive recordings limit the usefulness SLR measures in future investigations into the fast visuomotor system in disease or across the lifespan. While some subjects may simply not express SLRs, the stimuli and behavioral paradigms used previously may not have been ideal to evoke the SLR. Past reports of SLRs have typically used paradigms wherein participants generate visually-guided reaches towards static, suddenly appearing targets^{6,9}. However, a fast visuomotor system is the most likely needed in scenarios where one must rapidly interact with a falling or flying object, leading the person to wonder if moving rather than static

stimuli may better evoke SLRs. Therefore, we have adapted a moving target paradigm used to study eye movements¹¹, and combined it with a pro/anti visually guided reaching task used to examine the SLR⁹. When compared to results from paradigms used previously^{6,8,9}, it was found that SLRs in the emerging target paradigm evolved sooner, attained higher magnitudes, and were more prevalent across our participant sample. Overall, the emerging target paradigm promotes the expression of fast visuomotor responses to such a degree that objective EMG measures can be made reliably with surface recordings, potentiating study within clinical populations and across the lifespan. Further, the emerging target paradigm can be modified in many different ways, promoting more thorough investigations into the sensory, cognitive, and motor factors that promote or modify fast visuomotor responses.

PROTOCOL:

All procedures were approved by the Health Science Research Ethics Board at the University of Western Ontario. All participants provided informed consent, were paid for their participation, and were free to withdrawal from the experiment at any time.

1. Participant preparation

NOTE: A small sample of healthy, young participants was studied (3 female, 2 male; mean age: 26 years +/- 3.5). All participants were right-handed and had normal or corrected-to-normal vision, with no current visual, neurological, or musculoskeletal disorders. Participants with a history of musculoskeletal upper limb injury or disorders were excluded.

1.1 Apply EMG sensors to the targeted upper limb muscle involved in the reaching movement being studied. Here, EMG recordings were made from the clavicular head of the right pectoralis major muscle, which is recruited for cross-body (leftward) reaching.

NOTE: Recordings can be made from other muscles of the upper limb, or from the sternal or lateral portion of the pectoralis major muscle.

1.1.1 Visualize the target muscle by requesting an action known to recruit the muscle of interest. For the clavicular head of the pectoralis major muscle, ask the participant to relax their elbows at their sides and push their palms together. If having difficulty visualizing the target muscle, palpate the area of interest while having the participant repeatedly perform the requested action, and target areas with notable changes in the muscle for electrode placement.

NOTE: Visualization refers to the identification of target muscle, via seeing the muscle's shape through the overlying skin as the participant performs an action that recruits the muscle. Visualization aids localization of the target muscle.

1.1.2 Using alcohol swabs, clean the skin surface over the target muscle where the electrode will be placed, and also over the area where a ground electrode will be located.

1.1.3 Prepare the surface sensors by applying adhesives and electrode gel.

1.1.4 Ask the participant to perform the action associated with the muscle recruitment again, and adhere sensors over the muscle belly, positioning them to lie in parallel with the direction of the fibres of the targeted muscle. Place the ground electrode on the clavicle contralateral to the reaching arm. Secure sensors and ground electrodes to the surrounding skin with medical tape. Turn on the EMG system to allow for EMG collection throughout the experiment.

NOTE: After the placement of the EMG electrodes, EMG data is collected passively and continuously throughout the experiment via the EMG system and saved as an analog datastream for later analysis.

1.1.5 Check the quality of the EMG signal by using a desktop monitor or oscilloscope connected to the EMG system. To determine the suitable quality, have the participant perform a reaching movement into or opposite from the preferred direction of the muscle of interest, and ensure that EMG activity increases or decreases, respectively. If there is no activity at rest, then ensure that EMG activity does not increase for movement in the non-preferred direction.

NOTE: Muscle signal quality from surface electrodes depends on many characteristics (e.g., idiosyncratic distribution of adipose tissue, subject posture). The peak EMG activity associated with movement in the preferred (contracting) direction is recommended to be at least 2x the level of activity at rest but should be considerably higher.

1.1.6 Reposition the electrodes if necessary, to ensure that these activity levels are observed. Leave the viewing monitor or oscilloscope connected throughout the experiment to continuously monitor EMG output.

1.2 Set up the specific participant with the applied EMG sensors in a robotic reaching apparatus which allows reaching movements in a horizontal plane, and the application of force to the manipulandum.

NOTE: Adding force against the muscle of interest increases the background activity, allowing for the expression of the SLR as an increase or decrease in muscle activity following stimulus presentation in the muscle's preferred or non-preferred direction, respectively. A level of baseline activity is especially useful in the non-preferred direction, as baseline and non-preferred reaching activity would be indistinguishable without a background loading force. An applied force of 5N to the right and 2N of force down (opposite to a leftward presented target relative to the start position), throughout the entirety of the experiment can be sufficient. The force should remain constant throughout the experiment, so lower forces can be used if necessary.

1.2.1 Seat the participant in the experimental chair, prioritizing participant comfort with respect to the added forced against the limb to minimize changes of posture throughout the experiment.

2. Stimuli construction/ apparatus

2.1 Generate all experimental procedures and stimuli in the robotic reaching apparatus with a built-in visual display.

NOTE: Ensure that the robotic reaching apparatus is equipped with an interface between visual output and manipulandum motor output that permits simultaneous analog (e.g., manipulandum position, photodiode output) and EMG recordings. Ensure that this apparatus is equipped with software capable of running blocks of individual, pre-programmed trials with all preprogrammed visual components. The built-in visual display may be a standard monitor or customized high-quality projector; however, higher quality projectors are recommended to ensure temporal and visual resolution of the displayed target.

2.1.1 Generate the 4 primary components of the emerging target paradigm (see **Supplemental Figure 1**) via built in software that drives the visual display.

NOTE: Components should all be generated via built in software which projects the specified components onto the visual display during each data collection session. Each component is manually entered into the software, which transforms input coordinates for shapes into shapes seen on the visual display. A complete coding of all components and target motions is done prior to data collection, therefore, no experimenter intervention of the paradigm is required during the data collection, as the paradigm runs automatically based on the participant's responses. The following coordinates (reported in cm) are referenced in relation to the midpoint of the two robot manipulandum origins in the robotic reaching apparatus used to collect data from participants in the current manuscript. All components of the paradigm are visible to the participant throughout each trial, except for the start position which disappears after appearance of the moving target. A different apparatus may use a different reference frame.

2.1.1.1 Generate an inverted y path by manually inputting coordinates for six rectangles with the following coordinates (y: -19 (top of inverted y) or -34 (bottom of inverted y), x: -/+2 (inner, bottom inverted y), -/+8 (outer bottom inverted y); width .5 height: 20 (top) or 15 (bottom)).

2.1.1.2 Generate an occluder by manually inputting coordinates for one large rectangle (centered at: 0, -29; width: 35 height: 15) overlaying the center of the inverted y path. The color of this occluder may vary from trial to trial, providing an instruction to the participant.

NOTE: The occluder contains a notch cut out on the center bottom between the two outputs (0, -29; width: 5 height: 5). The participant is instructed to: "fixate the notch while a target is behind the occluder". Doing so ensures the eye is stable at target emergence. The occluder will either be colored red or green at the beginning of each trial.

2.1.1.3 Generate a moving target by manually inputting coordinates for one circle which will eventually move down the inverted y and behind the occluder (start: 0, -17; radius: 1; speed: 10 cm/s, speed behind occluder: 30 cm/s).

NOTE: The moving target (T1) is visible and stationary at the beginning of every trial.

2.1.1.4 Generate how the target will move in the software by specifying the x and y coordinates of target motion.

NOTE: The speed of target is calculated by the distance of the successive x and y coordinates. Proper presentation of target motion is dependent on the ability of the software and visual display to properly update each x and y position in rapid succession. In the software, change the status of the moving target to “invisible to participant” when the x and y position of the target have moved fully below the occluder until the x and y position have fully emerged from the occluder.

2.1.1.5 Generate a start position (0, -42; radius 1). The participant will need to acquire this position to initiate each trial.

2.1.2. Generate a real time cursor (RTC) representing the participant’s hand position on the screen in real time.

NOTE: The participant’s hand/arm was occluded during the experiment via an upward facing mirror reflecting downward-presented targets. This may be done via built in software functions specific to the apparatus, which places a target overtop of the continually updated x and y coordinates of the hand.

3. Procedure

3.1 Click the “Start” button on the associated software presented on the experimenter’s screen, which initiates the first trial and force generated by the robotic reaching apparatus applied to the participants upper limb.

NOTE: After the experimenter clicks start, no intervention is required by the experimenter, until between blocks where the experimenter must press start again. Experimenter intervention may also be required if the EMG signal being continually monitored changes, or the participant is unable to complete the experiment. All experiments should be stopped immediately if an emergency arises. Force applied to the participants hand is automatically stopped if the participant lets go of the handle via built in task programs. It is recommended that an apparatus with a button to terminate the experiment in emergency situations is used.

3.1.1 Verbally instruct the participant to start the first trial by bringing the RTC (indicated by the position of the manipulandum) into the start position (T0) for a variable duration of 1- 1.5 s. The occluder changes color to instruct the subject that the upcoming trial requires a pro- or anti-reach.

NOTE: Bringing RTC into T0 initiates each trial. If the participant exits the T0 start position before the prescribed time, the trial will start again once RTC is back in T0.

3.1.2 Ensure that the moving target (T1) which was stationary and visible to the participant at the top of the inverted y (2.1.1.3), begins movement towards the participant along the path of the inverted y, which was initiated by the participant bringing RTC into T0 in the previous step.

NOTE: When T1 starts moving, T0 disappears. No restrictions are placed on the participant's arm after this time, however, the participant is instructed to stay within the imagined confines of T0.

3.1.3 Ensure that T1 moves behind the occluder and is invisible to the participant. During this interval, the participant maintains hand position at imagined T0.

3.1.4 Ensure that T1 travels behind the occluder at a constant speed of 30 cm/s along the y axis towards the participant. Once T1 reaches half the length of the occluder, it bifurcates along one of the inverted y outputs with an additional x velocity component. Thus, speed along the y axis is kept constant. The target vanishes for a constant delay of ~0.5 s, with the delay depending on the size of the occluder and the speed of T1 motion.

3.1.5 When T1 reaches the edge of the occluder closest to the participant, ensure that the software program does not present T1 as emerging by sliding past the edge of the occluder, as doing so would initially present a "half-moon" stimulus to the visual system. Instead, check that the software program keeps T1 invisible until the full target has emerged, and then presents it to the participant.

NOTE: This is done to control for visual processing effects of partial stimuli, especially if different speeds of targets are used which would cross the boundary at different times. A partial emergence of a target (e.g., half moon stimulus) produces a target composed initially of a higher spatial frequency, which based on previous results would lead to increased SLR latency and decreased magnitude¹⁰.

3.1.6 Check that the software program presents T1 to a randomized side at one of the two inverted y paths while the participant's hand remains stationary at T0.

NOTE: Simultaneous with the emergence of T1 from below the occluder, a secondary target is presented in the corner of the screen, at a location covered by a photodiode. This target presented to the photodiode is not seen by the subject but provides an analog signal to a photodiode integrated in the robotic reaching device. This photodiode signal allows for the precise alignment of target appearance with muscle activity and ensures no lags or delays are present within the robotic reaching apparatus.

3.1.7 When T1 emerges from the behind the occluder, see if the participant is able to generate a visually guided reach depending on the color of the occluder. When the occluder is green, ask the participant to intercept T1 with the RTC. When the occluder is red, ask the participant to move the RTC away from T1.

NOTE: A green occluder color (2.1.1.2) indicates a pro reach (i.e., towards the occluder) and a red color indicated away from moving target T1 (i.e., an anti-reach). In the anti-reach condition, a correct interception is not based on the mirror image of T1, but rather the horizontal distance relative to T0.

3.1.8 Depending on their reaching behavior, provide feedback as either a 'hit' (correct interception), 'wrongway' (incorrect direction for pro/anti reach), or 'miss' (neither correct nor incorrect responses detected) during the inter-trial interval. This feedback consists of text written on the occluder.

3.1.9 Make sure that T1 and T0 reappear at their respective original locations 200 ms after the participant's reach behavior is completed. Start the next trial when the participant brings the RTC to T0.

3.2 Ask each participant to perform 4 blocks of 100 trials, yielding 100 reaches per condition. Randomize the trial types intermixed with pro or anti-reaches after left and right stimuli. Each block takes approximately 7.5 min to complete.

NOTE: It is recommended that each condition consists of a minimum of ~80 repeats when using surface recordings, as the next analysis step relies on data from many trials for SLR detection.

3.2.1 Minimize the participant movement between each block to ensure consistency of recordings. After verbal confirmation that the participant is ready to begin the next block, initiate the next block and continue to monitor participant performance and EMG output.

NOTE: Continued monitoring of EMG output via a desktop monitor by the experimenter may be required for detecting issues with surface EMG recordings. For example, during extended periods of reaching movements, surface EMG electrodes may become unstuck from the participant's skin due to sweating.

3.3 Collect data from a control static paradigm to enable comparison of data to that obtained in the emerging target paradigm.

NOTE: This may be done before or after the emerging target paradigm. To create a control static paradigm, repeat steps 2.1.1.3, 2.1.1.5, 2.2, 3.1, 3.1.1, 3.1.7, 3.2 and 3.2.1; however, in step 2.1.1.3, do not code T1 starting at the top of the screen and moving towards the participant. Instead, position T1 to appear either to the left or right of T0. Furthermore, T0 is now either red or green akin to the occluder used in the emerging target paradigm. The trial proceeds as described below.

3.3.1 Verbally instruct the participant to bring the RTC into T0 to start the first trial, which is in the same location as in the emerging target paradigm.

3.3.2 Make sure that the software program presents T0 as either red or green to indicate a pro or anti reach respectively. Randomize the hold period of 1-2s for the participant to hold the RTC in T0.

3.3.3 Make sure that the software program presents a static target either to the left or right, 10 cm from T0. Randomize the target side across trials.

3.3.4 As in the emerging target paradigm, ask the participant to reach towards a target if T0 is green, and reach in the diametrically opposite direction away from a target if T0 is red. The next trial proceeds after contact with a target or anti-target location.

3.4.5 Ensure that each participant performed 4 blocks of 100 trials, yielding 100 reaches per condition. Trial types were randomly intermixed.

4. Analysis

4.1 Analyze all data in offline custom scripts and discard error trials.

NOTE: Error trials are defined by incorrect reach directions (3.5 cm), long RTs (>500 ms) indicating presumed inattentiveness or short RTs (<120) indicating anticipation.

4.1.1 Derive the reaction time (RT) for reaching movements for each trial by identifying the time at which movement exceeded 8% of the peak tangential velocity.

NOTE: Other methods to define RT may be used.

4.1.2 To analyze the muscle activity, use offline scripts to convert the EMG signals to source microvolts, remove any DC offset, rectify the EMG signal, and filter the signal with a 7-point moving average filter.

4.1.3 Use a time-series receiver-operating characteristic (ROC) analysis to detect the presence and latency of the SLR^{6, 7}.

NOTE: Alternative methods for determining the time-locked nature of SLR activity may be used.

4.1.3.1 To perform the time-series ROC analysis, segregate EMG data based on the side of target presentation and trial condition (**Figure 1a** shows left versus right data for pro-reaches).

4.1.3.2 Calculate the area under the ROC curve for the two populations, for every time sample (1 ms) from 100 ms before to 300 ms after the target presentation (e.g., **Figure 2c**).

NOTE: ROC value of 0.5 indicates chance discrimination, whereas values of 1 or 0 indicate perfectly correct or incorrect discrimination relative to target presentation, respectively.

4.1.3.3 Determine discrimination latency as the first of 8 of 10 consecutive points that exceeded a value of 0.6 (**Figure 2c** indicated by vertical red or blue lines).

NOTE: Threshold, and number of points exceeding threshold may change depending on the quality and quantity of surface or intramuscular EMG recordings, and a bootstrapping analysis may be used to objectively determine confidence intervals. Past work has shown that a value of 0.6 equates approximately to a 95% confidence interval¹².

4.1.4 To determine the presence of an SLR on pro-reach trials, use an RT-split analysis (see **Figure 1, 8**), whereby steps 4.1.3.2 and 4.1.3.3 are performed separately on the early and late half of reaches based on RT (**Figure 1a** purple trials, and green trials).

4.1.4.1 Plot the early discrimination time and mean early RT as one point, then plot late discrimination time and mean late RT as a second point on the same plot. Connect these two points with a line (**Figure 1c**). An SLR is detected when the slope of this line exceeds 67.5° .

NOTE: For this line, a slope of 90° would indicate that EMG discrimination times are perfectly locked to stimulus presentation (as EMG activity is initiated at the same latency, regardless of ensuing movement time), whereas a slope of 45° would indicate that EMG discrimination are perfectly locked to movement onset. In practice, a cut-off slope of 67.5° (halfway between 45° and 90°) is used to detect whether an SLR was present (slope $> 67.5^\circ$) or not (slope $< 67.5^\circ$); as this indicates that EMG activity is more locked to stimulus rather than movement onset.

4.1.5 If SLR presence is determined, define the SLR latency by the discrimination latency from all trials (4.1.3.3).

4.1.6 Define the SLR magnitude as the difference between left and right mean EMG traces (e.g., **Figure 2c** dark red versus light red traces, or dark blue versus light blue traces) from SLR latency to 30 ms post discrimination latency.

NOTE: Magnitude time values may be extended or shortened.

REPRESENTATIVE RESULTS:

Stimulus locked responses (SLRs) are brief bursts of muscle activity time locked to the stimulus onset that evolve well before the larger volley of muscle recruitment associated with movement onset. The time-locked nature of the SLR produced a 'banding' of muscle activity visible at ~ 100 ms when viewing all trials sorted for reaction time (RT) (**Figure 1a**, highlighted by grey boxes). As shown in **Figure 1a**, SLRs was dependent on target location, with SLRs on the right pectoralis major consisting of an increase or decrease in muscle recruitment following leftward or rightward target presentation, respectively. SLRs were detected with an RT split analysis (methods 4.1.4), whereby separate time-series ROC analyses were performed on early and late RT trials (**Figure 1b**- purple versus green). This analysis indicates whether EMG onset was invariant to stimulus or movement onset, which was determined by the slope of the line connecting early and late discrimination times plotted as a function of RT (**Figure 1c**). Previous studies of the SLR using

static stimuli reported detection rates across all participants below 70%^{8,9}. Here, a comparison was made to the effectiveness of an emerging target paradigm in evoking SLRs to that obtained using a paradigm with static targets.

In the emerging target paradigm (**Supplemental Figure 1**), subjects reached towards emerging moving targets instead of stationary targets. **Figure 2** shows data from two subjects reaching toward a stationary target (first and third rows) or moving targets that emerge beneath an occluder (second and fourth rows). Participant 1 does not exhibit an SLR in the static paradigm, but exhibits a clear SLR in the emerging target paradigm; SLRs were apparent as a vertical band of activity in the trial-by-trial plots (**Figure 2a**) ~100 ms after stimulus onset in the emerging target but not static paradigm. The SLR was also apparent in the mean EMG traces (**Figure 2b**) for participant 1 in the emerging target but not static paradigm (red traces in top two rows of **Figure 2b**). Participant 1 provided an example of someone who does not exhibit an SLR in a static paradigm used previously in the literature, but who does exhibit an SLR in the emerging target paradigm. In contrast, while participant 2 exhibited an SLR in both the static and emerging target paradigms, the magnitude of the SLR was much greater in the emerging target paradigm, with magnitudes approaching that attained just before movement onset.

We compared the properties of the SLRs observed in the emerging targets versus static paradigm across the sample, examining data collected in the pro-reach condition. As shown in **Figure 3a** (green lines), and consistent with the representative results in **Figure 2**, SLR magnitude was considerably larger in the emerging target versus static paradigm, with recruitment magnitudes in the interval 80-120 ms after stimulus onset increasing fivefold on average. In contrast to such systematic changes in SLR magnitude, the latency of detected SLRs did not differ in the static versus emerging target paradigm (**Figure 3a**, purple lines). As shown in **Figure 3b** (blue bars), SLRs were detected in all five participants in the emerging target paradigm (i.e., a prevalence of 100%), but only in three participants in a paradigm with static targets (i.e., a prevalence of 60%, resembling previous reports^{8,9}). Observing SLRs on all participants in the emerging target paradigm was even more impressive considering that we relied on non-invasive surface EMG recordings, whereas previous reports have generally relied on invasive intramuscular EMG recordings. Importantly, while reach RTs tended to be much shorter in the emerging target versus static paradigm (**Figure 3b**, black lines), SLRs do not simply arise in the emerging target paradigm due to expedited RTs. For example, the data for Participant 1 in **Figure 2** exhibited prominent SLRs in the emerging target but not static paradigm for overlapping ranges of reach RTs. Finally, we also examined how the instruction to move away from the emerging target influenced the SLRs. As found previously with static targets⁹, SLR magnitudes in the anti-reach condition were muted compared to that in the pro-reach condition (**Figure 3c**, blue lines; see also mean EMG traces in **Figure 2**, **Figure 4**). This shows that emerging target paradigm can be used to study aspects of cognitive control, which in this case related to consolidation of the instruction to move either toward or away from an emerging target.

We show data recorded from all five participants in **Figure 4**, in order to illustrate the variability in the characteristics of SLRs recorded in the static versus emerging target paradigms in the pro- and anti-reach conditions (the grey boxes in **Figure 4** depict the SLR interval). As with participant

1 (shown in upper two rows in **Figure 2**), participant 5 also exhibited an SLR in the emerging target but not static paradigm in the pro-reach condition. As with participant 2 (shown in lower two rows in **Figure 2**), participants 3 and 4 also exhibited considerably larger SLRs in the emerging target versus static paradigms in the pro-reach condition. Two other features of the data shown in **Figure 4** deserve emphasis. First, in participants 3, 4 and 5, we observed a larger SLR in the anti-reach variant of the emerging target task, with the time-series ROC peaking above 0.6 before assuming levels near 0. An SLR toward the stimulus in an anti-reach condition has been observed previously⁹, and we have related this to the brief movement of the hand toward the stimulus in an anti-reach variant of an on-line correction task³. Second, in the pro-reach condition in the emerging target task, a distinct separation was observed between the SLR and ensuing movement-aligned activity in some participants (e.g., participants 1, 3 and 5; see how time-series ROC drops briefly after peaking during the SLR interval), but found that the SLR blended into movement-aligned activity in others (e.g., participants 2 and 4). As noted below, this relates to the design of algorithms for detecting the SLR.

Overall, the emerging target paradigm is more effective at evoking SLRs and short RTs than paradigms using static targets. This is demonstrated by increases in SLR prevalence, magnitude, and shorter latency RTs with respect to static targets.

FIGURE AND TABLE LEGENDS:

Figure 1: SLR detection. Example of an SLR from a representative participant, illustrating the detection criteria for SLRs. **(a)** Trial-by-trial recruitment for right pectoralis major muscle for right or left reaches in the pro-reach condition. Each row is a different trial. Intensity of color conveys the magnitude of EMG activity. Trials were sorted by reach RT (white boxes) and aligned to stimulus onset (black line). The SLR appeared as a vertical banding of activity highlighted by grey boxes; note how EMG activity increased or decreased (time-locked ~90 ms) after leftward or rightward stimulus presentation, respectively. Purple or green bars indicate the trials contributing to the early or late RT groups, respectively. **(b)** Time-series ROC analysis indicating time of EMG discrimination for early (purple) and late (green) trials shown in (a). **(c)** For the early (purple) and late (green) groups, mean RT was plotted as a function of ROC discrimination. The slope of the line connecting these two points is 83.7° , indicating that EMG activity was more aligned to stimulus presentation than movement onset.

Figure 2: Representative results. Data from participants 1 and 2 showing the variability in the presence or absence of SLRs in the static (1st and 3rd rows), and the consistency of SLR presence in the emerging target paradigms (2nd and 4th rows). **(a)** Trial-by-trial recruitment for right pectoralis major muscle for these participants (same format as Figure 1a). Conditions exhibiting an SLR are outlined in purple (2nd, 3rd and 4th rows). **(b)** Mean \pm SE of EMG activity for both pro (red) and anti (blue) reaches, segregated by the side of stimulus presentation (fainter traces used for movements in the non-preferred direction). **(c)** Time-series ROC analysis for pro (red) and anti (blue) reaches shown in (b). SLR epoch highlighted in grey box; horizontal dashed lines at 0.4 and 0.6. Vertical colored lines (if present in pro condition) show the discrimination time for pro- (red) or anti- (blue) reach trials.

Figure 3: Effects of an emerging target paradigm on SLR characteristics and reach RT. (a) SLR latency (purple) and magnitude (green) for pro reaches in static versus emerging target paradigms. Latency defined as first 8 out of 10 continuous data points surpassing ROC threshold of 0.6 (see methods). Magnitude of SLR was defined as the integrated area over 30 ms after SLR discrimination between the mean EMG activity on left versus right trials. All magnitudes were normalized to the maximum for the participant across conditions (e.g., a value of 1 indicates the maximal response). (b) SLR prevalence and reach RT. (c) SLR magnitude and latency results from pro and anti-reaches in the emerging target paradigm. * denotes significance at $p < .05$ compared to static or anti condition based on unpaired t-test.

Figure 4: Mean EMG and time-series ROC analyses for all participants. Left column of plots: Mean \pm SE of EMG activity for both pro (red) and anti (blue) reaches, segregated by the side of stimulus presentation (fainter traces used for movements in the non-preferred direction). Right column of plots: Time-series ROC analysis for pro (red) and anti (blue) reaches shown in (left column of plots). SLR epoch highlighted in grey box; horizontal dashed lines at 0.4 and 0.6. Vertical colored lines (if present in pro condition) show the discrimination time for pro- (red) or anti- (blue) reach trials.

Supplementary Figure 1: Top view of task in the robotic reaching device. Large white dot on lower left side represents the photodiode. White target (T1) is shown exiting the inverted 'y' path to the left. White dot to the right of T1 represents RTC in the midst of a visually guided reach. The occluder is shown here as green, indicating a pro reach was required. T0 not shown, due to the simultaneous disappearance with target emergence.

DISCUSSION:

Humans have a remarkable capacity, when needed, to generate rapid, visually guided actions at latencies that approach minimal afferent and efferent conduction delays. We have previously described stimulus-locked responses (SLRs) on the upper limb as a new measure for rapid visuomotor responses^{6,9,10}. While beneficial in providing a trial-by-trial benchmark for the first aspect of upper limb muscle recruitment influenced by the visual stimulus, limb SLRs have not been expressed in all subjects and often relied upon invasive intramuscular recordings. Here, an emerging target paradigm (**Supplementary file 1**) is described and the results are compared to those obtained with static targets. The benefits of the emergent target paradigm are apparent within individual participants, as participants who do not express the SLR in a static paradigm express one in the emerging target paradigm (e.g., **Figure 2**, participant 1- 1st row versus 2nd row). Furthermore, SLRs expressed in the emerging target paradigm are much larger than in other paradigms, sometimes attaining magnitudes that are equivalent to volitional magnitudes (**Figure 2**, participant 2; **Figure 4**, participant 5). Thus, this paradigm has proven to be effective in increasing the magnitude (**Figure 3a**), detectability of the SLR (**Figure 3b**), and promoting shorter reach RTs by ~ 50 ms (**Figure 3b**), compared to a paradigm using static targets. The emerging target paradigm also has advantages over paradigms requiring mid-flight corrections⁴, where a new stimulus is presented while a reaching movement is already in mid-flight. EMG or kinetic changes to movements already in mid-flight can also occur during experiments which change the

visual feedback of current hand position, either alone or in conjunction with changes in target position¹³. While commonly used to study fast visuomotor responses, in such paradigms the EMG, kinetic, and/or kinematic activity driven in response to the new stimulus evolve on top of activity related to the original movement. In contrast, since the participant is in a stable posture at the time of stimulus emergence in the emerging target paradigm, SLRs are easily discerned, even on a trial-by-trial basis.

The three most critical aspects to the emerging target paradigm are the use of implied motion behind a barrier (3.1.3), certainty of the time of the target appearance (3.1.4), and full target emergence from behind an occluder (3.1.5). Of these three aspects, we speculate that the use of implied motion is the most important. Implied motion produces strong signals in motion-related areas in the dorsal visual stream that are indistinguishable from those produced by visible moving targets¹⁴. We speculate that, when combined with such implied motion, the sudden appearance of the emerging target below the obstacle creates a stronger visual transient than in the static target paradigm. Our implementation of the emerging target paradigm also incorporated a high degree of trial-by-trial certainty of the time at which the target would re-appear. The disappearance and subsequent emergence of the target behind the barrier may be akin to a ‘gap interval’ between offset of a central fixation or hold stimulus and presentation of a peripheral target, which also expedites reach reaction times¹⁵ and promotes the expression of express saccades¹⁶, which are another type of fast visuomotor response. Finally, it is important that the target emerging from behind the barrier is presented in its entirety, rather than being presented as sliding from behind the barrier. Were the target to slide past the barrier, the earliest stimulus available to the visual system would be a ‘half-moon’ stimulus that would lack the lower spatial frequencies known to promote earlier and stronger expression of limb SLRs¹⁰. In addition to these critical steps, it is important to position the outlets for the emerging targets at locations associated with the preferred or non-preferred direction of the muscle(s) under study. Introducing a background loading force to increase activity of the muscle of interest is also beneficial in the detection of limb SLRs.

In terms of troubleshooting, it is imperative to ensure that the time of target emergence is known on every trial, given the short latency of the limb SLR. This is particularly important for digital monitor displays, which may systematically induce variable delays in the time of stimulus presentation that could compromise accurate alignment of muscle activity to critical events. Prior to any implementation of the emerging target experiment, and regardless of the type of visual display, we encourage the use of multiple photodiodes to record the timing of stimulus appearance at multiple screen locations (e.g., at the unseen location referenced in 3.1.6, and at the locations where T1 will emerge). If the interval between stimulus appearance at these two locations is invariant across trials, then the photodiode at the unseen location can serve as a proxy for T1 appearance during the actual experiment, after adjusting for any lags specific to the different locations at which T1 may appear. We also encourage close ‘on-line’ monitoring of EMG activity during the experiment, to watch for any changes in background EMG activity prior to target emergence, or to changes in EMG activity associated with reaching movements in of opposite from the muscle’s preferred direction of movement.

There are a number of ways in which the emerging target paradigm could be modified and doing so can further the understanding of the sensory, cognitive, and movement-related factors that influence the fast visuomotor system. Here, we instructed the subjects to prepare to move toward (a pro-reach) or away (an anti-reach) from the emerging target. As expected from previous results⁹, consolidation of this instruction enabled subjects to dampen SLR magnitude without changing SLR timing. This shows that the neural centers mediating the SLR can be pre-set by higher-order areas establishing task set, prior to target emergence. There are numerous other dimensions in which the task could be modified to manipulate cognitive factors, for example by altering the predictability of target appearance in either time (i.e., making the timing of emergence less predictable) or space (i.e., biasing target emergence to one side or another, or providing endogenous cues to indicate the side of emergence). Manipulations of the sensory parameters of the emerging target (e.g., the speed, contrast, size, or color of the emerging stimulus, or the presence of competing distractors) will also provide insights into underlying substrates. Presenting a static rather than moving target below the barrier would also help parse the effects of target motion versus temporal predictability on the robustness of the limb SLR. Finally, from a motor perspective, the framework of the emerging target paradigm can be extended to bilateral reaching movements and establishing the presence of robust SLRs on upper limb muscles potentiates the investigation the distribution of such signals to other trunk or limb muscles.

One of the limitations associated with this paradigm, perhaps paradoxically, is the degree to which reach RTs were shortened. Our SLR detection criteria resembled that used previously¹², as we ran separate time-series ROC analyses for the shorter- or longer-than median RT groups. Doing so requires some degree of variance in reach RTs, and in practice we have found that reach RTs are shorter and less variable in the emerging target paradigm compared to the static paradigm (279 +/- 58 ms (static); 207 +/- 34 ms (emerging target)). Indeed, RTs were sometimes shortened to such a degree that the movement-related volley of EMG activity often blended into the SLR interval. Consequently, the time-series ROC often rose directly from values near 0.5 to values near 1.0, without displaying the brief decrease after the SLR that was required for detection in ref.⁸ (see **Figure 4**, participant 1,2,4,5). More importantly, the smaller RT variance is detrimental to the detection of slope (**Figure 1c**); whereby a lack of variability in RTs may lead to lower levels of detectable SLRs. We expect that the detection criteria for SLRs may continue to evolve and will likely have to be optimized to the specifics of the task at hand. Other task manipulations, perhaps by increasing the temporal uncertainty of target re-emergence or requiring that subjects wait to move for a short interval after target emergence (e.g., by waiting for the emerged target to change color), may help increase the mean and variance of reach RTs and separate recruitment during the SLR interval from that associated with movement onset. A second limitation, which has not been explored, may be that some participants may not exhibit an SLR in the emerging target paradigm. We recognize that our sample is small and future studies should employ the emerging target paradigm on larger populations.

In closing, the emerging target paradigm offers a more reliable technique for eliciting the SLR, when compared to paradigms using static targets. The framework of the emerging target paradigm will advance the study of rapid visuomotor responses, by providing a means to obtain

robust expression of upper limb SLRs. It is particularly noteworthy that all of the results reported here were obtained with surface recordings, as this will enable study of SLRs in populations that may be less amenable to intramuscular recording, like the young, the elderly, or the infirm. We also expect that the emerging target paradigm could be extended into animal studies in non-human primates and combined with neurophysiological techniques to explore potential neural substrates. Together with future work in humans that can rapidly explore the numerous sensory, cognitive, and motor dimensions of the task, the emerging target paradigm should potentiate hypothesis-driven explorations of the fast visuomotor system.

ACKNOWLEDGMENTS:

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DISCLOSURES:

The authors have nothing to disclose

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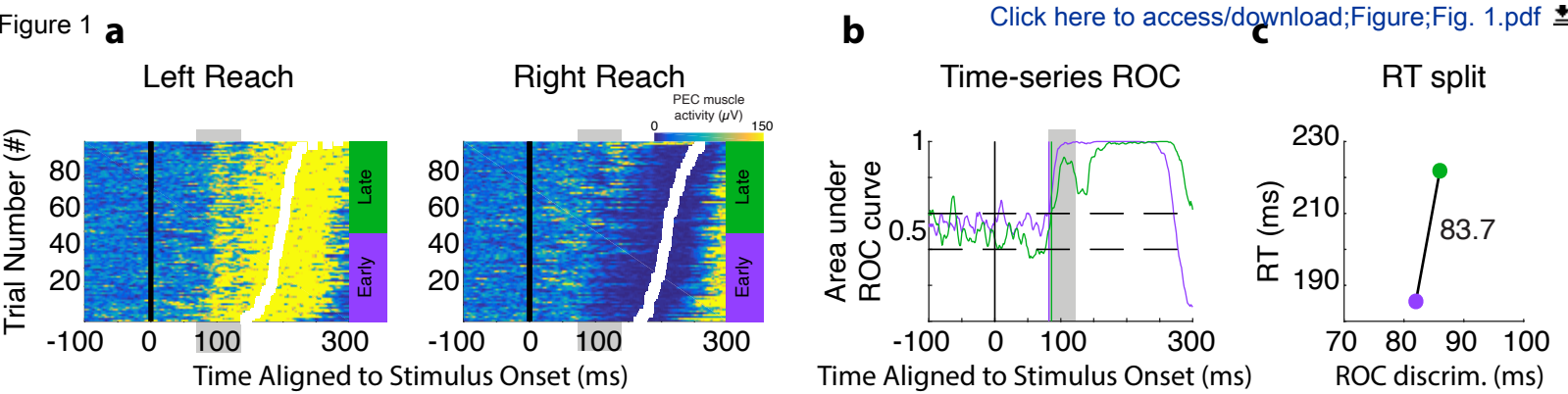


Figure 1-Kozak et al. 2020

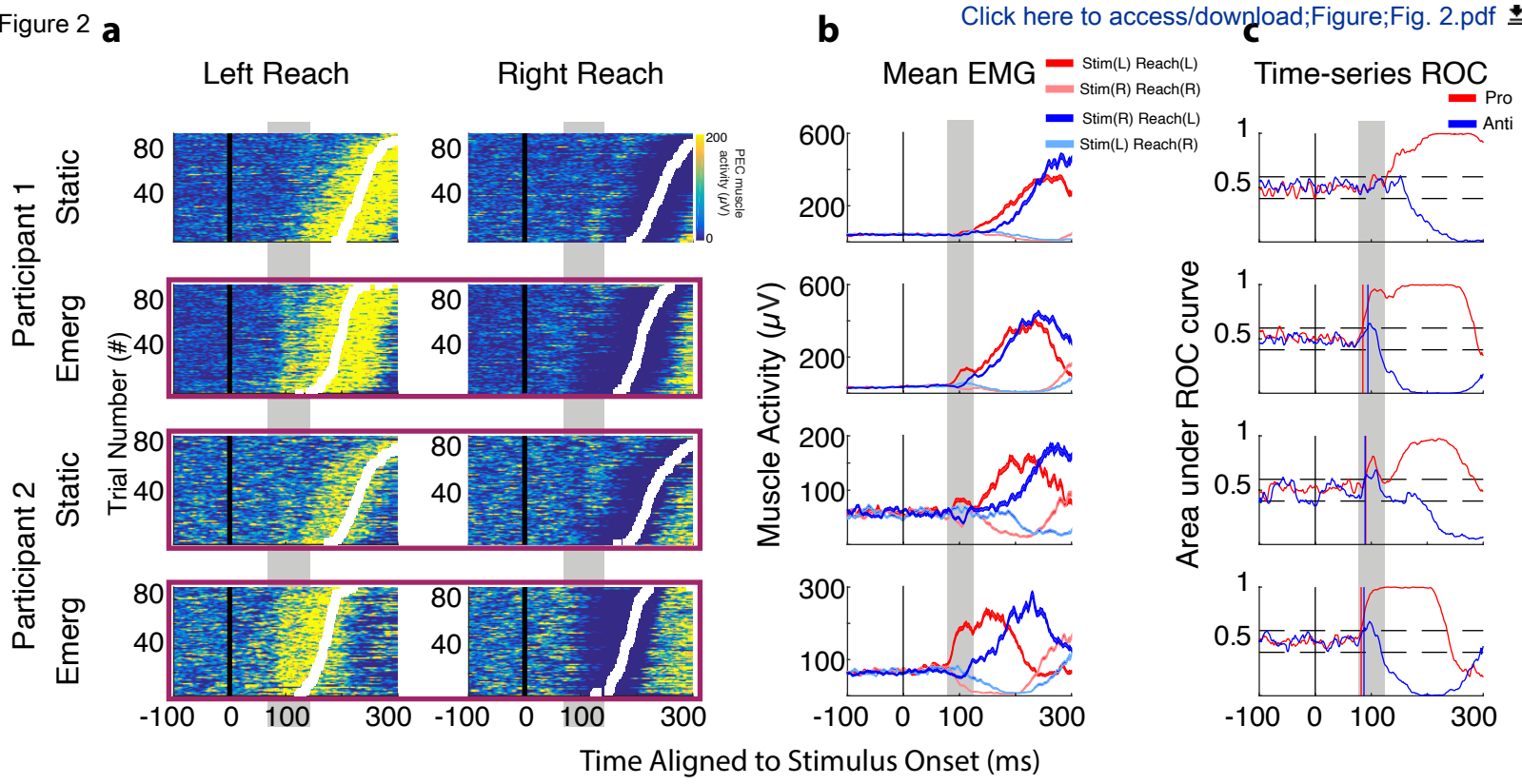


Figure 2-Kozak et al. 2020

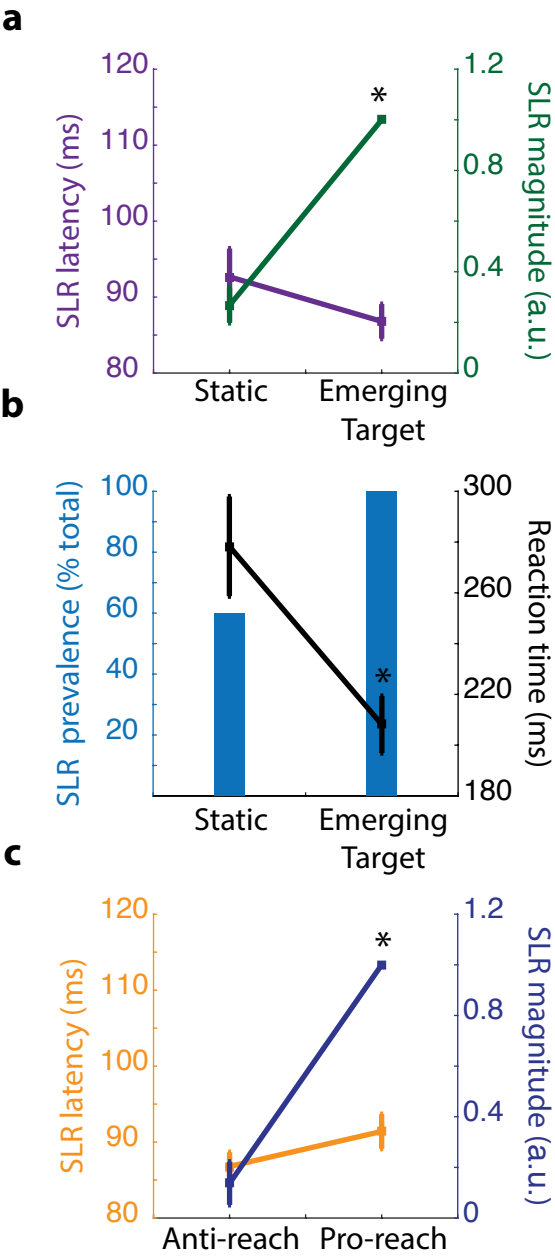


Figure 3-Kozak et al. 2020

Figure 4

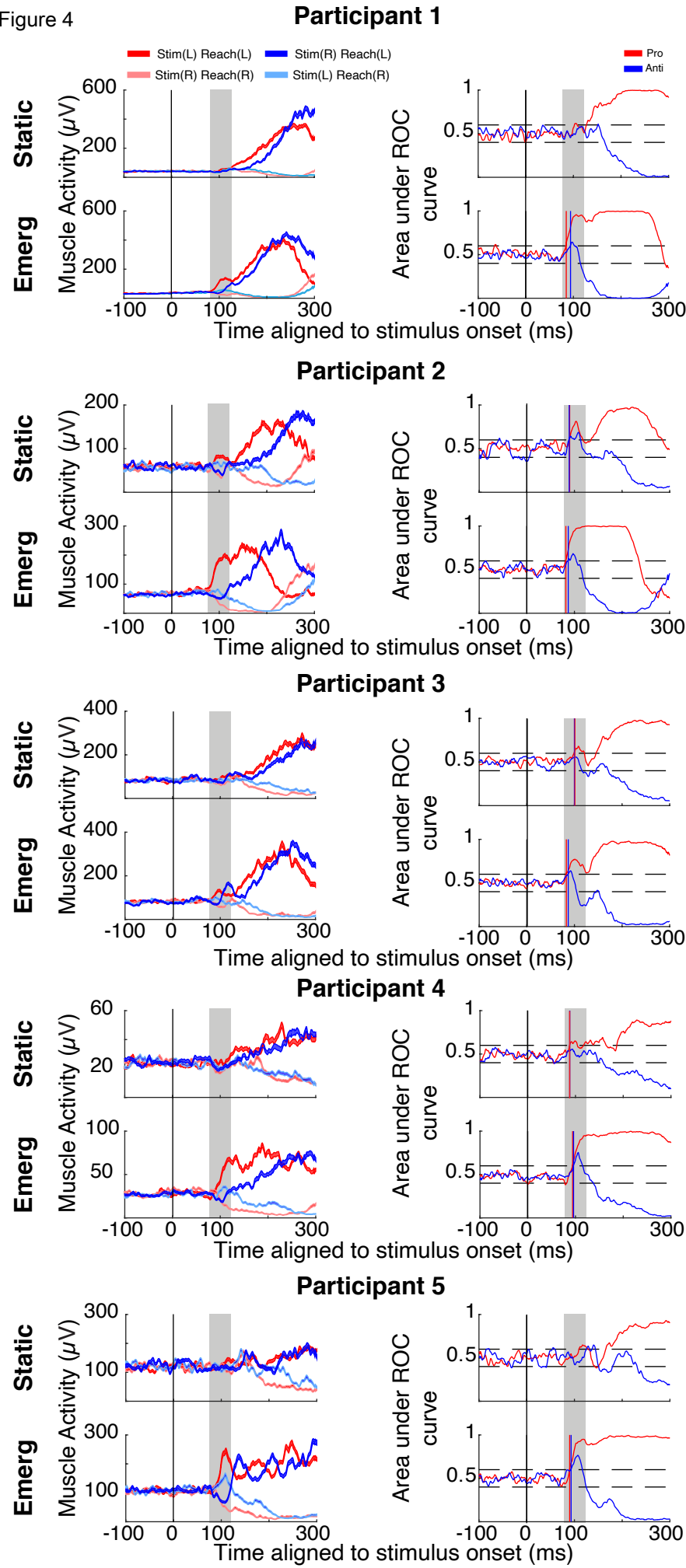


Figure 4-Kozak et al. 2020

Name of Material/ Equipment

Bagnoli-8 Desktop Surface EMG System
Kinarm End-Point Robot

MATLAB (version R2016a) Stateflow and Simulink applications

PROPixx projector

| Company | Catalog Number | Comments/Description |
|--|-------------------|--|
| Delsys Inc. Kinarm, Kingston, Ontario, Canada The MathWorks, Inc., Natick, Massachusetts, United States | | Another reaching apparatus may be used Another reaching apparatus may be used |
| VPIXX Saint-Bruno, QC, Canada | | This is a custom built addon for the Kinarm. Other displays may be used. Resolution: 1920 x 1080. Standard viewing monitors may also be used. |

Dear Dr. Bajaj

Thank you for your editorial review of JoVE61428R1, now titled “An Emerging Target Paradigm to Evoke Fast Visuomotor Responses on Human Upper Limb Muscles”. In the attached manuscript 61428_R2, we have used tracked changes to identify all of the edits. Here, we provide a point-by-point response, referring to the line changes. Our response is in bold, and the original comment is included in plain text if we felt this would help for the clarity of our response.

Please let us know if you have any questions on any of these points; I would also be happy to speak over the phone if needed.

With best regards,
Brian

Editorial comments:

1. We have reworded the title.

2. We now provide 7 distinct keywords or phrases.

3. We need a minimum of 1 page of highlight and a max of 2.75 pages including headings and spacings. I have adjusted the highlight to make it a cohesive story. Please check and approve.

Notes cannot be filmed so I have removed the highlight throughout. Please convert to action steps if this needs filming.

All the steps must be presented in imperative tense.

Please ensure that you answer the how question, how is this step performed.

We have checked and approved the highlighted text, and understand that notes cannot be filmed. We have also presented the steps in imperative tense, and endeavoured to answer the “how” question wherever possible throughout the protocol.

4. We now specify that the ground electrode is placed on the clavicle contralateral to the reaching arm. See Line 136-137.

5. As per the manufacturer’s recommendation? In between 1.1.4 and 1.1.5 please include a step to show that EMG data were acquired and include how this was done.

Please see the text on lines 138, and from 140-142. EMG data collection is standard across many different manufacturer platforms.

6. Will it be possible to include some EMG data?

Single trial EMG data is represented in Fig. 1. The novelty in the manuscript lies in the behavioral paradigm, hence showing raw EMG data at this point would not be beneficial. Further, the detection of SLRs depends on the analysis conducted after a full dataset is collected, as described in sections 2.1 and 4. If it helps, EMG data should be visualized during the video shoot on the desktop monitor.

7. We acknowledge that notes cannot be filmed.

8. The experiment is run on one participant at a time. See line 159.

Comments 9-11. How is this done? For all substeps under 2.1.1. How is this done? – manually? Through button clicks in the software? Scripts? Please include all mechanical actions, button clicks, command lines etc. if using scripts please include as supplementary files and reference here.

The components of the emerging target paradigm are designed in software. We struggled with this since there seems to be a contradiction between previous instructions not to specify particular equipment, and the request here to specify scripts that will only make sense with such specific equipment. We emphasize here and elsewhere that the novelty of the manuscript lies in the nature of the emerging target paradigm, which can be incorporated into any platform. Thus, rather than providing mechanical actions, button clicks, command lines, or scripts, we instead have described the manual construction of each of the four essential components of the task. See lines 185-233.

12. The real-time cursor (RTC) is generated in software, as noted in lines 242-244.

13. We have endeavoured to write all steps in imperative tense.

14. Please see line 248 for how to start the experiment.

15. The instructions are provided to the participant verbally. Line 260

16. How does this happen?

This is referring to what provides the signal to start the movement of T1. Please see lines 265, and 270.

17. Please see lines 275-276.

Comments 18 and 19. Please use imperative tense and describe how individual step is happening. Do you perform any button clicks in the associated software? Is this happening automatically? Do you set up any program? Please include all specific details. We can only film action steps in imperative tense. How is this done? Do you press some buttons to do this? Is it performed automatically?

This step is now presented in imperative tense. Target movement is described in section 2.1.1.4 (see lines 228-233).

20. Now written in imperative tense. See lines 296-297.

21. Is this done manually, or is any button click required?

Section 2 is now modified to state that the trial proceeds automatically.

22. We now specify the inter-trial interval on line 321-323.

23. One lines 341-367, we very briefly describe the steps needed to construct a paradigm that does not feature the emerging target components (which we describe as our “control static paradigm”). We opted to leave this text in, as this control paradigm provides the comparator for the results from the emerging target paradigm. We could take it out if necessary, but have put the steps in imperative tense.

24. We have endeavored to describe how each step is performed.

25. We now provide the method for calculating reaction time (lines 374-375).

26. Regarding offline scripts, the analytical steps are not novel, and we were instructed previously not to reference Matlab. We have explained the steps in generic terms that can be implemented easily in any analysis language.

27. We have deleted the reference to Fig 4 on line 460, so that Fig 4 is now referenced after Fig. 3

28. The figure legend for Fig 4 has been expanded.

