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A timeline of motor preparatory state prior to response initiation: Evidence from startle

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Abstract

Response preparation in simple reaction time (RT) tasks has been modelled as an increase in neural activation to a sub-threshold level, which is maintained until the go-signal. However, the amount of time required for response preparation following a warning signal (WS) is currently unclear, as experiments typically employ long foreperiods to ensure maximal preparation. The purpose of the present experiments was to examine the time course of motor preparation in a simple RT task when given a limited amount of time to engage in preparatory processing. In Experiment 1, participants completed wrist extension movements in a simple RT paradigm with a short (500ms) fixed foreperiod, and a long (8.5-10.5s) inter-trial interval. To probe response preparation, a startling acoustic stimulus (SAS), which involuntarily triggers the release of sufficiently prepared responses, was randomly presented during the foreperiod at one of six equally spaced time points between 0 and 500 ms prior to the go-signal. Results showed that the long inter-trial interval was not always effective at preventing participants from engaging in preparatory processing between trials; thus, in Experiment 2 participants performed wrist flexion or extension movements in an instructed delay paradigm, where the required movement was cued by the WS. Results showed that the proportion of startle trials where the intended response was elicited by the SAS at short latency significantly increased until 100 ms prior to the go-signal, indicating response preparation can take up to 300-400 ms following the WS in a simple RT task with a short fixed foreperiod.

Keywords: reaction time, motor preparation, startle, StartReact, electromyography

Abbreviations: ECR: extensor carpi radialis, EMG: electromyography, IS: imperative stimulus, ITI: inter-trial interval, MEP: motor evoked potential, RM ANOVA: repeated measures analysis of variance, RT: reaction time, SAS: startling acoustic stimulus, SCM: sternocleidomastoid, TMS: transcranial magnetic stimulation, WS: warning signal.

Introduction

In a simple reaction time (RT) task the response to be made is known in advance of the imperative stimulus (IS), and RT is consistently found to be significantly shorter in comparison to choice RT tasks where the required response is unknown until the IS (Donders, 1969). It has been proposed that these RT benefits are the result of advance knowledge of the required response, which allows for planning, or "programming," of the muscle commands to be completed prior to the IS (Keele and Posner, 1968). This advance preparation seen in simple RT tasks can be explained using a neural accumulator model of movement preparation (Hanes and Schall, 1996). In this model, preparation involves increasing activation within a network of neurons to a level below movement threshold, while

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response initiation involves the input of additional activation to surpass this threshold. In a simple RT task, individuals engage in preparation processes during the foreperiod, after which the prepared response is held at a high level of readiness/activation until the IS occurs, at which point additional cortical input provides sufficient activation to surpass movement threshold, resulting in movement execution (for a review see Carlsen et al., 2012).

The use of neuroimaging and neurostimulation techniques has allowed researchers to more closely examine preparation processes occurring during the foreperiod of instructed delay and simple RT tasks, including the time course of motor preparation. For example, studies employing electroencephalography in movement tasks have characterized waveforms thought to represent an increase in cortical activity related to progressive movement preparation (Walter et al., 1964; Deecke et al., 1976). One of these, the Contingent Negative Variation, appears following a warning cue and is characterized by a slowly increasing neural activity wave (Walter et al., 1964), with a later component that can begin as early as 1.5 s prior to movement onset that is thought to represent a release of response inhibition (MacKinnon et al., 2013). Similarly, studies using functional magnetic resonance imaging have also shown that preparation-related neural activity can begin to increase as early as 1 s prior to presentation of the anticipated IS (Thickbroom et al., 2000). Transcranial magnetic stimulation (TMS) has been used to provide an index of corticospinal excitability during motor preparation by measuring the size of the motor evoked potential (MEP) produced in response to a single TMS pulse. Application of TMS during a 1.5 s foreperiod in a simple RT task showed that the size of the MEP in the agonist increased for the first 500 ms following the warning signal (WS), before leveling off until presentation of the IS (Kennefick et al., 2014). The increase in MEP size during the foreperiod was attributed to an increase in excitability of the corticospinal tract as a result of progressive preparation of the upcoming movement.

While these studies suggest 500 to 1500 ms may be required to increase neural activation to a level sufficient for the response to be executed quickly following the IS, more recent studies have suggested a much shorter timeline for response preparation is possible. For example, Haith et al. (2016) investigated the independence of motor preparation and initiation processes by estimating the time required for motor preparation and the time of movement initiation in two different RT conditions. In their free RT condition, which resembled a choice RT task, mean RT across all participants was 212 ms. In the forced RT condition, participants were required to initiate the movement at a specified time, with the correct target provided at varying times before the required initiation. In this condition, participants were able to initiate the response at much shorter latency than in the free RT condition, with results indicating that participants only needed about 130 ms to prepare the correct response. While these data contrast previous estimates of 500-1500 ms required for response preparation when provided sufficient time, the much shorter response preparation time of 130 ms reported by Haith et al. (2016) is indicative of the minimal time required for response preparation. However, the short latency responses reported by Haith et al. (2016) included many trials with movement errors, indicating that at least some additional preparation time beyond the reported 130 ms may be required to ensure the correct response is initiated.

Another technique that can be used to study advance preparation is the use of a startling acoustic stimulus (SAS). Presentation of a SAS concurrent with the IS in a simple RT task leads to significant reductions in RT, while still resulting in a similar movement in terms of EMG profile and kinematics - a phenomenon that has been termed the StartReact effect (Valls-Solé et al., 1999; Carlsen et al., 2004b). In studies where a SAS was presented when the response was not known in advance (e.g. a choice RT task), the StartReact effect was not observed, which suggests that a SAS only leads to the involuntary triggering of prepared responses (Carlsen et al., 2004a; Carlsen et al., 2008). As such, a SAS provides researchers a method of investigating the conditions where pre-programming occurs, as well as a timeline of response preparation (see

Valls-Solé et al., 2008 for reviews; Carlsen et al., 2012; Marinovic and Tresilian, 2016).

Here we describe two experiments that were conducted to examine the average state of motor preparation at various times following a warning cue in RT tasks with a short fixed foreperiod. More specifically, we investigated the amount of time required to sufficiently prepare a motor response, such that it would be involuntarily triggered at short latency by a SAS. This was accomplished by presenting the SAS at various time points following the WS. In Experiment 1, participants performed a targeted wrist extension in response to a visual go-signal in a simple RT paradigm with a long and variable inter-trial interval (ITI) and a short (500 ms) fixed foreperiod. While previous studies have employed a long foreperiod to ensure maximal time for preparatory processes, a 500 ms fixed foreperiod can be accurately estimated by participants (Hasbroucq et al., 1999), and leads to maximum effectiveness of preparatory processes (Bertelson, 1967; Touge et al., 1998). Indeed, previous research has suggested that 300 - 500 ms represents the required foreperiod duration for optimizing preparatory processing (Bertelson, 1967; Alegria, 1974). Thus, a 500 ms foreperiod was used to allow an investigation of how rapidly responses are prepared in a simple RT paradigm when given limited time to engage in preparatory processing. To discourage participants from maintaining a continuous state of response specific preparation, a long ITI was used as motor preparation is typically only maintained for brief periods of time, and longer time periods reduce the accuracy of temporal predictions (Hasbroucq et al., 1997; Ulrich et al., 1998).

In Experiment 1 it was expected that participants would be forced to engage in rapid response preparation, beginning from a low baseline level of response specific activation, allowing for a determination of the time required for a sufficient preparatory state to be reached. It was hypothesized that once the movement was sufficiently prepared it would be triggered at a short latency by the SAS, and that the proportion of StartReact responses elicited would dramatically increase as the SAS was presented later in the foreperiod, providing insight into the time course of motor preparation. It was predicted that the time required for response preparation would be longer than that observed by Haith et al. (2016), as participants would be unlikely to engage in any form of advance preparation prior to the WS. In addition, longer RTs would be expected as only responses that were fully prepared would be triggered by the SAS at short latency, whereas the errors seen in trials with the fastest RTs in the work of Haith et al. (2016) suggest partial or incomplete preparation. However, in the present paradigm the short foreperiod was expected to encourage participants to prepare the response as quickly as possible, providing novel insight into the minimum time required to increase neural activation to a level sufficient for the response to be triggered at short latency by the SAS. A second Experiment was conducted to explore some of the limitations of Experiment 1 (see below) and provide further insight into the timeline of the motor preparatory state prior to response initiation.

Experiment 1 Experimental Procedures

Participants. Seventeen adults with normal or corrected-to-normal vision participated in Experiment 1. Six participants did not exhibit a consistent startle reflex in response to a SAS presented with the go-signal (see data reduction and analysis for inclusion criteria details); thus, their data were excluded from the primary data analysis, resulting in a final sample size of 11 participants (5 M, 6 F; M_{age} = 28, SD = 11). All participants provided written informed consent prior to participating. The experiment was conducted in accordance with the ethical guidelines of the Health Science Research Ethics Board at the University of Ottawa, and conformed to the latest version of the Declaration of Helsinki.

Experimental apparatus and task. Participants were seated approximately 1.5 m from a 24-inch LCD computer screen (Asus VG248; 144 Hz refresh) with the right forearm placed in a custom-made manipulandum. The arm was placed such that the shoulder was abducted approximately 30° and the elbow was flexed approximately 90°, resulting in the forearm resting parallel to the floor

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with the palm facing inward. The forearm was fixed in this position using Velcro straps placed just distal to the elbow joint and just proximal to the wrist joint, allowing movement solely around the wrist joint. Participants began with their wrist resting in a relaxed position at 20° of flexion, and were required to perform a 20° targeted wrist extension in response to a visual go-signal. The goal of the task was to react to the go-signal as quickly and as accurately as possible. To encourage participants to react quickly a points system rewarded participants for fast RTs (RT < 280 ms) and penalized them for slow RTs (RT > 350 ms). These points were not analyzed, and did not correspond to any real-world value following the experiment as they were only provided to increase motivation to prepare and respond quickly.

Procedure. Each trial began with a blank gray screen being displayed for 8.5 – 10.5 s (representing the ITI), followed by a visual WS consisting of a 3mm outline of a 6.3 cm black square appearing in the middle of the computer screen. The WS remained on the screen for the duration of the 500 ms fixed foreperiod, at which point the visual IS occurred, consisting of the square being filled in bright green. Participants were instructed to respond as quickly and accurately as possible to this visual IS. A 500 ms fixed foreperiod was chosen to maintain consistent time intervals between the WS and SAS, and between the SAS and visual IS, as well as to limit the use of different timing strategies across participants. In addition, a 500 ms foreperiod was used to ensure participants had sufficient time to complete preparation prior to the visual IS on all trials, while still being short enough to require preparatory processing to be initiated shortly following the WS. After completion of the movement, feedback including RT, movement accuracy (degrees over/under shoot relative to the target), and the points earned/lost for the preceding trial were displayed for 3000 ms until the beginning of the next trial. Trial events and stimuli were hardware timed using data acquisition hardware and customized LabVIEW software (National Instruments Inc.).

Participants began the experiment by completing one block of 12 practice trials to become familiar with the task. This block of practice trials was identical to the experimental trials, with the exception that a SAS was never presented. The experimental session consisted of 150 trials, sub-divided into five blocks of 30 trials. In 20% of trials (six per block) a SAS (120 dB, 25 ms, white noise waveform) was randomly presented at one of six time points: concurrent with the IS (ST-0), 100 ms prior to the IS (ST-100), 200 ms prior to the IS (ST-200), 300 ms prior to the IS (ST-300), 400 ms prior to the IS (ST-400) or concurrent with the WS (ST-500). Participants were told that they may hear a loud sound, but that it was irrelevant to the task and to maintain focus on the visual stimulus. The SAS was generated using digital to analog hardware (National Instruments PCIe-6321), and amplified and presented by a loudspeaker (MG Electronics M58-H, frequency response 300 Hz - 11 kHz, rise time <1 ms) located 30 cm behind the participant at ear level. Two trials in each block of 30 (6.67%) were designated as catch trials, where the WS was presented without the presentation of the visual IS. Participants were told not to respond in these trials, and these were included to prevent participants from anticipating the IS rather than reacting to it (Klemmer, 1956). Participants only responded on a total of 11% (12/110) of catch trials, indicating that participants were reacting to the onset of the visual IS rather than timing their response to the fixed foreperiod. Trials were presented pseudo-randomly, such that a SAS was never presented on the first two trials of a block, a SAS was never presented on two trials in a row, and one SAS trial at each time point was presented in each block.

Upon completing the experimental trials, participants completed a practice and experimental block of a more commonly employed version of a simple RT task (i.e., short ITI with a longer variable foreperiod). Trials in this traditional simple RT block involved the same motor task as the previous blocks, except that the ITI was 3.5 s, and a variable foreperiod of 2 – 2.5 s spanned the warning and go-signals. Participants performed a practice block consisting of 10 trials without a SAS, followed by one testing block of 25 trials including 18 control trials, 5 SAS trials (where the SAS was presented concurrent with the visual IS), and 2 catch trials (no IS). This block of trials was included as it was thought that many participants would not exhibit consistent startle responses in the paradigm employed in this experiment, which aimed to have participants begin each trial with a low level of preparatory activation. Furthermore, in the present experiment startle and StartReact were used to assess preparation; however, under typical conditions (e.g. quiet sitting) the startle reflex habituates quickly, but is strongly potentiated by concurrent motor preparation (Carlsen et al., 2003). As such, it was expected that some participants may exhibit StartReact responses less often than normal due to the paradigm used, particularly at the earliest SAS presentation times. Thus, these trials were included to distinguish if a low probability of observing a consistent startle reflex was due to: 1) a low level of motor preparation in the task (Carlsen et al. 2011), or 2) an inherently low responsiveness to startle (as observed in approximately 10-20% of the population; Abel et al. 1998). The proportion of startle reflex responses seen in these trials was then used as part of the inclusion criteria for determining which participants were sufficiently responsive to startle to be included in the final data analysis (see *Data Reduction and Analysis* below).

Recording equipment. Surface EMG was collected from the superficial muscle bellies of the right extensor carpi radialis (ECR), right flexor carpi radialis, and left sternocleidomastoid (SCM). EMG was collected using bipolar preamplified (gain = 10) surface electrodes (Delsys Bagnoli DE-2.1) connected via shielded cabling to an external amplifier (Delsys Bagnoli-8). The electrodes were attached to the skin with double-sided adhesive tape and oriented such that the separation between sensors was parallel to the muscle fibers. A grounding electrode (Dermatrode HE-R) was also placed on the right lateral epicondyle. In order to minimize electrical impedance, all four electrode sites were cleaned with abrasive skin prepping gel and alcohol wipes prior to the attachment of electrodes. Angular displacement data for the wrist was collected via a potentiometer attached to the axis of rotation of the custom manipulandum. Raw band-passed (20 - 450 Hz) EMG and raw potentiometer data were digitally sampled at 4000 Hz (National Instruments PCIe-6321) using a customized LabVIEW program and stored for offline analysis. Data collection was initiated by the computer for each trial 1000 ms prior to presentation of the imperative stimulus and continued for 3000 ms.

Data reduction and analysis. SAS trials where there was no detectable SCM activation, defined as a burst in SCM EMG within 50-120 ms of the SAS, were discarded, as this is considered to be a robust and reliable indicator of a startle reflex that is sufficient to elicit a StartReact response (Carlsen et al., 2011b). Participants who failed to show SCM activation on at least 60% of SAS trials in either of the conditions where the SAS was presented concurrently with the go-signal (i.e., either the ST-0 condition or during the traditional simple RT SAS trials), were excluded from the primary analysis. As stated in the Participants section, this resulted in the exclusion of 6 of the original 17 participants from analysis as they only exhibited SCM activation in an average of 21% (range 9 – 40%) of these SAS trials. In the remaining participants, SCM activation was observed on a total of 202/330 SAS trials, resulting in a total SAS trial inclusion rate of 61%. Trials where participants exhibited evidence of anticipation (RT < participant condition mean – 2.5 SD, or RT < 50 ms; 68 trials) were also discarded. In addition, trials with particularly slow RTs (due to inattentiveness) were excluded from analysis. Specifically, in the control and ST-0 conditions inattentiveness was defined as RTs > participant condition mean + 2.5 SD. For trials where the SAS was presented prior to the go-signal trials were discarded due to inattentiveness if RT was 2.5 SD above each participant's control mean, as participants may have responded to either the SAS or the control IS. This resulted in the exclusion of 32 trials due to inattentiveness. Across all participants, 12 trials were discarded due to participants responding on a catch trial. Finally, 11 trials were excluded due to movement errors such as not responding, moving too slowly or performing multiple extension/flexion movements. This resulted in an overall trial inclusion rate of 84% (1668/1975), with excluded trials distributed evenly across participants (max = 34, min = 7). Note that participants with the highest error rates were those who did not exhibit SCM activation on a large proportion of SAS trials, resulting in high numbers of 'No SCM' errors.

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EMG burst onset for all muscles was defined as the point where rectified and filtered (25 Hz lowpass elliptic filter) EMG activity surpassed two standard deviations above baseline level, defined as the mean EMG in a 100 ms window preceding the WS, and remained elevated for a minimum of 20 ms. Premotor RT was defined as the time between presentation of the IS (or SAS on trials where the SAS preceded the IS) and the initial EMG onset in the right ECR. As such, premotor RT relative to the SAS on trials where participants exhibited a startle response were considered to be involuntarily triggered, whereas premotor RT relative to the go-signal in control trials were considered to be voluntary responses. EMG burst offset was defined as the point where EMG activity fell below 20% of the maximum amplitude of that EMG burst, with time between EMG onset and offset considered burst duration. EMG traces were displayed on a computer monitor with EMG onset and offset markers computed using a customized LabVIEW algorithm. The EMG markers were then manually adjusted to correct for any possible errors due to the strictness of the algorithm (Hodges and Bui, 1996). Peak EMG amplitude was defined as the greatest amplitude occurring within 100 ms of EMG burst onset, and peak displacement was defined as the greatest movement amplitude reached throughout the movement.

Finally, StartReact responses were defined based on each individual's mean premotor RT in the ST-0 condition. SAS trials in each startle condition where an SCM burst was seen, and premotor RT with respect to the SAS was within 2.5 SD of each individual's mean in the ST-0 condition were classified as a StartReact response. Thus, the proportion of StartReact responses elicited in each condition represents the proportion of SAS trials where an SCM burst was elicited *and* participants exhibited early response triggering. The point at which there was no further significant increase in the proportion of StartReact responses was used to indicate the point where preparation was completed on most trials, and this time was used as the estimate of time taken by participants for response preparation.

Statistical analyses. Due to the low number of startle trials in the present experiment, all statistical analyses, with the exception of proportion data, were performed on participant medians. Premotor RT, initial ECR burst duration, and initial ECR burst peak amplitude in control trials and startle trials where the SAS was presented with the go-signal were analyzed using separate 2 (Task: short ITI, long ITI) x 2 (Acoustic stimulus: none, SAS) repeated measures analysis of variance (RM ANOVA) to determine if StartReact responses were different between the two RT tasks used in the present experiment.

For the long ITI task, non-parametric analyses were used to compare proportion data due to the low number of trials per condition. As such, the proportion of SAS trials where an SCM response was elicited and the proportion of SAS trials where a StartReact response was elicited were analyzed using separate one-way, six factor (SAS time: 0, -100, -200, -300, -400, -500) Friedman's ANOVAs to determine if the time of SAS presentation had an effect on the incidence of eliciting a startle reflex or StartReact response. Premotor RT and peak displacement in StartReact trials were analyzed using separate one-way, six factor (SAS time: 0, -100, -200, -300, -400, -500) RM ANOVAs to determine if the time of SAS presentation affected the premotor RT or movement performance observed for StartReact responses. Finally, not all participants exhibited a startle reflex in all conditions, resulting in five missing cells (5/66) in the analyses of the proportion of StartReact responses, premotor RT and peak displacement. These missing values were filled using a linear-regression based multiple imputations procedure in SPSS (IBM Inc.), as discarding participants without a value for each measure reduces power, and using the group mean artificially reduces variability. The significance value for all statistical tests was set at $p < .05$, and where appropriate, partial eta squared (η^2_p) and r values are reported as measures of effect size. All significant differences were analyzed using either Tukey's Honestly Significant Difference (HSD) post-hoc tests (for parametric analyses) or Wilcoxon-Signed Rank tests with a Bonferroni correction (for non-parametric analyses) to determine the locus of any significant differences. All analyses were performed using the statistical software package SPSS 21 for Windows (IBM Inc., Armonk, NY, USA).

Experiment 1 Results

StartReact effect (SAS presented at Go). For the two different RT paradigms (long ITI versus traditional RT with short ITI), analysis of premotor RT between control trials and startle trials where the SAS was presented at the go-signal revealed a significant main effect of Acoustic stimulus, F(1,10) = 300.95, $p < .001$, $\eta^2_p = .968$, as well as a significant main effect of Task, F(1,10) = 5.161, $p = .046$, $\eta^2_p = .340$. However, these effects were superseded by a significant interaction between these factors, F(1,10) = 7.995, $p = .018$, $\eta^2_p = .444$. Post-hoc tests indicated that startle RTs in both the long (98.8 ms, SD = 23.8) and short (99.0 ms, SD = 16.9) ITI tasks were significantly shorter than control RTs (see Figure 1 A & B for representative individual data from the long ITI task for a control and startle trial, respectively), with no significant difference between the tasks for SAS trials. However, in the control conditions premotor RT was significantly shorter in the long ITI task (201.8 ms, SD = 23.8) than the short ITI task (225.5 ms, SD = 32.9), potentially owing to the experimental differences between the two tasks or participant fatigue at the end of the experiment. Analysis also revealed a significant main effect of Acoustic stimulus on initial ECR peak amplitude, F(1,10) = 5.435, p = .042, $\eta^2_p = .352$, indicating that initial ECR peak amplitude was significantly larger in startle (0.302 mV, SD = 0.152) than in control trials (0.268 mV, SD = 0.126). There was no significant main effect of Task (p = .835), or any interaction between the factors (p = .431). Finally, there was no significant difference due to Task (p = .250) or Imperative stimulus (p = .875) for initial ECR burst duration. Taken together, these results indicate that similar StartReact responses were elicited during the simple RT paradigm irrespective of ITI length, despite differences in control premotor RT.

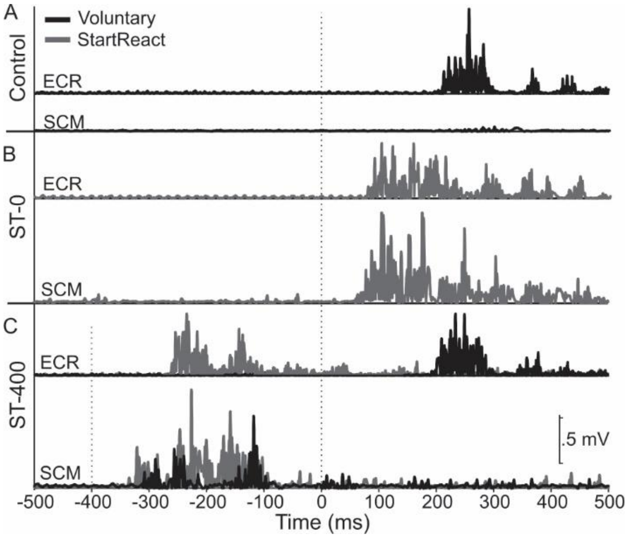

Fig. 1. *Rectified EMG traces of a typical participant in: A) the control (non-startle) condition, B) the ST-0 condition, where the SAS was presented with the go-signal, C) the ST-400 condition, where the SAS was presented 400 ms prior to the go-signal. Black traces represent voluntary responses to the go-signal, and grey traces represent StartReact responses to the SAS. ECR = extensor carpii radialis; SCM = sternocleidomastoid.*

Proportion of SAS trials resulting in startle reflexes and StartReact responses. There were no significant differences between SAS presentation times in the proportion of SAS trials where a startle reflex was elicited, χ^2_F (5) = 5.232, p = .388. In the ST-500, ST-400, ST-300, ST-200, ST-100 and ST-0 conditions the proportion of SAS trials where a startle related burst of activity in SCM occurred was .51 (SD = .34), .55 (SD = .37) .69 (SD = .30), .98 (SD = .34), .71 (SD = .35), and .67 (SD = .30) respectively. In contrast, the percentage of SAS trials in each condition where a *StartReact* response was elicited showed a main effect of SAS presentation time, χ^2_F (5) = 18.920, p = .002, (Figure 2). Post-hoc Wilcoxon Signed Ranks tests (using a Bonferroni correction resulting in an adjusted alpha of .003) revealed that there was a significantly greater proportion of StartReact responses in both the ST-0 condition (T = 0, z = -2.809, p = .002, r = -.847) and in the ST-100 condition (T = 0, z = -2.941, p = .001, r = -.887), compared to the ST-500 condition. There was also a trend

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towards a larger proportion of StartReact responses in the ST-300 condition than in the ST-500 condition (T = 1, z = -2.722, p = .004, z = .821). Finally, there was also a trend towards a significantly greater proportion of StartReact responses in the ST-100 condition than in the ST-200 condition, but this did not meet the adjusted significance level (T = 4.5, z = -2.538, p = .009, r = -.765). Overall, these results indicate that while there was no difference in the proportion of startle reflexes elicited across SAS presentation time, as the time of SAS presentation approached the presentation of the IS, the likelihood of eliciting a StartReact response increased.

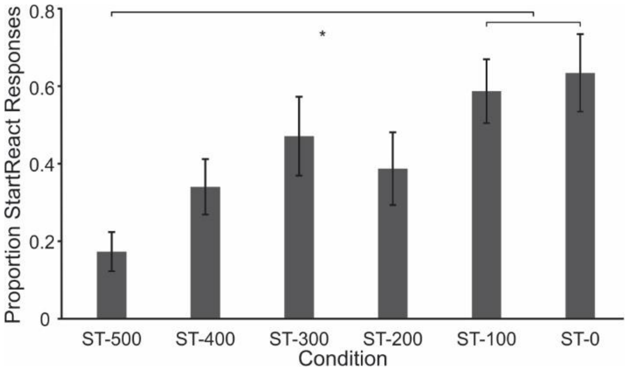

Fig. 2. *Mean (SE) proportion of StartReact responses elicited across participants in each condition where a SAS was presented in Experiment 1. Conditions represent the SAS being presented at time intervals (-500 ms, -400 ms, -300 ms, -200 ms, -100 ms, 0 ms) prior to, or concurrent with, the go-signal. Asterisks (*) denote significant (p < .05) differences.*

Premotor RT. The results of the analysis of the proportion of StartReact responses (Figure 2) confirmed an increased likelihood of short latency response triggering with later SAS presentation times, indicative of increasing motor preparation as the foreperiod progressed. In order to evaluate the premotor RT data, it was necessary to determine if the participant was involuntarily responding at short latency in response to the SAS, or voluntarily responding later to the visual go-signal (as instructed). For example, Figure 1C displays representative individual data showing a StartReact response in the ST-400 condition (grey line; note the presence of SCM and ECR activity shortly following the SAS), as well as a trial from the ST-400 condition where there was an early SCM burst in response to the SAS but a later voluntary response was made to the IS (black line).

To determine if these data represented bimodal distributions, a continuous non-linear regression was performed on premotor RT for each condition where the SAS was presented prior to the go-signal (see Frankland & Zumbo 2002 for details). Figure 3A displays a histogram of the proportion of premotor RTs in 50 ms bins for each of the experimental conditions. Analysis revealed a significant bimodal distribution in the ST-100 (F(6,8) = 7.835, *p* = .0052, R^2 = .779), ST-200 (F(6,14) = 4.196, *p* = .0127, R^2 = .54), ST-300 (F(6,16) = 3.44, *p* = .0224, R^2 = .294), ST-400 (F(6,19) = 6.37, *p* = .001, R^2 = .535) and ST-500 (F(6,24) = 10.376, *p* < .001, R^2 = .614) conditions. These results indicate that the presentation of a SAS during the foreperiod resulted in two distinct response patterns: early responses initiated to the SAS, and late responses initiated to the IS. To further characterize these distributions, involuntary RTs were defined as StartReact responses elicited by presentation of the SAS, while voluntary RTs were defined as RTs greater than 50 ms following the IS (see data reduction and analysis section above). Figure 3B displays the mean premotor RT for the involuntary (i.e., SAS RT trials) and in each condition. Analysis of premotor RT for SAS trials where a StartReact response was elicited (involuntary) revealed a significant effect of the time of SAS presentation on premotor RT, F(5, 50) = 4.156, p = .003, η^2_p = .294. Post-hoc analysis indicated that RT was significantly shorter in the ST-0 condition than in the ST-400 and ST-200 conditions. There was also a significant linear trend for time, F(1,10) = 12.594. p = .005, η^2_p = .557, indicating that the response latency of the StartReact effect decreased as the SAS was presented later in the foreperiod.

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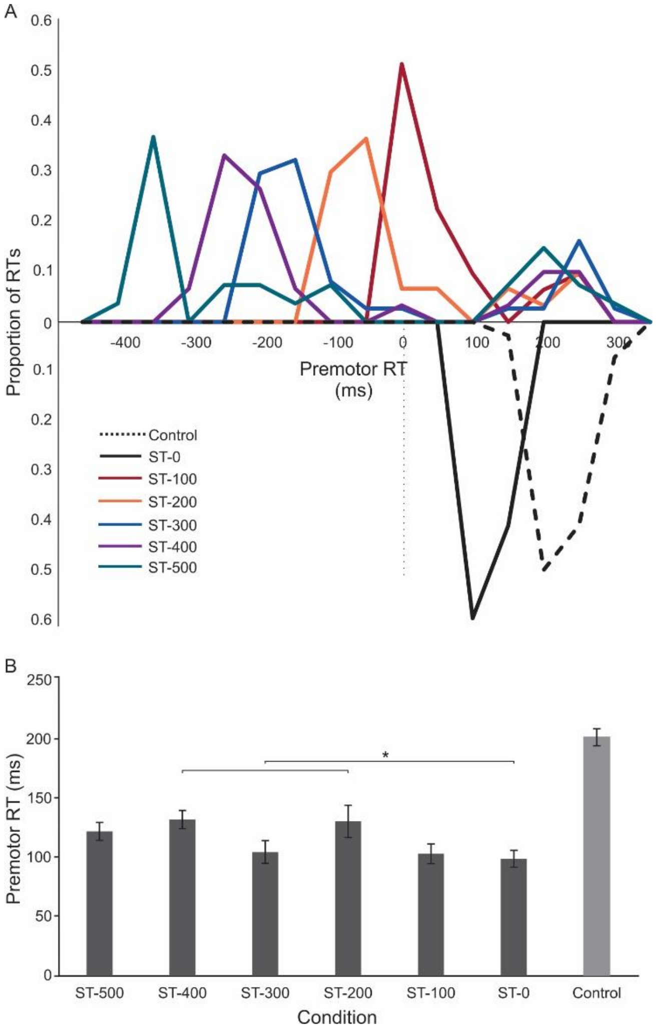

Fig. 3. *A) Histogram displaying proportion of premotor RTs falling in 50 ms bins for each experimental condition in Experiment 1. Conditions represent the SAS being presented at time intervals (-500 ms, -400 ms, -300 ms, -200 ms, -100 ms, 0 ms) prior to, or concurrent with, the go-signal. Traces for the control and ST-0 condition are shown downwards to enhance clarity and readability of the figure. B) Mean (SE) premotor reaction time (RT) observed across all experimental conditions in Experiment 1. Dark grey bars represent StartReact responses, and the light grey bar represents voluntary responses in the control condition. Asterisks (*) denote significant (p < .05) differences for SAS RT.*

Peak Displacement. Analysis revealed no effect of the time of SAS presentation on peak displacement (p = .082), and a linear trend analysis for time only approached conventional levels of statistical significance, F(1,10) = 4.155, p = .069, η^2_p = .294. Across participants the mean peak displacement was 29.6 (13.5), 29.7 (8.9), 31.7 (9.0), 32.2 (11.9), 35.6 (13.2), and 39.4 (10.4) in the ST-500, ST-400, ST-300, ST-200, ST-100 and ST-0 conditions, respectively. In control trials, mean peak displacement was 29.7 (6.6). While between-subject variability was reasonably high, mean within-subject standard deviation of peak displacement in the control and ST-0 conditions was 5.5 (1.9) and 7.1 (3.9), respectively.

Experiment 1 Discussion

The purpose of the present experiment was to examine the time course of the state of response preparation in a simple RT task with a short fixed foreperiod. In the present experiment, a long ITI and short fixed foreperiod were used, with the goal of having minimal preparatory activation when the WS was presented, then rapidly increasing activation during the foreperiod so that the response would be ready for execution upon presentation of the IS. A SAS was then presented at various time points throughout the short foreperiod to determine the point in time following a WS where a response was sufficiently prepared to be involuntarily triggered by the SAS (i.e., elicit a StartReact response). Results showed that StartReact responses were elicited at all SAS presentation times; however, as the SAS was presented later in the foreperiod the proportion of StartReact responses elicited increased up until 400 ms following the WS (100 ms prior to the IS, see Figure 2), after which there was no further increase in the proportion of StartReact responses elicited. These results suggest that in a simple RT task with a short (500 ms) foreperiod, the intended response is sometimes sufficiently prepared such that it can be elicited by a SAS after only 100 – 200 ms, but often response preparation took longer.

While the results of Experiment 1 suggest a possible timeline for response preparation with a short fixed foreperiod, the presence of StartReact responses seen in the ST-500 condition confounds the ability to make definitive conclusions based on the present data, as it is clear that most participants held elevated levels of response preparation during the long (8.5–10.5s) ITI on at least some trials. Indeed, seven out of eleven participants exhibited StartReact responses when the SAS was presented concurrent with the WS, resulting in an overall proportion of StartReact responses of 17% (range 0-50%). These results suggest that participants at times were able to either 1) maintain an elevated level of preparatory activation throughout the ITI, or 2) estimate the end of the ITI with reasonable accuracy and increased preparatory activation prior to the WS. It has been well established that as the length of a variable foreperiod increases, RT decreases due to increased probability of the IS occurring, a phenomenon termed the aging foreperiod effect (Niemi and Näätänen, 1981; Vallesi et al., 2007). In a similar way, participants may have begun to increase preparatory activation to a certain degree once sufficient time had passed in the ITI, as there would be an increasing likelihood of the WS (and subsequent short foreperiod) occurring as the ITI “aged.” Thus, while the results seen in Figure 2 suggest that it can take up to 400 ms for the motor response to become sufficiently prepared to be consistently elicited by a SAS, some participants appear to have used alternative strategies to reduce RTs in the present paradigm and further experimentation was warranted to examine the timeline of the motor preparatory state prior to response initiation.

Experiment 2 Introduction

The results of Experiment 1 suggest that even though a long ITI was implemented between trials, some participants had an elevated response specific preparatory state when the WS was presented; thus, an accurate estimate of the time required for response preparation to occur based on these data may be misleading. In order to address this limitation, Experiment 2 examined the time course of response preparatory state through the use of an instructed delay task. On any particular trial participants were required to perform *either* a targeted flexion or extension movement of the wrist, which was indicated by the WS. It was reasoned that if participants did not know which movement was to be performed prior to the WS they would not be likely to engage in any response-specific preparatory processing. As in Experiment 1, a short (500 ms) fixed foreperiod was used to maximize the efficiency of preparatory processing (Bertelson, 1967; Touge et al., 1998). A SAS was presented at various time points prior to the go-signal to probe the level of response preparation, with the expectation that once the movement was sufficiently prepared the movement would be triggered by the SAS. Due to this paradigm change, it was hypothesized that, in contrast to the results of Experiment 1, StartReact responses would not be seen when the SAS was presented concurrent with the WS. As such, the proportion of StartReact responses seen when the SAS was presented later in the foreperiod would provide insight into the time required for response preparation beginning from a minimal level of preparatory activation.

Experiment 2 Experimental Procedures

Participants. Seventeen participants with normal or corrected-to-normal vision, and no obvious upper body abnormalities participated in this experiment. Five participants did not exhibit consistent SCM activation in response to presentation of a SAS; thus, data from these participants were excluded from the final data analysis. This resulted in a final sample size of 12 participants (7 F, 5M; M_{age} = 25, SD = 7). All participants provided written informed consent prior to participating in the experiment, which was conducted in accordance with the ethical guidelines of the Health Science Research Ethics Board at the University of Ottawa, and conformed to the latest version of the Declaration of Helsinki.

Experimental apparatus and task. The experimental apparatus was identical to that of Experiment 1; however, the task was modified. Participants began each trial with their wrist in a neutral position (neither flexed nor extended), and were required to perform either a targeted 20^{o} wrist extension or flexion movement in response to a visual go-signal. The movement to be completed on each trial was indicated by the WS on each trial, and participants were informed that the goal was to react as quickly and accurately as possible. As in Experiment 1, a points system rewarded participants for fast RTs (RT < 180 ms) and penalized participants for slow RTs (RT > 280 ms); however, these points were not analyzed.

Procedure. The experimental procedure was similar to that of Experiment 1 with a few important modifications. All trials began with the presentation of a trial start cue, consisting of an auditory stimulus (100 ms, 200 Hz, 82 dB) accompanied by black outlines of two 6.3 cm squares on either side of a fixation cross appearing on the computer monitor, indicating to participants the onset of a new trial. Following a variable time period of 2000 – 2500 ms the WS was presented, which consisted of one of the two squares being filled in green. This WS indicated to participants which movement to perform in response to the upcoming IS; the right square being filled in corresponded to a wrist extension movement, while the left square being filled in corresponded to a wrist flexion movement. The WS remained on the screen for the duration of the 500 ms fixed foreperiod, followed by presentation of the auditory IS (82 dB, 25 ms, 1000 Hz sine wave). The difference in IS modality between experiments (visual for Experiment 1, auditory for Experiment 2) was due to the use of a visual WS in Experiment 2 that consisted of the representative square turning green to indicate the required response. While it is well known that stimulus modality affects voluntary RT (Woodworth, 1938; Carlsen et al., 2011a), no comparisons were made between experiments for non-startle RTs and preparation levels were assessed by the response to an identical SAS presented at time points following a visual WS in both experiments. Participants were instructed to respond as quickly and accurately as possible to the auditory IS.

To become familiar with the task, participants completed a practice block of 21 trials, 10 each of the flexion and extension movements, as well as one catch trial. These practice trials were identical to the experimental trials, with the exception that there were no SAS trials. The experimental session consisted a total of 185 trials, broken into five blocks of 37 trials. In each block, participants completed 18 flexion trials with the control IS, 12 extension trials with the control IS, six extension trials with a SAS, and one catch trial. A SAS was only presented for the extension movement to allow for better comparison with the results of Experiment 1, as well as to limit the number of SAS trials participants were exposed to. As in Experiment 1, the SAS was randomly presented at one of six time points, resulting in the same six SAS conditions, with one being presented at each time point in each block. Catch trials where the IS never occurred were also included in Experiment 2, and throughout the experiment participants only responded on 5% (3/60) of catch trials, suggesting that participants were reacting to the IS and not simply anticipating the end of the fixed foreperiod. In contrast to Experiment 1, a block of trials of a simple RT task was not completed following the experimental trials, as the instructed delay paradigm used was very similar to a traditional simple task, and it was expected that typical StartReact responses would be seen. The recording equipment used to collect EMG and kinematic data were identical to Experiment1.

Data reduction and analysis. To confirm that StartReact responses were elicited, premotor RT, initial ECR burst duration, and initial ECR burst peak amplitude were compared between the extension trials with the control IS and extension trials where SAS was presented concurrent with the IS (ST-0). These data were analyzed using separate paired-samples t-tests. The remaining data reduction and analysis procedures, as well as the remainder of the statistical analyses, were identical to those employed in Experiment 1. As stated in the Participants section, this data reduction procedure resulted in the exclusion of five participants, as they did not exhibit a sufficient proportion of trials (50%) with SCM activation in the ST-0 condition. In the remaining participants, 62 SAS trials were discarded due to lack of SCM activation. In addition, 64 trials were discarded for RTs that were too fast, five trials were discarded for RTs that were too slow, seven trials were discarded due to participants failing to respond, and nine trials were discarded due to movement errors (e.g. performing the wrong movement, performing multiple movements). These excluded trials were distributed evenly across participants (min = 4, max = 19; M = 12.5, SD = 5.4), and their exclusion resulted in an overall inclusion rate of 93% (2073/2220).

Experiment 2 Results

Confirmation of StartReact responses. Analysis of premotor RT between control trials for the extension movement and startle trials where the SAS was presented with the go-signal (ST-0) revealed a significant difference between conditions, T (11) = 7.097, p < .001, r = .91, d = 2.32. Premotor RT was significantly faster in the ST-0 condition (96.2 ms, SD = 18) than in the control extension condition (142.2 ms, SD = 21). Analysis also revealed a significant difference for initial ECR peak amplitude between the control extension and ST-0 conditions, T (11) = 3.924, p = .002, r = .76, d = .67, indicating that initial ECR peak amplitude was significantly larger in the ST-0 condition (0.293 mV, SD = 0.14) than in the control extension condition (0.214 mV, SD = 0.099). Finally, there was no significant difference in initial ECR burst duration between the control extension and ST-0 conditions (p = .12). These results indicate that typical StartReact responses were seen in the instructed delay paradigm used in Experiment 2.

Proportion of SAS trials resulting in startle reflexes and StartReact responses. Analysis of the proportion of SAS trials where a startle reflex was elicited revealed a significant main effect of time, χ^2_F (5) = 18.731, p = .002. However, post-hoc tests using Wilcoxon Signed Ranks tests and a Bonferroni correction revealed that these differences were not significant when correcting for multiple comparisons (all p-values > .003). The proportion of trials where a startle reflex was evoked was .59 (SD = .36), .83 (SD = .20) .71 (SD = .33), .83 (SD = .25), 95 (SD = .10), and .90 (SD = .14) in the ST-500, ST-400, ST-300, ST-200, ST-100 and ST-0 conditions, respectively. The percentage of SAS trials where a StartReact response was elicited can be seen in Figure 4. Analysis revealed a significant main effect of time of SAS presentation, χ^2_F (5) = 39.307, p < .001. Post-hoc Wilcoxon Signed Ranks tests using a Bonferroni correction for multiple comparisons revealed that the proportion of StartReact responses elicited in the ST-200 condition was larger than in the ST-500 condition (T = 0, z = -2.823, p = .002, r = -.815). There was also a significantly greater proportion of StartReact responses elicited in the ST-100 condition than in the ST-400 (T = 0, z = -2.825, p = .002, r = .82) and ST-500 (T = 0, z = -3.077, p < .001, r = .89) conditions. Finally the proportion of StartReact responses in the ST-0 condition was significantly greater than in the ST-300 (T = 0, z = -2.947, p = .001, r = -.85), ST-400 (T = 0, z = -2.821, p = .002, r = -.81) and ST-500 (T = 0, z = -3.084, p < .001, r = .89) conditions. There was a trend towards a greater proportion of StartReact responses in the ST-100 condition than in the ST-300 condition (T = 0, z = -2.692, p = .004, r = -.78), as well as in the ST-300 condition than in the ST-500 condition (T = 3, z = -2.674, p = .005, r = -.77), but these did not reach the adjusted significance level. All other comparisons between conditions were not significant when correcting for multiple comparisons (all p-values > .0033). These results indicate that while there were no reliable differences in the proportion of startle responses elicited across time of SAS presentation, the proportion of StartReact responses (SAS trials with SCM activation and fast RTs) increased as the SAS was presented later in the foreperiod.

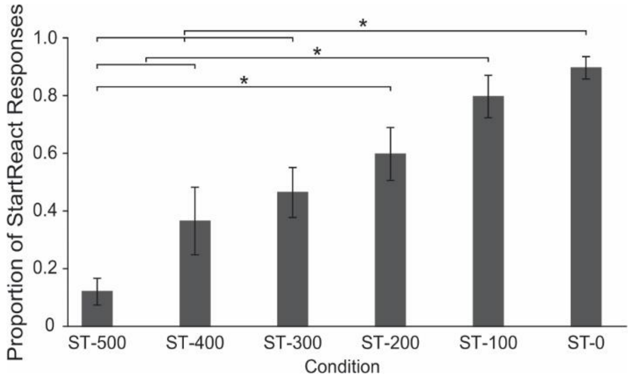

Fig. 4. *Mean (SE) proportion of StartReact responses elicited across participants in each condition where a SAS was presented in Experiment 2. Conditions represent the SAS being presented at time intervals (-500 ms, -400 ms, -300 ms, -200 ms, -100 ms, 0 ms) prior to, or concurrent with, the go-signal. Asterisks (*) denote significant (p < .05) differences.*

Premotor RT. As in Experiment 1, a continuous non-linear regression was performed on premotor RT for each condition where the SAS was presented prior to the go-signal to determine if the distributions exhibited bimodality (Frankland and Zumbo, 2002) of involuntary (StartReact) and voluntary responses. A histogram of the proportion of premotor RTs in 50 ms bins for each of the experimental conditions can be seen in Figure 5A. Analysis revealed a significant bimodal distribution in the ST-400 condition, $F(6,5) = 21.0$, $p = .002$, $R^2 = .945$, as well as a trend towards a bimodal distribution in the ST-500 condition, $F(6,9) = 3.118$, $p = .062$, $R^2 = .164$. There was no significant bimodal distribution in the other conditions (all p-values > .05). Analysis of premotor RT in SAS trials where a StartReact response was elicited (Figure 5B) revealed a significant main effect of time, $F(5, 55) = 5.557$, $p < .001$, $\eta^2_p = .336$. Post-hoc analysis using Tukey's HSD test indicated that premotor RT was significantly faster in the ST-0 and ST-100 conditions as compared to the ST-400 and ST-500 conditions. There was also a significant linear trend for time, $F(1,11) = 13.920$, $p = .003$, $\eta^2_p = .559$. These results indicate that as the SAS was presented later in the foreperiod the response latency of the StartReact effect tended to decrease.

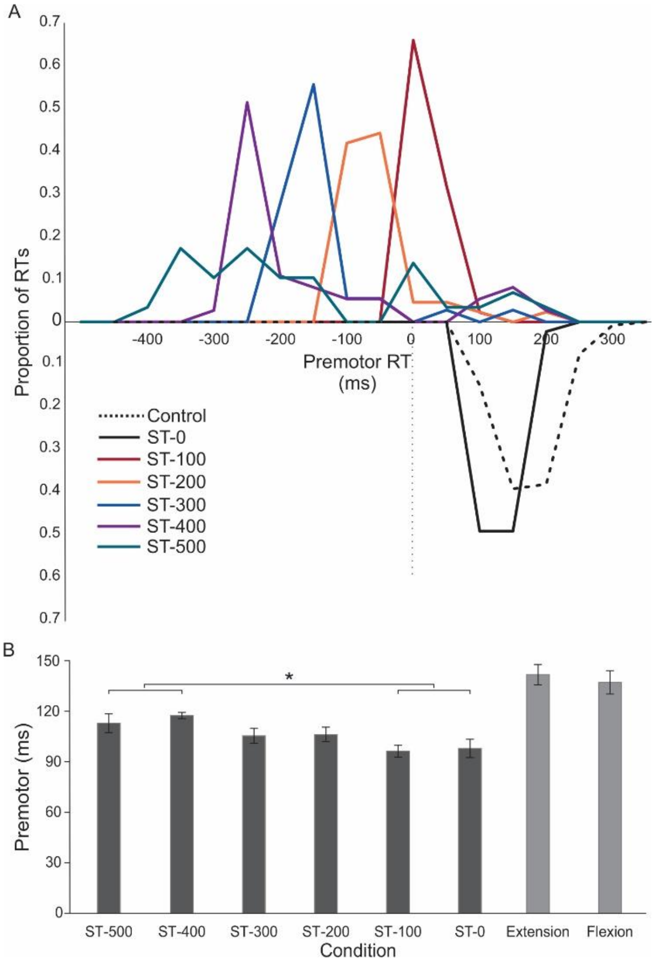

Fig. 5. *A) Histogram displaying proportion of premotor RTs falling in 50 ms bins for each experimental condition in Experiment 2. Conditions represent the SAS being presented at time intervals (-500 ms, -400 ms, -300 ms, -200 ms, -100 ms, 0 ms) prior to, or concurrent with, the go-signal. Traces for the control and ST-0 condition are shown downwards to enhance clarity and readability of the figure. B) Mean (SE) premotor reaction time (RT) observed across all experimental conditions in Experiment 2. Dark grey bars represent StartReact responses, and light grey bars represent voluntary responses in the control extension and flexion conditions. Asterisks (*) denote significant (p < .05) differences for SAS RT.*

Peak Displacement. Analysis revealed that peak displacement in the SAS conditions was not normally distributed; thus, non-parametric tests were used to assess the effect of time of SAS presentation on peak displacement. Results indicated a significant main effect of SAS presentation time on peak displacement, χ^2_F (5) = 30.513, p < .001. Post-hoc Wilcoxon-Signed Ranks test with a Bonferroni correction for multiple comparisons revealed that peak displacement was significantly smaller in the ST-500 condition than in all other conditions (for all comparisons, T = 0, z = -3.059, p < .001, r = -.88). Mean peak displacement in the ST-500, ST-400, ST-300, ST-200, ST-100, ST-0 and control conditions was 19.9 (5.4), 35.8 (6.5), 30.7 (11.5), 33.2 (10.1), 34.9 (8.4), 34.1 (8.4), and 28.0 (10.2), respectively. Raw displacement traces of the movement performed across conditions by a representative participant are displayed in Figure 6. As in Experiment 1, between-subject variability was relatively high, so within-subject standard deviation of peak displacement was calculated for the control (5.7, SD = 3.1) and ST-0 (4.8, SD = 1.2) conditions, revealing that participants were performing similar movements throughout the experiment.

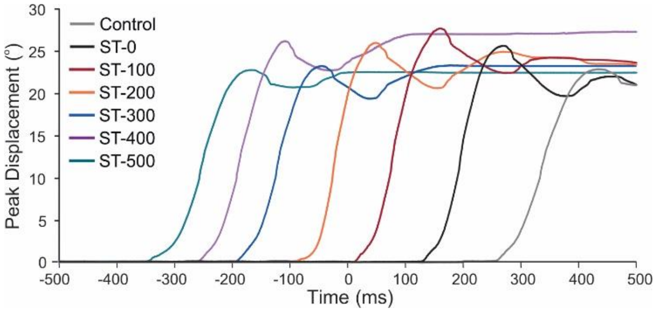

Fig. 6. *Raw movement traces from a typical participant across all experimental conditions in Experiment 2. Startle (ST) trials are shown, separated by the time of SAS presentation prior to the go-signal (e.g., ST-100 = SAS presented 100 ms prior to go-signal).*

Experiment 2 Discussion

The purpose of Experiment 2 was to further examine the time course of the response preparatory state following the WS in an instructed delay paradigm that required fast response preparation. In this paradigm, participants were required to perform either a flexion or extension movement of the wrist on each trial, with the required movement being indicated by the WS. In contrast to Experiment 1, the required movement for each trial was not known in advance of the WS; thus, it was expected that participants would not engage in any response specific preparation prior to presentation of the WS. This would require participants to rapidly prepare the motor response beginning from a minimal level of preparatory activation, allowing an investigation of the amount of time required for the response to become sufficiently prepared such that it could be involuntarily triggered by a SAS. Similar to Experiment 1, results showed that when the SAS was presented only 100 ms following the WS (ST-400; see Figure 4), the SAS sometimes led to the early triggering of the response, although this low proportion was not significantly greater than when the SAS was presented concurrent with the WS (ST-500) (*p* = .07, greater than the *p* = .0033 required when correcting for multiple comparisons). However, with later SAS presentations, the proportion of StartReact responses elicited was significantly larger 300 ms following the WS, where a response was triggered by the SAS more than 50% of the time (ST-200). Furthermore, for later SAS times the proportion of responses elicited by the SAS became significantly larger than the ones preceding it at 300 ms intervals (i.e. ST-100 > ST-400, ST-0 > ST-300). These results suggest that when provided only a 500 ms foreperiod, responses can be sufficiently prepared to be triggered by a SAS after only 100 ms. However, approximately 300 ms are required until the response is sufficiently prepared more than 50% of the time, and approximately 400 ms are required until no further increase in the proportion of StartReact responses are observed. While there is considerable variability in the preparation process both within and between subjects (see Figure 4), as well as between this and other paradigms, the present results suggest that maximal preparation occurs within 300 – 400 ms of presentation of the WS in a simple RT task with a short fixed foreperiod.

While Experiment 2 was designed to prevent participants from engaging in any response-specific preparation prior to presentation of the WS, and thus eliminating StartReact response in the ST-500 condition, a small proportion of responses were nevertheless involuntarily triggered by the SAS in this condition. As participants were unaware of the required movement prior to the WS, this result suggests that some participants may have been employing a strategy of guessing which movement to perform on the upcoming trial, allowing sufficient response specific preparation to occur such that the movement could be triggered early by a SAS presented with the WS. However, inspection of data from the ST-500 condition revealed that StartReact responses were only seen in four participants, resulting in a total of six (12%) StartReact responses. Furthermore, analysis of peak displacement revealed that responses involuntarily triggered by the SAS in the ST-500 condition were significantly smaller than responses in all other SAS conditions. This suggests that while a few participants exhibited StartReact responses in the ST-500 condition these were both qualitatively and quantitatively different than those seen in conditions where the SAS was presented later in the foreperiod. Finally, movement errors were only seen in seven trials across all experimental conditions, suggesting that participants were likely not employing a strategy of guessing the movement to be performed on the upcoming trial. As such, it appears that the use of an instructed delay task was able to address the primary limitation of Experiment 1, allowing for greater confidence in the results of Experiment 2 with respect to the time course of response preparation in a simple RT task with a short fixed foreperiod.

General Discussion

Previous research examining the time course of response preparation has provided conflicting results with respect to the amount of time that is necessary to prepare a motor response, with estimates ranging from 130 ms to over 500 ms, depending on the paradigm used. The present experiments used a SAS to probe the state of response preparation at various times in two RT tasks with short fixed foreperiods. Although the experiments employed slightly different paradigms to examine minimum preparation time, the results were consistent between studies. Our data indicated that while a low percentage of StartReact responses could be elicited as early as 100 ms following the WS, the proportion increased as the SAS was presented later in the foreperiod (Figures 2 & 4). We observed approximately a 50% StartReact response rate at 200 ms following the WS, and this proportion continued to increase until approximately 300 – 400 ms following the WS. Thus, these results appear to represent a cumulative distribution of the time taken to prepare an accurate response that is ready for initiation, when only 500 ms is available to prepare.

These results indicate a preparatory timeline similar to that found by Kennefick et al. (2014), who showed that mean corticospinal excitability increased in the first 500 ms following a WS before leveling off for the remaining 1000 ms until presentation of the IS. The somewhat shorter time taken to reach this preparatory state in the present experiments may be due to the use of a shorter foreperiod (500 ms versus 1500 ms), which required participants to prepare rapidly following the WS. However, our time required for response preparation (300 – 400 ms) is considerably longer than the 130 – 210 ms required to prepare a response in a choice RT task suggested by Haith et al. (2016). The greater length of preparation time in the present experiment may be explained by the difference in how preparation is defined in these experiments. The estimate of 300 – 400 ms required for response preparation is based on when there was no further increase in the proportion of StartReact responses seen (Figures 2 & 4). The work of Haith et al. (2016) revealed similar findings, with an asymptote of successful responses reached at ~300 ms. If we instead chose to define preparation as the point where a 50% incidence of StartReact responses is seen, the average time required for response preparation would be ~200 ms, similar to the mean RT of 212 ms reported by Haith et al. (2016).

Another possible reason for the shorter estimate of preparation time in the Haith et al. (2016) experiment is that participants were instructed to emphasize fast RTs, potentially initiating responses that were only partially prepared. Indeed, the shortest latency responses reported by Haith et al. (2016) included numerous errors suggesting that complete response preparation was not achieved. The suggestion of early initiation of "incomplete" responses also appeared to occur in the current studies. In both experiments, a substantial proportion of StartReact responses were observed 100 – 200 ms following the WS (ST-400 & ST-300 respectively; see Figures 2 & 4). These results imply that in some instances participants were able to quickly achieve a sufficient preparatory state to elicit a StartReact response. Based on the time required for processing of external stimuli and the transmission of motor commands, 100 ms is likely close to the physiological limits of the sensorimotor system. These results suggest that occasionally the preparatory state of the response was at a level sufficient to elicit StartReact responses in 100 ms, but participants engaged in further preparatory processing during the foreperiod such that approximately 300 – 400 ms were often used for response preparation to be completed.

The present experiments used a SAS to probe the time course of motor preparation, so it was important to ensure that typical StartReact responses were observed in order to draw conclusions based on the proportion of StartReact responses seen. In both experiments, participants exhibited consistent SCM activation in response to a SAS, and movement-related EMG patterns were similar in StartReact and voluntary responses, although it was found that peak displacement and EMG amplitude were greater for the initial agonist burst in SAS trials. This is occasionally seen in startle trials, and is hypothesized to be due to the generation of a larger initiation signal on SAS trials, or to summation of activation from the startle volley and voluntary response (Siegmund et al., 2001; Carlsen et al., 2013; Smith and Carlsen, 2018). This paradigm allowed participants up to 500 ms to prepare motor responses, with the SAS involuntarily triggering sufficiently prepared responses. Based on the difference in the proportion of StartReact responses seen as the time after the warning signal increased, it appears that this is a novel, effective method of probing response preparation.

While it may seem contradictory for the preparation time we observed in a simple RT task to be similar to RTs previously reported in a choice paradigm, the finding that they are similar is not surprising. In a choice RT paradigm, preparatory processing occurs following the go-signal (Donders, 1969), whereas a simple RT paradigm allows for preparatory processes to occur prior to the go-signal, resulting in a shorter response latency. However, the present experiment investigated the time required to prepare a response in a simple RT paradigm beginning from resting (non-prepared) state and thus preparation time should be similar to that observed in a choice RT paradigm. As previously mentioned, it is well known that choice RT is longer than simple RT; however, in the present experiment the instructed delay RTs of Experiment 2 were faster than the simple RTs of Experiment 1. This seemingly abnormal finding can be explained by the fact that Experiment 1 employed a visual IS, whereas Experiment 2 employed an auditory IS, and the differences in processing speeds of these two sensory streams are known to result in significantly faster RTs to auditory stimuli than to visual stimuli (Woodworth, 1938; Carlsen et al., 2011a).

A final note of interest concerns the distribution of premotor RTs in the conditions where the SAS was presented prior to the IS. In Experiment 1, when the SAS was presented during the foreperiod, premotor RTs were bimodally distributed, falling primarily into two distinct clusters: one representing rapid, involuntarily triggered responses to the SAS, and one representing voluntary responses to the control IS (Figure 3A). This suggests that if the response was sufficiently prepared, it was elicited shortly after the SAS, otherwise, participants waited until the IS as instructed, with few responses occurring in between these extremes. Drummond et al. (2016) observed a similar dichotomous effect of a SAS on RT in a stop-signal task, where participants exhibited reduced preparation due to the possibility of having to inhibit the response. In contrast to the results of Experiment 1, the

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pattern of premotor RTs in Experiment 2 only exhibited a bimodal distribution in the ST-400 and ST-500 conditions (Figure 5A). In the other conditions where a SAS was presented prior to the control IS, participants mostly responded to the SAS and only occasionally responded to the IS. This discrepancy from Experiment 1 is likely due to the use of an acoustic imperative stimulus in Experiment 2, rather than a visual one. As participants were waiting to respond to an acoustic stimulus, they may have responded to the SAS presented prior to the go-signal more frequently, regardless of the level of preparation achieved.

In conclusion, the results of the present experiments suggest that in a simple RT paradigm, response preparatory activity continues for up to 300 - 400 ms following presentation of the WS. The current findings suggest that the mean level of preparatory activation is low when only a short amount of time has elapsed since a WS (i.e., 100 ms); however, a more accurate characterization may be that at short intervals following a WS there is a low probability of preparatory activation being elevated to a level sufficient for response initiation, and that this probability increases with increasing time after the WS until it reaches asymptote at some point between approximately 300 and 400 ms. These results are consistent with previous work examining minimum preparation time, but were achieved using a novel methodology, which lends further support to a timeline where preparation begins as soon as 100 ms following a warning cue, and continues up until approximately 300 – 400 ms have elapsed. This time period represents the amount of time required such that response preparation can be completed on most trials, and can be consistently triggered involuntarily by the SAS.

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