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# Startle reveals decreased response preparatory activation during a stop-signal task

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**Drummond NM, Cressman EK, Carlsen AN.** Startle reveals decreased response preparatory activation during a stop-signal task. *J Neurophysiol* 116: 986–994, 2016. First published June 8, 2016; doi:10.1152/jn.00216.2016.—In a stop-signal task participants are instructed to initiate a movement in response to a go signal, but to inhibit this movement if an infrequent stop signal is presented after the go. Reaction time (RT) in a stop-signal task is typically longer compared with that in a simple RT task, which may be attributed to a reduced readiness to initiate the response caused by the possibility of having to inhibit the response. The purpose of this experiment was to probe the preparatory activation level of the motor response during a stop-signal task using a startling acoustic stimulus (SAS), which has been shown to involuntarily trigger sufficiently prepared responses at a short latency. Participants completed two separate tasks: a simple RT task, followed by a stop-signal RT task. During both tasks, an SAS (120 dB) was pseudorandomly presented concurrently with the go signal. As expected, RT during the simple RT task was significantly shorter than during the stop-signal task. A significant reduction in RT was noted when an SAS was presented during the simple RT task; however, during the stop-signal task, an SAS resulted in either a significant speeding or a moderate delay in RT. Additionally, the subset of SAS trial responses with the shortest RT latencies produced during the stop-signal task were also delayed compared with the short-latency SAS trial responses observed during the simple RT task. Despite evidence that a response was prepared in advance of the go signal during a stop-signal task, it appears that the amount of preparatory activation was reduced compared with that achieved during a simple RT task.

stop-signal task; startling acoustic stimulus; preparation; reaction time; inhibition

**NEW & NOTEWORTHY**

*In this study a startling acoustic stimulus (SAS) was used to probe preparatory activation during a stop-signal task. Results indicated that although the response was prepared in advance during a stop-signal task, reaction time (RT) was delayed for both control and SAS trials compared with a simple RT task. The increased RT typically observed in a stop-signal task is therefore attributed to a reduced level of preparatory activation associated with the possibility of having to inhibit the response.*

IN A STOP-SIGNAL TASK participants are instructed to plan and initiate a response as fast as possible to a go-signal, but to inhibit this planned movement in response to an infrequent stop-signal presented at variable delays after the go (Logan et al. 1984). Similar to a simple reaction time (RT) task, in a stop-signal task only one response is initiated in response to the go signal. Since the seminal work of Donders (1969), significant evidence has supported the proposal that when a single response is required, it can be selected and planned in advance of the go signal (Carlsen et al. 2012; Leuthold et al. 2004). When interpreted within the context of a neural activation model (Hanes and Schall 1996), this advance preparation allows the activation level related to the motor response to be maintained close to the threshold needed for the response to be initiated, thus reducing the time to achieve threshold following presentation of the go signal and resulting in a fast RT. Yet for stop-signal RT tasks, results have consistently shown that RT on go trials is longer compared with RT in a simple RT task (by ~100–200 ms) despite a theoretical capability for advance response preparation that should be similar to that for a simple RT task (Verbruggen and Logan 2009). The increase in RT observed in a stop-signal task may arise as a result of a reduced amount of preparatory activation related to the motor response, due to the possibility of having to inhibit the response. Indeed, the amount of advance preparatory activation has previously been shown to affect performance in RT tasks. For example, lateralized readiness potential (LRP) amplitude measured before the go signal, which is viewed as an index of motor preparatory activation (Coles 1989; Kutas and Donchin 1980), has demonstrated that larger LRPs correspond with faster RTs (Gratton et al. 1988; Leuthold et al. 1996).

Another way to probe the preparatory activation level of the motor response during a stop-signal task is to use a loud (120 dB) startling acoustic stimulus (SAS). During a simple RT task it has been shown that not only does an SAS cause a reflexive startle response (Brown et al. 1991; Landis et al. 1939), but if a motor response is sufficiently prepared, an SAS can also trigger the prepared action involuntarily, producing very short RTs while preserving the kinematics and electromyographic (EMG) features of the movement (Carlsen et al. 2004b, 2012; Castellote et al. 2007; Siegmund et al. 2001; Valls-Solé et al. 1999, 1995). This phenomenon is suggested to arise as a result of the SAS increasing the activation in neural circuits related to the motor response beyond the threshold necessary for initiation. The neural mechanism underlying this effect is currently a matter of debate, with studies supporting both a subcortical storage and release mechanism (Castellote and Valls-Solé 2015; Honeycutt and Perreault 2012; Nonnekes et al. 2014; Sanegre et al. 2004; Valls-Solé et al. 1999), as well as a mechanism involving the subcortically mediated triggering of a cortically stored motor command (Alibiglou and MacKinnon 2012; Maslovat et al. 2014; Stevenson et al. 2014). This early involuntary response initiation is not seen, however, in circumstances where there is a limited ability to prepare the

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response in advance (e.g., a choice RT task; Carlsen et al. 2004a; Maslovat et al. 2012). Thus an SAS can be used to probe response preparation before a go signal by examining whether the expected response is triggered at a short latency. The latency at which the response is triggered by the SAS may also provide insight into the amount of preparatory activation, with decreased preparation associated with longer control and SAS response latencies (Drummond et al. 2015).

In the current experiment an SAS was presented concurrently with the go signal to determine whether the response was prepared in advance of the go signal during a stop-signal task. It was hypothesized that if the longer go-trial RTs observed during a stop-signal task were due to an inability to prepare the response, the SAS would not result in short-latency RTs. If, however, longer RTs on go trials were the result of a reduced level of preparation, the presentation of an SAS would trigger the early release of the prepared response, albeit at a longer latency compared with the same response triggered during a simple RT task.

## MATERIALS AND METHODS

*Participants.* Data were collected from 14 healthy participants [7 men, 7 women; mean age 26.0 yr (SD 3.6 yr)] with no sensory or motor dysfunction. However, four participants failed to show a reflexive startle response (see *Data reduction* for details) in the majority of the startle trials in the simple RT task, and thus their data were excluded from the analyses (see Carlsen et al. 2011 for more details regarding recommended inclusion criteria). Data are presented from the remaining 10 participants [5 men, 5 women; mean age 25.7 yr (SD 3.7 yr)]. All participants provided written informed consent and had normal or corrected-to-normal vision. The study was approved by and conducted in accordance with the ethical guidelines set by the University of Ottawa's Research Ethics Board and conformed to the latest revision of the Declaration of Helsinki.

*Apparatus and task.* Participants sat facing a 24-in. liquid crystal display (LCD) computer monitor with their right arm resting in a manipulandum that restricted movement to wrist flexion and extension. The forearm was parallel to the floor with the palm facing inwards and was secured with Velcro straps placed proximal to the wrist and distal to the elbow. Participants completed two separate tasks in a serial fashion that were performed in a single session lasting ~1 h 30 min. First, participants completed a simple RT task with instructions to react as fast as possible to a visual go signal (see *Instrumentation and stimuli* for details) by performing a targeted 20° wrist extension movement from a neutral position (wrist neither flexed nor extended). Participants then completed a stop-signal task with instructions to react as fast as possible to the go signal but to try to withhold the response if a stop signal was presented. Participants were told that the probability of being able to stop when a stop signal appeared was ~50%, and therefore they should not wait for a stop signal to appear. Feedback was provided on the computer monitor after each trial and consisted of RT on that trial and accuracy with respect to the target. Points were given by means of a payoff matrix such that in the simple RT task, the payoff was designed to solely reward fast RTs (≤200 ms), whereas in the stop-signal task, the payoff was designed to equally reward fast RTs (≤ 50 ms) and correct responses (i.e., going on go trials and stopping on stop trials). A displacement RT >500 ms in either task resulted in a deduction of points and a "Too slow!" message displayed on the screen. The simple RT task was always administered before the stop-signal task to avoid any influence of experience with a previous stop-signal task on the preparatory activation during simple RT task performance (Monsell 2003; Waters-Metenier et al. 2014).

*Instrumentation and stimuli.* To start each trial, a white square (8 × 8 $cm^2$) was presented in the middle of the screen and a visual warning signal "GET READY!" was displayed for 1,000 ms. This was followed by a variable foreperiod (2,000-2,500 ms) and presentation of the imperative go signal (the square turned green). For the simple RT task, participants performed 10 practice trials followed by 20 testing trials.

Stimulus presentation during the stop-signal task was similar to that during the simple RT task, with the exception that on 25% of trials, a stop signal (the square turned red) was presented following the go signal. The time between the go and stop signals (stop-signal delay, SSD) was dynamically varied on the basis of individual responses by using a tracking procedure: SSD started at 200 ms and increased or decreased by 25 ms following a successful or failed stop, respectively (Logan et al. 1997). Thus the tracking procedure ensured an individualized SSD, thereby compensating for differences between participants in task performance (see Verbruggen and Logan 2009 for a review). Participants performed 10 practice trials in the stop-signal task followed by 100 testing trials.

An SAS consisting of a 120-dB, 25-ms white noise waveform (equal power from 1 Hz to 22 kHz) was presented concurrently with the go signal via a loudspeaker (MG Electronics M58-H; rise time <1 ms) located behind the participant's head in 25% of the simple RT trials (5/20 trials) and 20% of the go trials in the stop-signal task [15/75 go trials (or 15/100 total trials)]. The SAS was always presented on go trials because we expected a response to be initiated regardless of the effect of the SAS. This design avoids the issue of classifying any possible involuntary triggering of the response by SAS on a stop trial as an "error." Moreover, go trials in which an SAS was presented were always preceded immediately by another go trial to prevent a preceding stop trial from influencing preparation in the subsequent SAS trial, because several studies have found that go RT is modulated when preceded by a stop-signal trial (see Verbruggen and Logan 2009 for a review). Acoustic stimulus intensity was confirmed with the use of a precision sound-level meter located at the same distance from the loudspeaker to the ears (30 cm; Cirrus Research CR:162C; A-weighted, impulse-response mode). Participants were told that during the testing trials, a loud auditory stimulus would be presented randomly but that this noise was irrelevant to the task and they should continue performing the task as instructed. The SAS was presented pseudorandomly such that no two consecutive trials included an SAS, no SAS was presented in the first two trials, and no SAS was presented before, on, or after a stop trial. Given the trial-type breakdown, there was an increase in probability of a stop trial or SAS trial following two or more consecutive go trials. However, because participants were unaware of how many SAS, stop, and go trials there were, it is unlikely that participants were able to predict when an SAS or stop trial would occur.

Surface electromyography (EMG) data were collected from the muscle bellies of the right extensor carpi radialis longus (ECR), right flexor carpi radialis (FCR), and left sternocleidomastoid (SCM; as an indication of a startle reflex) using bipolar preamplified surface electrodes (Delsys DE 2.1) connected to an external amplifier system (Delsys Bagnoli-8). Wrist angular position data were collected using a potentiometer attached to the central axis of the manipulandum. On each trial, band-passed (20–450 Hz) EMG and raw position data were digitally sampled at 4 kHz (National Instruments PCIe-6321) for 3 s, beginning 1 s before the go signal, using a customized program written with LabVIEW software (National Instruments) and were stored for offline analysis.

*Data reduction.* Surface EMG burst onsets in ECR and SCM were defined as the point at which the filtered EMG (2nd-order elliptic filter) first began a sustained (>20-ms duration) rise 2 SD above baseline levels (mean EMG activity 100 ms before the go-signal onset). To distinguish startle-related SCM activity from other SCM activity, SCM onset had to occur within a time window between 30 and 170 ms after SAS onset (indicative of the reflexive startle response; see Carlsen et al. 2011). Similar to Kumru et al. (2006), who presented a startle in a go/no-go RT task, the startle SCM time

window was extended to 170 ms following the SAS to determine whether inhibitory processes related to stop-signal task performance affected the onset and size of the startle response. The magnitude of the SCM response was quantified as the integrated EMG profile over the first 100 ms of muscle activity (Q100). The proportion of go trials resulting in a startle response (SCM+) within simple RT and stop-signal tasks was calculated by dividing the observed number of SCM+ SAS trials by the total number of SAS trials. The proportion of SAS SCM+ "early" responses was calculated by dividing the number of observed "early" SCM+ responses by the total number of SCM+ SAS trials (see *Statistical analyses* and RESULTS, *Response latency*, for details regarding "early" responses).

Premotor RT was defined as the time between the go signal and EMG onset in the ECR muscle. Go trials during the simple RT and stop-signal task with a premotor RT greater than 3 SD above the participant's mean were considered erroneous and removed from the analysis (0.75% and 1.5% of trials, respectively). Peak displacement was defined as the greatest displacement achieved during the movement, and final position corresponded to the angular position of the wrist with respect to the home position at the first time point at which angular velocity fell below 8°/s and remained below for at least 150 ms. A response with a peak displacement $\geq 2°$ was defined as an initiated response, whereas anything $<2°$ was considered a stop response. Additional measures that were specific to the stop-signal task included probability of successful stopping and mean SSD (time between go signal and stop signal). To estimate the duration of the stop process, the mean method was used (see Logan et al. 1984), which calculates stop-signal RT (SSRT) by subtracting the mean of the inhibition function (i.e., SSD where $P_{respond} = 0.5$) from the mean RT observed in control go trials on a per-participant basis.

*Statistical analyses.* To investigate the effect of a startling stimulus on kinematic and EMG variables, only SAS trials in which a startle response was observed in SCM (i.e., SCM+) were included in these analyses (see Carlsen et al. 2011 for rationale). Premotor RT was analyzed using a 2 stimulus (control vs. SAS) × 2 task (simple RT vs. stop-signal task) repeated-measures analysis of variance (RM ANOVA). Premotor RT for SAS trials in the stop-signal task was found to be distributed bimodally (see RESULTS, *Response latency*), and thus premotor RTs were classified on the basis of whether or not they were faster than each participant's own fastest control RT in the stop-signal task (see RESULTS, *Response latency*). These responses were separated out and classified as "early" responses. For the simple RT task, all SAS trials with SCM activity (SCM+) were considered "early" responses. Premotor RT and response kinematics (final position and peak displacement) for these "early" responses were compared with those in control trials using 2 stimulus (control vs. SAS) × 2 task (simple RT vs. stop signal) RM ANOVA. The proportion of go trials in which the SAS elicited an EMG response in the SCM (SCM+) and the proportion of SCM+ trials that resulted in the early release of a movement were compared between the simple RT and stop-signal tasks using Student's paired *t*-tests. Before analyses were performed, proportion data were subjected to an arcsine square root transformation (Osborne 2010). Differences with a probability $<0.05$ were considered to be significant, and Tukey's honestly significant difference post hoc tests were administered to determine the locus of any significant differences. Partial eta squared ($\eta_p^2$) and *r* values are reported to provide estimates of effect size.

## RESULTS

*Stop-signal task performance.* Across participants the probability of successful stopping was found to be 37.3% (SD = 5.4). As well, the mean SSD was 105 ms (SD = 47), whereas mean SSRT was 291 ms (SD = 57). Though simply descriptive, these data demonstrate that participants were performing the task correctly and are in line with norms for manual stop-signal task performance (Stuphorn and Emeric 2012).

*Startle response.* Analysis of the proportion of SAS trials in which a startle response was elicited revealed that the SAS led to a greater proportion of SCM+ responses during the simple RT task compared with the stop-signal task [$t(9) = 2.649$, $P = 0.027$, $r = 0.66$; see Fig. 1, gray bars]. In fact, the mean within-subject difference between the proportion of SCM+ trials during the simple RT task (mean 0.94, SD 0.09) and stop-signal task (mean 0.75, SD 0.27) was 0.19 (SD 0.23). Analysis of the onset and size (Q100) of the startle response revealed no differences between the simple and stop-signal tasks [$t(9) = 1.212$, $P = 0.256$, $r = 0.37$, and $t(9) = 0.906$, $P = 0.389$, $r = 0.08$, respectively].

*Response latency.* Analysis of response latency confirmed significant main effects for task [$F(1,9) = 38.841$, $P < 0.001$, $\eta_p^2 = 0.812$] and stimulus [$F(1,9) = 28.871$, $P < 0.001$, $\eta_p^2 = 0.762$], as well as a significant interaction [$F(1,9) = 5.011$, $P = 0.05$, $\eta_p^2 = 0.358$; see Fig. 2]. Post hoc analysis of the interaction revealed that control RT during the simple RT task (mean 211.42 ms, SD 21.45 ms) was significantly faster ($P = 0.01$) than control RT during the stop-signal task (mean 302.60 ms, SD 30.66 ms). In addition, RT in SAS trials during the simple RT task (mean 89.09 ms, SD 16.07 ms) was significantly faster ($P < 0.01$) than control RT in the simple RT task. Furthermore, SAS trials in the simple RT task demonstrated significantly faster ($P < 0.01$) RTs than SAS trials during the stop-signal task (mean 251.70 ms, SD 101.48 ms). No significant difference was observed between SAS RT and control RT in the stop-signal task ($P = 0.18$).

A large amount of variability was observed in the SAS RT data during the stop-signal task (see Fig. 3, light gray lines), and thus an analysis was undertaken to assess and quantify bimodality in the data. A continuous nonlinear regression was performed on individual participant data which was trans-

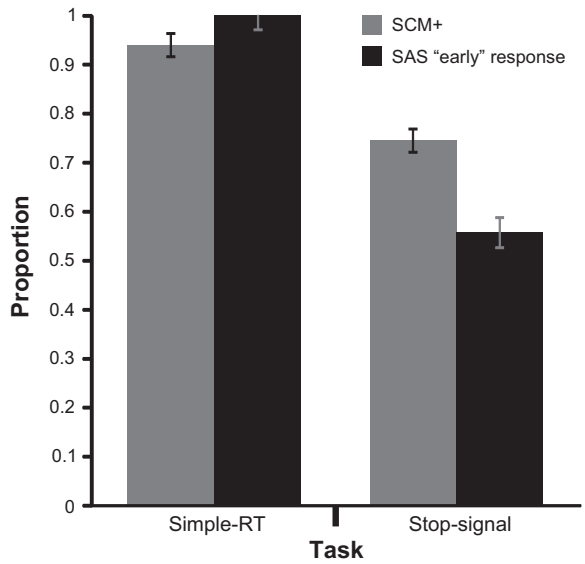


Fig. 1. Mean proportion of startle trials that resulted in a startle response (SCM+; gray) and mean proportion of trials showing a startle response that resulted in the "early" release of the response (SAS "early" response; black) as a function of task. See RESULTS, *Response latency*, for definition of an "early" response. Error bars denote within-subject 95% confidence intervals of comparisons between the simple RT and stop-signal tasks (Morey 2008).

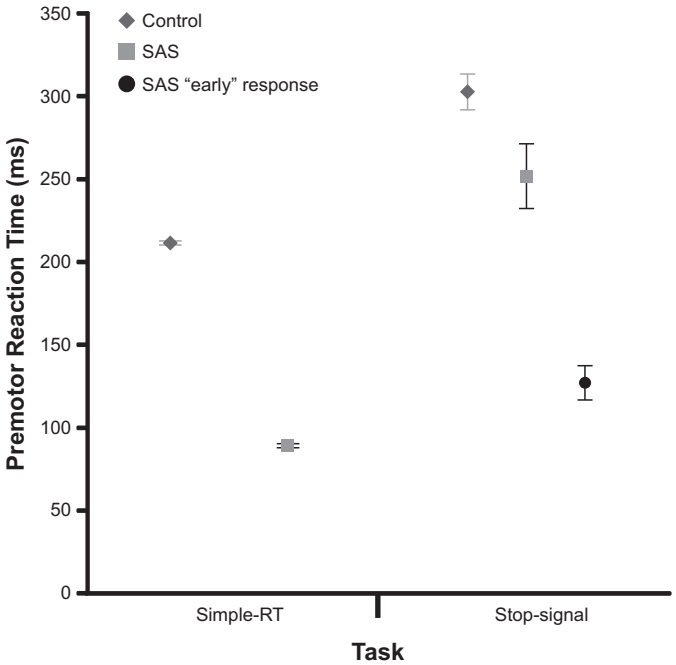


Fig. 2. Mean premotor reaction times for each task separated by trial type. Control trials are shown in dark gray (diamonds), startle trials (SAS) are shown in light gray (squares), and stop-signal task SAS trials that resulted in the early release of the movement (SAS "early" response) are shown in black (circle). See RESULTS, *Response latency*, for definition of an "early" response (see also Fig. 3, black line). Error bars denote within-subject 95% confidence intervals (Morey 2008) computed within task (i.e., simple RT and stop-signal RT tasks, respectively).

formed into $Z$ scores relative to each participant's own mean control RT during the stop-signal task (Fig. 4; see Frankland and Zumbo 2002 for details). The analyses revealed a significant bimodal distribution [$F(1,14) = 55.556$, $P < 0.001$, $R^2 = 0.947$, $SS_{error} = 0.003$]. The first identified distribution had a mean $Z$ score of $-2.023$ (SD 0.477), and the second distribution had a mean $Z$ score of 1.144 (SD 1.204). Results indicate that the presentation of the SAS had a dichotomous effect on RT during the stop-signal task, with responses falling into either a fast or a (moderately) delayed response distribution relative to control RT. Trials were separated into two groups for each participant on the basis of whether the observed RT fell above or below that participant's fastest RT in control trials; those below were defined as "early" responses (individual cutoff shown in Fig. 3 by horizontal black line) and were compared with the RT of all SAS trials from the simple RT task. It was reasoned that these "early" responses may be more reflective of an involuntarily triggered response by the SAS during the stop-signal task and would thus allow for a more equitable comparison to SAS-trial RTs seen in the simple RT task. Analysis again revealed main effects for both task [$F(1,9) = 43.193$, $P < 0.001$, $\eta_p^2 = 0.828$] and stimulus [$F(1,9) = 871.012$, $P < 0.001$, $\eta_p^2 = 0.990$], as well as a significant interaction [$F(1,9) = 31.286$, $P < 0.001$, $\eta_p^2 = 0.777$]. Post hoc analysis revealed that all means were significantly different (all $P$ values $<0.01$) such that "early" SAS RTs (mean 127.07 ms, SD 32.57 ms) was significantly faster than control RT during both simple RT and stop-signal tasks. However, the "early" SAS RTs in the stop-signal task were nevertheless significantly slower than the SAS RTs in the simple RT task (see Fig. 2).

Analysis of the proportion of the SAS trials which resulted in the early release of the response revealed that a greater proportion occurred during the simple RT task (mean 1.0, SD 0.0) compared with the stop-signal task [mean 0.56, SD 0.30; $t(9) = 5.697$, $P < 0.001$, $r = 0.88$; see Fig. 1, black bars].

*Response kinematics.* " Early" responses observed in response to a SAS were analyzed to determine if they exhibited any differences in kinematics compared with control trial go responses for both the simple RT and stop-signal tasks. Analysis of final position revealed a significant main effect of task [$F(1,9) = 14.957$, $P = 0.004$, $\eta_p^2 = 0.624$], as well as a significant interaction [$F(1,9) = 7.593$, $P = 0.022$, $\eta_p^2 = 0.458$], but no main effect of stimulus [$F(1,9) = 0.583$, $P = 0.465$, $\eta_p^2 = 0.061$]. Post hoc analyses revealed that the final position achieved during the stop-signal task control trials (mean 22.70°, SD 2.27°) and early SAS responses (mean 18.18°, SD 3.62°) were not different, but indicated that final

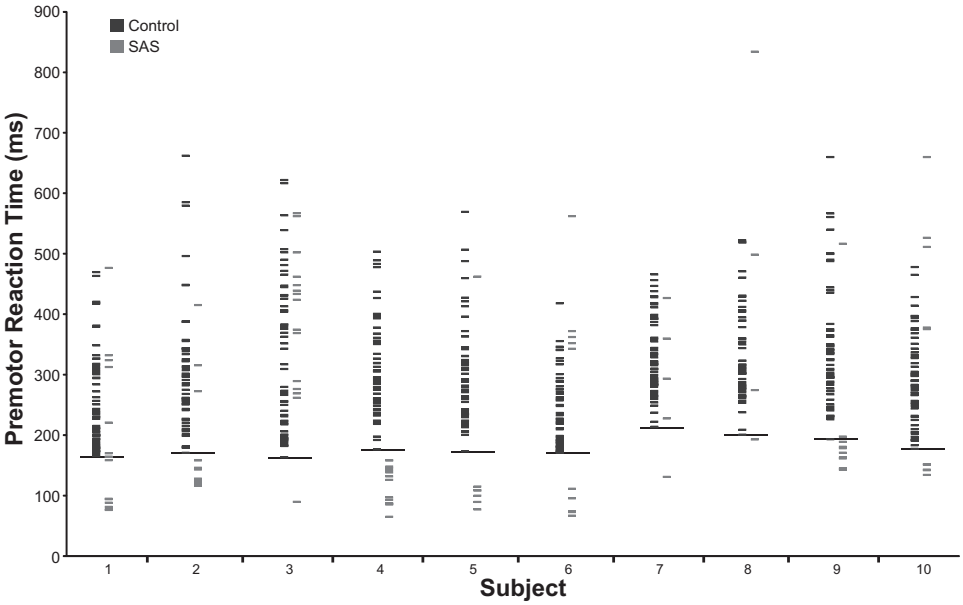


Fig. 3. Individual trial premotor reaction time observed during the stop-signal task for each participant. Control trials are shown in dark gray and startle trials (SAS) in light gray. Black horizontal lines indicate participants' fastest control trial RT and were used as a cutoff to define "early" responses (see RESULTS, *Response latency*, for details).

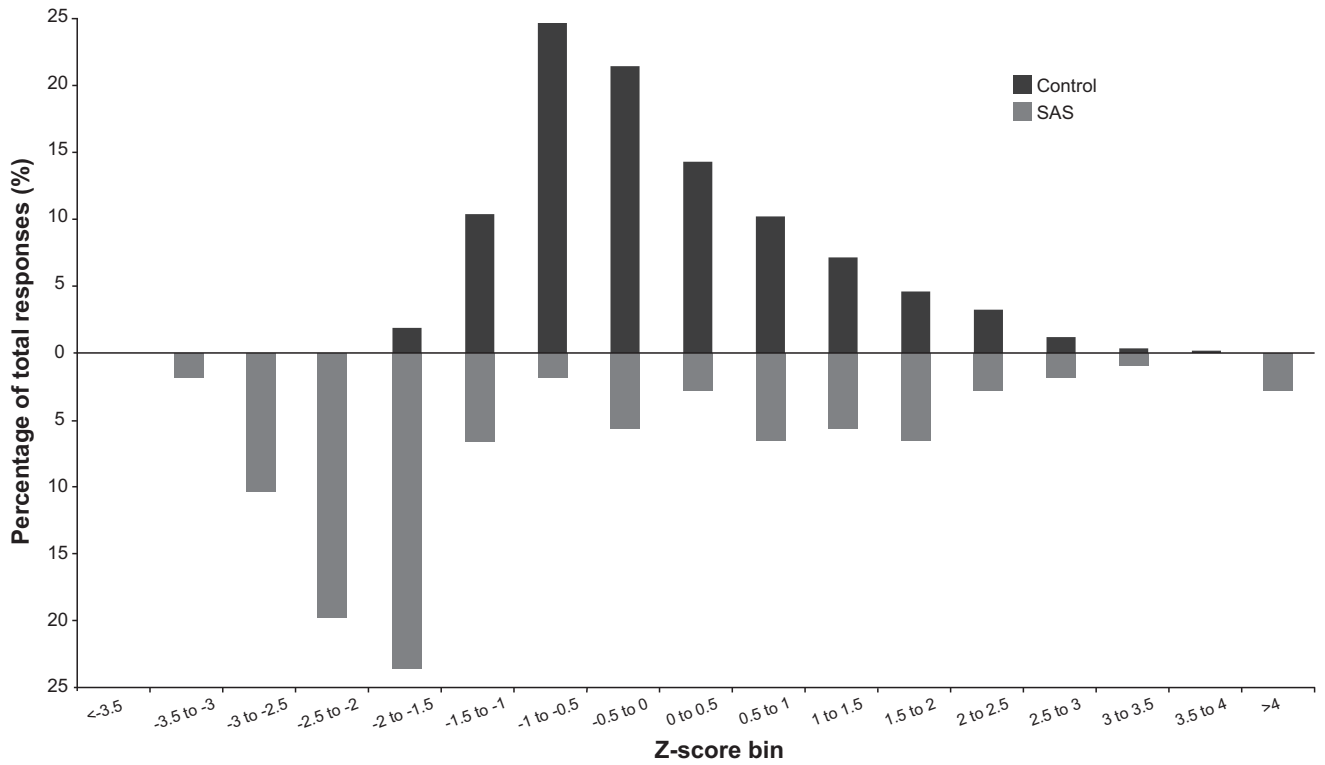


Fig. 4. Percentage of total responses as a function of *Z*-score bin for control trials (*top*, dark gray) and startle trials (SAS; *bottom*, light gray). Note the bimodal distribution within SAS data, with the SAS either reducing RT (mean *Z* score −2.023) or delaying RT (mean *Z* score 1.144) relative to mean control RT.

position for early SAS responses were significantly smaller ($P < 0.01$) than the final position achieved during simple RT task control trials (mean 27.97°, SD 7.09°) and simple RT SAS responses (mean 30.26°, SD 7.27°). Analysis of peak displacement also revealed a significant main effect of task [$F(1,9) = 21.938$, $P = 0.001$, $\eta_p^2 = 0.709$] and a significant interaction [$F(1,9) = 19.213$, $P = 0.002$, $\eta_p^2 = 0.681$], but no main effect of stimulus [$F(1,9) = 0.751$, $P = 0.409$, $\eta_p^2 = 0.077$]. Post hoc comparisons revealed no differences ($P = 0.23$) in peak displacement between control and early SAS responses in the stop-signal task (mean 25.86°, SD 2.17°); however, all others were significantly different ($P < 0.05$) such that peak displacement was always smaller in the stop-signal task compared with the simple RT task (mean 36.99°, SD 4.56°) and peak displacement was larger during simple RT task SAS responses (mean 40.21°, SD 4.63°).

## DISCUSSION

The purpose of the present study was to investigate preparatory activation of the motor response during a stop-signal task by presenting a startling acoustic stimulus (SAS) concurrent with the go signal. Previous work has demonstrated that with sufficient response preparation, an SAS can involuntarily trigger the "early" release of the prepared movement (Carlsen et al. 2004b, 2012; Castellote et al. 2007; Siegmund et al. 2001; Valls-Solé et al. 1995, 1999). The results from the simple RT task in our experiment replicate these findings, demonstrating significant speeding (Δ 122 ms) of premotor RT during SAS trials compared with control (see Fig. 2). Furthermore, these SAS RTs were of sufficiently short latency (89 ms) that it is unlikely that normal voluntary cortical processes were employed in initiation of the response (Carlsen et al. 2012). It appears that in the simple RT task, preparatory response activation was sufficiently high to allow for short-latency involuntary triggering by the SAS. Further support for this high level of advance preparation during the simple RT task comes from the high proportion of startle responses and "early" responses observed (Fig. 1). Results from the stop-signal task indicate that participants were performing the task correctly, and as expected, premotor RT in the control trials (303 ms) was significantly slower compared with that in the simple RT task (211 ms). In contrast to the simple RT task, no significant shortening in mean response latency was observed between SAS and control trials in the stop-signal task (see Fig. 2), suggesting that the response was not (highly) prepared in advance. However, further analysis of these stop-signal task SAS trials revealed the presence of two RT distributions within the data, consisting of *1*) fast (or "early") and *2*) moderately delayed responses (Figs. 3 and 4). The "early" SAS response distribution indicates that on a trial-to-trial basis the response was sometimes sufficiently prepared to be triggered early by the SAS. However, these early SAS-triggered responses during the stop-signal task were nevertheless elicited later (mean 127 ms) compared with the SAS triggered responses in the simple RT task (mean 89 ms), which suggests responses were prepared, but at a decreased level of preparatory activation compared with the simple RT task. Kinematic analyses revealed no differences in the overt responses produced between SAS and control trials within each task, suggesting that the "early" startle-triggered response was indeed the planned "go" motor response. Differences in kinematics found between tasks are likely due to practice effects; that is, because the simple RT

task was carried out first (see *Apparatus and task*), participants became more accurate and efficient at preparing and performing the 20° targeted response as time progressed, resulting in performance benefits during the second (stop signal) task. The stop-signal task also exhibited a decrease in the proportion of startle reflex responses observed in SCM, suggesting that during some stop-signal task trials, preparation was dramatically reduced such that it may be considered absent. Together, these findings suggest that longer RTs typically observed on "go" trials during the stop-signal task compared with a simple-RT task may be attributed to a decrease in response preparatory activation.

*Reduced preparatory activation.* A startle response (i.e., SCM+) was elicited in a majority of stop-signal SAS trials (75%), suggesting that the motor system was engaged in at least some advance preparation (Carlsen et al. 2012; Waters-Metenier et al. 2014). However, a null effect of SAS on RT when a startle reflex was elicited (SAS trials vs. control trials) during the stop-signal task suggests that the level of preparation was too low for the voluntary response to be consistently triggered early by the SAS. On closer inspection, the RT data revealed that the SAS had two contrasting effects. As shown in Fig. 3, premotor RT observed during SAS trials within each participant was not stereotyped; instead, responses appeared to fall into one of two distributions. Analysis of *Z*-score-transformed SAS RT data confirmed the presence of bimodality within the RT distribution, revealing a "fast response" distribution occurring at a mean of −2.02 SD from control RT and a "moderately delayed response" distribution occurring at a mean of +1.14 SD (Fig. 4). The presence of this fast-response distribution suggests that on a number of trials the level of preparation was sufficiently high to enable startle to involuntarily trigger the prepared response. Since the additional activation that startle provides to the motor system is presumably constant across SAS trials (Maslovat et al. 2014), the latency at which responses are involuntarily triggered by startle can provide insight into the level of preparatory activation achieved. It was hypothesized that if the increased RT observed during the performance of a stop-signal task was the result of a reduced level of voluntary preparation, SAS-triggered responses during the stop-signal task would have a longer RT compared with the same responses triggered during a simple RT task. Indeed, results showed that "early" SAS-triggered responses during the stop-signal task (mean 127 ms) were elicited later compared with the SAS triggered responses in the simple RT task (mean 89 ms), indicative of a decreased amount of preparatory activation during the stop-signal task (see Fig. 2). In addition to differences in startle-triggered response latency, evidence of reduced preparation during the stop-signal task can be seen by the reduced proportion of SAS trials that resulted in the early triggering of the response during the stop-signal task (56%). In contrast, the simple RT task showed a high level of advance preparation as evidenced by a high probability of the SAS triggering an early release of the response (100%) (Fig. 1).

Modulation of preparatory activation between tasks is likely a result of the strategy chosen to comply with task demands. The goal in the simple RT task was to initiate a response as fast as possible, and given that the go signal was always presented, participants were able to hold preparatory activation very close to threshold to decrease the time needed to initiate the response when the go signal was inevitably presented, resulting in fast RTs. In contrast, instructions in the stop-signal task were to initiate a response as fast as possible in the go trials and to withhold a response in the stop trials, requiring participants to balance the speed of the go response with the possibility of having to inhibit it, resulting in slower RTs. Our results suggest that during the stop-signal task, the level of preparatory activation was not held as close to initiation threshold by way of a strategy to deal with the potential of having to inhibit response initiation. In this way, the time needed for go activation to reach threshold would be increased to allow inhibitory processes sufficient time to inhibit response output if necessary. In support of these results, Ko et al. (2015) showed that EEG-derived measures of preparatory activation corresponded with stopping success during a choice-selective stopping task. Specifically, during stop trials in which participants failed to stop, there was a larger amount of voluntary preparatory activation compared with trials in which participants successfully stopped (Ko et al. 2015). Thus our results support the suggestion that the ability to inhibit motor output depends not only on the speed and strength of inhibitory processes but also on the amount of voluntary preparatory activation related to the go response.

Alternatively, the decrease in proportion of early responses as well as the increased SAS RT observed in the stop-signal task startle trials may be due to inhibition imposed by the motor system, rather than a reduced preparatory activation level per se. Two distinct mechanisms for inhibitory control have been proposed to exist: reactive and proactive inhibition (Aron 2011). Reactive inhibition has been described as a late correction mechanism, relying on the detection of a stimulus that signals the stopping of a planned or ongoing response, resulting in global nonspecific suppression of the motor system suggested to be implemented via the hyperdirect pathway (see Aron 2011 for pathway details). In contrast, proactive inhibition is thought to use task- and goal-relevant information to inhibit the motor system before the movement is performed, allowing one to plan for the possibility of stopping (Braver 2012). Proactive inhibition results in the suppression of response channels specific to the motor representation that may be or is being stopped, which is suggested to be implemented via the indirect pathway (see Aron 2011 for pathway details).

According to these definitions, a proactive inhibitory mechanism would have been invoked to account of the present results because preparatory activation was probed with an SAS concurrent with the go signal (i.e., before any possibility of being able to react to any stop signal). However, several lines of evidence suggest that reactive inhibition was likely used for stopping the response (if necessary) in the current stop-signal task. First, proactive inhibition is typically only observed during the performance of a selective stop-signal task, where advance information is provided indicating that one limb in a bimanual response may have to be stopped in the upcoming trial (e.g., "maybe stop right"; Cai et al. 2011; Majid et al. 2012). In contrast, reactive inhibition is typically observed during the performance of more traditional stop-signal tasks, such as the one used in the current study, which do not provide a precue (Majid et al. 2012). Although there is some evidence that both reactive and proactive inhibition might be engaged in the same traditional stop-signal task, proactive inhibition has only been seen on trials directly following stop-signal trials,

which showed longer response times (Chen et al. 2010). Given that in the current study the SAS was never presented following a stop-signal trial, this limits the potential for proactive inhibition to have influenced SAS RT results. Second, proactive inhibition has consistently been shown to result in longer RTs during go trials and longer stopping times (i.e., increased SSRT) during stop-signal trials compared with reactive inhibition (Greenhouse et al. 2012; Jahfari et al. 2011). Based on task demands of the current stop-signal task (i.e., react as fast as possible to the go signal but try to inhibit/withhold the response if a stop signal was presented), the use of a reactive mode of inhibition would be strategically beneficial for task performance compared with proactive inhibition in the current task.

In other inhibitory tasks such as a go/no-go task, it may be more strategically beneficial to use proactive inhibition to inhibit the motor system in advance of the imperative stimulus to prevent a false go response on no-go trials. Although speculative, results from go/no-go studies employing an SAS to probe preparatory activation concurrent with the go signal (Carlsen et al. 2008; Kumru et al. 2006) are more consistent with the use of proactive inhibition as opposed to a more reactive mode of inhibition in the current study. Specifically, during a go/no-go task, presentation of an SAS resulted in a significant decrease in the magnitude of the startle response (area of SCM EMG activity) compared with a choice RT task (Kumru et al. 2006). In addition, in a go/no-go task an SAS was not effective for involuntarily triggering the early release of the response (Carlsen et al. 2008). These results suggest that a high level of proactive inhibition may be placed on the motor system in these types of tasks to facilitate the withholding of a response in the case of a no-go stimulus.

In contrast to the SAS results obtained in a go/no-go task (Carlsen et al. 2008; Kumru et al. 2006), the present experiment found no differences in the onset or integrated area (Q100) of the startle response between simple and stop-signal tasks and showed that an SAS can often involuntarily trigger the early release of the response (albeit with a delayed onset and reduced frequency compared with a simple RT task). This again suggests a reactive mode of inhibitory control was likely used in the present task as opposed to proactive inhibition. These differences in the effects arising from a SAS between stop-signal and go/no-go tasks suggest that different modes of inhibition are likely used for each type of task (reactive inhibition in typical stop-signal tasks vs. proactive in go/no-go tasks). Importantly, reactive inhibition alone cannot account for the delay in "early" SAS RTs observed in the stop-signal task compared with the simple RT task, because inhibition would only occur after the presentation of a stop signal and thus could not influence the SAS trials (Badry et al. 2009; Majid et al. 2012). Taken together, the current evidence suggests that performance during a typical stop-signal task is likely governed by a decrease in voluntary response preparatory activation preceding the go signal, followed by a primarily reactive mode of inhibitory control in response to a stop signal.

*Absence of response preparation.* Although the presentation of an SAS during the stop-signal task often resulted in a startle response in SCM, it nevertheless occurred significantly less often (−19%) than during the simple RT task (Fig. 1). This decrease in the incidence of SCM activation provides additional insight into the processes occurring before response initiation, as well as the source of the increase in RT observed during the stop-signal task. One potential explanation for the decrease in the proportion of startle responses observed between tasks is that because the stop-signal task was always performed following the simple RT task, participants may have habituated to the SAS over the course of the experiment. This account is unlikely because, unlike previous studies documenting startle habituation (Abel et al. 1998; Valls-Solé et al. 1997), we found no reduction in the size (Q100) of the startle response between the simple RT and stop-signal tasks.

The more probable explanation for the decreased incidence of SCM activation observed during the stop-signal task is that participants were simply not preparing (or at least not enough preparatory activation had accrued) before the go signal. Previous studies have shown that readiness to perform a response during a simple RT task prevents habituation of the reflexive startle reaction, presumably due to enhanced excitability of the motor pathway (see Carlsen et al. 2011 for a review; Valls-Solé et al. 1997). In contrast, a diminished probability of eliciting a startle response in SCM can be found in tasks in which advance response-specific motor preparation is largely limited (e.g., choice RT task). Evidence for an absence of advance preparation can be seen in the distribution of control RTs during the stop-signal task, with a large within-participant range observed (mean RT range 357 ms, SD 85 ms) compared with that observed in the simple RT task (mean 71 ms, SD 27). This increased within-participant range observed during the stop-signal task compared with the simple RT task is likely due to larger variations in the amount of preparation trial to trial. Support for this assertion comes from previous studies that have shown that variations in baseline preparatory activation levels and the rate of rise of activation can account for the variability in RT (see Gold and Shadlen 2007; Munoz and Everling 2004 for review). Moreover, preparatory activation level has been suggested to be more predictive than rate of rise of trial-to-trial variability of RTs (Connolly et al. 2005; Dorris and Munoz 1998; Dorris et al. 1997; Everling and Munoz 2000; Lecas et al. 1986; Riehle and Requin 1993). Thus in the present study, the decreased incidence of observing a reflexive startle response together with the large range of control trial RTs observed during performance of the stop-signal task suggests that preparation preceding the go signal was not homogenous across trials and provides evidence that on a small proportion of trials participants did not sufficiently prepare the go response in advance. This dramatic reduction of advance preparation on a small proportion of trials provides a parsimonious explanation for the positively skewed data typically observed during the performance of the stop-signal task (Verbruggen and Logan 2009).

*Conclusion.* In summary, the use of a startling acoustic stimulus (SAS) provides novel insight regarding the motor preparatory state of the go response during a stop-signal task. Results from SAS trials during the performance of a stop-signal task indicate that although participants were often preparing a go response, the level of that voluntary preparatory activation was likely reduced compared with that achieved during a simple RT task. These findings suggest that longer RTs typically observed during a stop-signal task may be attributed to a decrease in preparatory activation of the voluntary response. Reducing the level of preparatory activation relative to threshold for response initiation may be one strategy to deal with the potential of having to inhibit response initia-

tion. Participants appear to trade off preparatory activation, resulting in more time to allow inhibitory processes to inhibit response output if a stop signal is presented. On a small proportion of stop-signal trials, this preparation appears to be dramatically reduced such that it may be considered absent. This heterogeneity in preparatory strategy also provides a neural explanation for the range and distribution of RTs typically observed in the stop-signal task literature.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

N.M.D., E.K.C., and A.N.C. conception and design of research; N.M.D. performed experiments; N.M.D., E.K.C., and A.N.C. analyzed data; N.M.D., E.K.C., and A.N.C. interpreted results of experiments; N.M.D., E.K.C., and A.N.C. prepared figures; N.M.D. drafted manuscript; N.M.D., E.K.C., and A.N.C. edited and revised manuscript; N.M.D., E.K.C., and A.N.C. approved final version of manuscript.

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