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Startle neural activity is additive with normal cortical initiation-related activation

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HIGHLIGHTS

- Subjects performed a simple reaction time (RT) task in response to an auditory cue.
- A startling acoustic stimulus (SAS) was presented during the RT interval.
- Results indicated that both voluntary and SAS initiation process jointly occur.
- We argue that an additive model of initiation-related activation can explain the results.

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ABSTRACT

The current study examined the process of response initiation in a simple reaction time (RT) task using a startling acoustic stimulus (SAS), which has been shown to trigger a prepared movement through an involuntary initiation pathway. The SAS was presented within the RT interval (concurrent with, and 25, 50, 75, 100, and 125 ms following the "go" signal), with the observed response latency used to examine the relative contributions of voluntary and involuntary activation to response initiation. Our results clearly indicate that both voluntary and startle-related initiation activation jointly contribute to the observed RT. The data support a model in which startle-related neural activity is additive with voluntary cortical initiation-related activation. This result also provides indirect support for the hypothesis that both voluntary and SAS-related involuntary activation involve a similar process of response output.

1. Introduction

In a simple reaction time (RT) paradigm it is unknown exactly when the response is to be performed, but knowing the required response in advance allows response selection and preparation to occur prior to the "go" signal. However, in these situations, RT values are considerably longer than what would be expected for pure stimulus detection, with the additional time interval thought to involve response initiation processes. The processes of response preparation and initiation have been recently described using a neural accumulation model [1] in which the preparation of a movement can be conceptualized as increasing activation of a neural network of cortical neurons to some level below threshold [13]. Initiation of the movement is then achieved through additional activation of the network beyond the "ignition point," leading to motor output (see [4] for a similar model involving saccade initiation).

The purpose of the current experiment was to probe the neural activation underlying the process of response initiation in a simple RT paradigm by using a loud acoustic stimulus, capable of eliciting a startle reflex. Previous work involving a startling acoustic stimulus (SAS) has shown that a pre-programmed movement can be triggered at a shorter latency by a SAS presented concurrent with the "go" signal via a faster, brainstem-mediated initiation process. In a normal (non-SAS) RT trial, the "go" signal is processed in sensory structures such as the primary auditory or visual cortices, leading to movement initiation through voluntary increases in neural activation. However, in a SAS trial, it is thought that a response that has been prepared in advance is initiated involuntarily by activation provided by neural circuits associated with the startle reflex. Thus a SAS can be used to determine if and

Abbreviations: ECR, extensor carpi radialis; FCR, flexor carpi radialis; RT, reaction time; SAS, startling acoustic stimulus; SCM, sternocleidomastoid.

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when substantial response preparation has occurred by examining whether the expected response was triggered at short latency (see [1,2,11] for recent reviews).

In the current study we presented the SAS at regular intervals *after* the "go" signal but before response onset (i.e., during the RT interval) to examine the effect of a SAS presented during the voluntary initiation process. Although the neural pathways involved in the startle reflex are well known, it is currently unclear how the SAS interacts with neural circuits to trigger the prepared response. One explanation for a shortened response latency in SAS trials involves increased activation of the reticular structures that are responsible for the startle reflex, suggesting sufficient detail of the movement characteristics are stored and triggered from subcortical structures including the brainstem and spinal centres [12]. For response initiation this subcortical triggering hypothesis would predict a "horse-race" between processes where response initiation would either occur from brainstem structures (resulting in startle-like RTs, relative to when the SAS was presented) or from cortical structures (resulting in control-like RTs), depending on whether the voluntary or SAS activation reached the prepared response first. Alternatively, it has recently been proposed that SAS may result in shortened response latency by the startle increasing motor cortical activation via an ascending reticulo-thalamo-cortical circuit; a faster pathway that results in the movement being initiated earlier from the same cortical neural network [1]. This hypothesis would predict that the voluntary and involuntary initiation processes may occur simultaneously, jointly contributing to response initiation-related activation.

2. Materials and methods

2.1. Participants

Data are presented from fifteen healthy participants (9F, 6 M; 24 ± 5 years) with no sensory or motor dysfunctions, who showed a consistent reflexive reaction to the SAS (see below). All participants gave written informed consent and reported normal hearing. This study was approved by and conducted in accordance with the ethical guidelines set by the Behavioural Research Ethics Board at the University of Ottawa and conformed to the latest revision of the Declaration of Helsinki.

2.2. Apparatus and task

Participants sat in a chair facing a 17 in. LCD computer monitor with their right arm resting in a custom manipulandum that restricted movement to wrist flexion and extension, with the forearm parallel to the floor and the palm facing inwards. The shoulder was abducted approximately 15°, and the arm was secured using Velcro straps placed proximal to the wrist and distal to the elbow. The task for the participant was to perform a ballistic 20° wrist extension movement from neutral (wrist neither flexed nor extended) "as quickly as possible" following an auditory imperative "go" stimulus. Feedback was provided on the computer monitor after each trial consisting of RT on that trial and accuracy with respect to the target. A points scheme was also provided to encourage fast RTs.

2.3. Instrumentation and stimuli

A warning tone (100 ms, 200 Hz) was followed by a variable foreperiod (2000–2500 ms), and finally an imperative "go" signal consisting of an 82 dB, 25 ms, 1000 Hz sine wave that was generated using digital to analog hardware (PCI-6024E, National Instruments). The signal was amplified and presented via a loudspeaker (MG Electronics M58-H, frequency response 300 Hz–11 kHz, rise time <1 ms) located 30 cm directly behind the participant at head height. Participants performed 5 blocks of 30 RT trials that emphasized fast reaction times in response to the sound.

In 20% of trials a startling acoustic stimulus (SAS), consisting of a 120 dB, 25 ms, white noise waveform (equal power from 1 Hz to 22 kHz), was presented at six different delay intervals (0, 25, 50, 75, 100, 125 ms) *following* the "go" signal. Stimulus intensity was confirmed using a precision sound level metre located at the same distance from the loudspeaker to the ears (Casella model CEL-254, A-weighted scale, impulse setting). Participants were told that on some trials they would hear a loud "static noise" sound that could be ignored. The SAS was presented pseudorandomly such that no two consecutive trials included a SAS, no SAS was presented in the first 2 trials of each block, and each SAS delay interval occurred in a random order, once in each 30 trial block. Participants performed up to two practice blocks of 10 trials (without SAS) to familiarize themselves with the task and equipment.

Surface electromyographic (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) muscles using bipolar preamplified surface electrodes connected to an external amplifier system (Delsys Inc.). Wrist angular position data were collected using a potentiometer attached to the central axis of the manipulandum. On each trial, unfiltered EMG and position data were digitally sampled at 1 kHz (National Instruments PCI-6024E via BNC-2090) for 3 s beginning 500 ms prior to the "go" signal using a customized programme written with LabVIEW software (National Instruments Inc.).

2.4. Data reduction and analysis

Peak displacement and velocity were defined as the points at which displacement and velocity decreased following displacement onset (angular displacement of more than 0.2°). Surface EMG burst onsets in all muscles were defined as the point at which the EMG first began a sustained rise 2 standard deviations above baseline levels (see [2] for details). Premotor RT was defined as EMG onset in the ECR muscle. To determine startle response incidence, trials were separated by whether or not an EMG burst was observed in SCM within 120 ms following SAS onset (indicative of startle related activity, see [2]). In order to investigate the effect of a startling stimulus on kinematic and EMG variables, only SAS trials where a startle response was observed in SCM were included in these analyses [2].

2.5. Statistical analyses

The proportion of trials in which an EMG response in SCM was elicited by the SAS was analyzed using a one-way, 6 factor (SAS delivery: 0, 25, 50, 75, 100, 125 ms), repeated measures analysis of variance (ANOVA), to determine if SAS presentation time led to any differences in startle response incidence. Prior to analysis proportion data were subjected to an arcsine square root transform to correct for violations to normality [7]. Similarly, premotor RT, peak displacement, time to peak displacement, peak velocity and time to peak velocity were analyzed using one-way, 7 factor (SAS delivery: none, 0, 25, 50, 75, 100, 125 ms), repeated measures ANOVA, to determine if there were differences in EMG onset and quality of movement produced. Greenhouse–Geisser corrected degrees of freedom were used to correct for any violations of sphericity. Differences with a probability of less than .05 were considered to be significant. Partial eta squared (η_p^2) is reported to provide an estimate of the proportion of the variance that can be attributed to the tested factor. Tukey's HSD

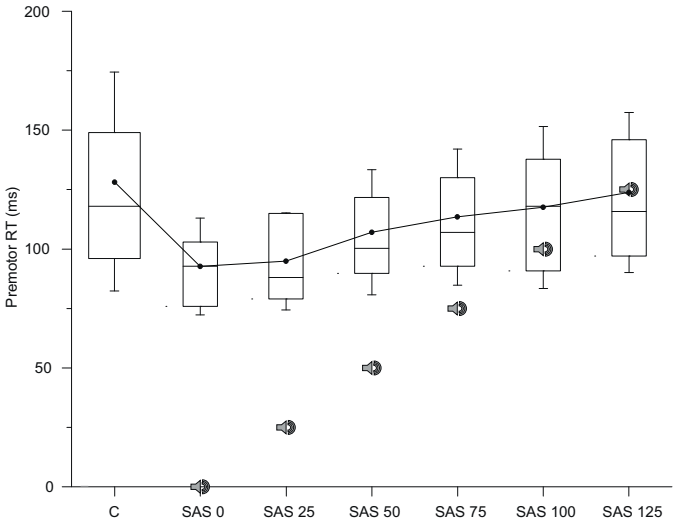

Fig. 1. Premotor reaction time (RT) boxplots. RT distributions are shown for control trials (C) and trials where the startling acoustic stimulus (SAS; speaker icon) was presented 0–125 ms following the "go" signal. Box boundaries represent the first and third distribution quartiles (horizontal line = median RT). Points connected by the line inside the boxplot represent mean RTs (error bars = 1 SD).

post-hoc tests were administered to determine the locus of the differences.

3. Results

3.1. Startle response

Analysis of the proportion of trials in which a startle response was detected revealed a main effect of SAS presentation time $F(5,70) = 8.628$, $p < .001$, $\eta_p^2 = .381$. Post-hoc analysis showed that presenting the SAS coincident with, and 25 ms following the "go" led to a higher proportion of trials where a SCM EMG burst was elicited ($88.0 \pm 14.7\%$ and $88.0 \pm 21.1\%$ respectively) compared to when the SAS was presented at 100 and 125 ms following the "go" ($62.6 \pm 30.1\%$ and $57.3 \pm 36.1\%$ respectively). When the SAS was presented at 50 or 75 ms following the "go" a SCM burst was elicited in $80.0 \pm 23.9\%$ and $72.0 \pm 23.7\%$ of trials respectively.

3.2. Response latency

Response latency analysis confirmed a significant main effect for SAS delivery time, $F(6,84) = 19.928$, $p < .001$, $\eta_p^2 = .587$. While not all comparisons will be highlighted here, post-hoc decomposition of the main effect with Tukey's HSD showed the mean difference required to be considered significant ($p < .05$) was 14.7 ms. In general, later responses were observed following later SAS presentations (see Fig. 1) and when a SAS occurred coincident with, or up to 50 ms following the "go" signal, a significantly ($p < .05$) earlier onset of voluntary response-related EMG was observed compared to control. Of particular note was that no difference was found in voluntary response onset between a SAS presented at 0 or 25 ms following the "go" (mean difference = 3.3 ms), and there was no difference in response onset between non-startle trials and when a SAS was presented at 75, 100, or 125 ms.

3.3. Voluntary response characteristics

Kinematic variables were analyzed to determine if a similar response occurred when participants were startled. A main effect of SAS delivery time was found for peak displacement only, $F(6,84) = 4.751$, $p < .001$, $\eta_p^2 = .253$, which post-hoc analyses revealed significantly greater displacement when the SAS was presented at 0 ($30.6 \pm 5.3^\circ$) and 25 ms ($30.1 \pm 5.4^\circ$) following the "go" compared to control ($25.9 \pm 3.5^\circ$). No significant differences were found in peak velocity ($p = .137$), time to peak velocity ($p = .481$), or in time to peak displacement ($p = .177$).

4. Discussion

The purpose of this study was to examine the neural activation underlying the process of response initiation by presenting a startling acoustic stimulus (SAS) during the RT interval for a known response. Previous work has shown that the use of a SAS can result in the short latency triggering of a prepared movement through an involuntary initiation process, although there is currently debate surrounding the mechanism by which this occurs. It may be that the prepared response is stored subcortically and carried out via reticulospinal pathways [12], or stored cortically and executed via corticospinal pathways [1]. A "horse-race" model in which either the voluntary or involuntary initiation processes determines the observed latency of response would predict that latencies of SAS-triggered responses would increase commensurate with SAS delay, up until they were no longer faster than those in control (non-SAS) trials (127 ms); that is, at some point the normal cortical response initiation pathway would be faster than a delayed SAS pathway. However, data from the current study suggest that both the voluntary initiation-related activation *and* SAS-related involuntary activation interacted at a cortical level and contributed to the early response onsets as the speeding effect of startle was much greater than would be predicted by a horse-race model. For example, when the SAS was delivered concurrent with the "go" signal, a response latency of 90 ms was observed, similar to previously reported values [1]; however, when the SAS was delivered 25 ms later, the latency of the response only increased by ~3 ms – resulting in a response latency relative to SAS presentation of only 68 ms (Fig. 1). Similarly, when the SAS was presented 50 or 75 ms following the "go" signal, the wrist extension response was only delayed by 16 ms and 25 ms respectively, compared to a SAS presented coincident with the "go" (i.e., latencies relative to the SAS of 56 ms and 40 ms – see Fig. 1). Because the observed RTs were faster than would be expected in response to either the control tone or SAS alone, our results strongly suggest that there is a cumulative effect of both voluntary and startle-related initiation processes that contribute to the shortened response latency.

In order to determine the relative contributions of voluntary and SAS-related activation, we calculated the amount of time that could be attributed to the initiation process in both startle and non-startle trials. This "initiation time" was defined as the time between when initiation-related cortical activation begins to increase and when a threshold is reached such that motor commands are sent to the muscles. During voluntary movement initiation, it has been estimated that it takes approximately 35 ms for an auditory stimulus to reach primary auditory cortex [3], at which time the cue is recognized and the response initiation activation begins to increase. Furthermore, studies employing transcranial magnetic stimulation have shown that approximately 25 ms are required for nerve conduction from primary motor cortex to the arm muscles [9]. Applying these values to the observed control trial data, we assumed initiation-related activation would begin to rise from baseline 35 ms following the "go" signal (Fig. 2, point A) and reach threshold at 102 ms (observed mean premotor RT of 127 ms minus 25 ms for conduction time – Fig. 2, point B), resulting in 67 ms of "initiation time." In other words, if we use an arbitrary neural activation threshold of 100% (with 0% being a pre-stimulus baseline value), we can connect point A and B to create a slope where cortical activation reaches the threshold for motor output in

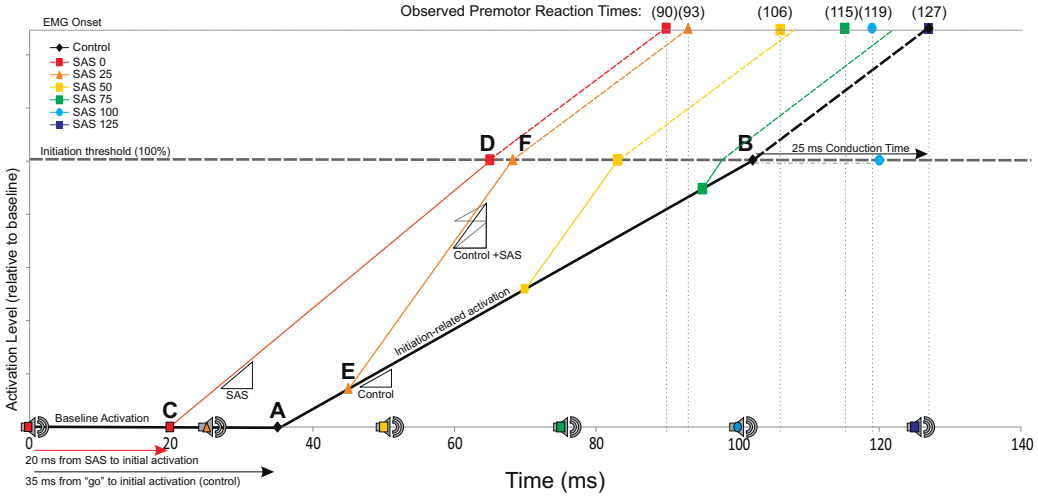

Fig. 2. Additive activation model (see also [10]). Time (ms) is on the horizontal axis and neural activation (arbitrary units) on the vertical axis. Horn icons with associated colour symbols show times when a startling acoustic stimulus (SAS) was presented. Observed mean premotor reaction time (RT) for the experimental conditions is shown at top. Sloped lines below the horizontal grey dashed line (response initiation threshold) represent calculated or predicted increases in initiation-related activation for each condition. Lines above threshold show 25 ms of nerve conduction. Point A represents when non-startle (control) initiation-related activation begins to rise above baseline, Point B represents when control trial activation has reached a threshold whereby motor commands are output from cortex to the muscles, with a calculated slope (black) drawn between Point A and B to represent non-startle initiation-related activation. The slope drawn between Points C and D (red) represents calculated initiation-related activation time when the SAS was presented coincident with the "go" signal. Conditions in which a SAS was presented in the RT interval are modelled by adding the control and startle slopes during the time frame when both processes are occurring simultaneously. Note that addition of control and SAS slopes (see triangles) results in RT predictions that correspond closely to observed values: for example, the SAS 25 condition shows an additive slope (orange) between points E and F, resulting in a predicted premotor RT value of 93 ms (identical to that observed). See Section 4 for further details.

67 ms (see black line, and control slope triangle, Fig. 2). For startle trials, the involuntary initiation-related activation is thought to begin to rise from baseline much sooner through a faster, startle-related reticulo-thalamo-cortical circuit, requiring approximately 20 ms following the presentation of the SAS (see [1] for detailed timing and pathway information) (Fig. 2, point C). Assuming a similar 25 ms conduction time to the muscles, we calculated that the threshold for motor output was reached at 65 ms (observed SAS premotor RT of 90 ms minus 25 ms for conduction time to the muscle – Fig. 2, point D). Connecting points C and D results in a SAS initiation slope that only requires 45 ms for the same 100% neural activation increase (see red line and SAS slope triangle, Fig. 2).

Using these calculated initiation times for both voluntary and involuntary initiation processes we examined whether an additive model of pre-response activation similar to that proposed by Siegmund et al. [10] might fit our results. This model posits that control and startle-related activation are summed prior to the response onset. For example, when the SAS was presented 25 ms following the "go" signal in the current experiment, we assumed that voluntary initiation-related activation would begin to increase as normal at 35 ms; at the same time, the involuntary SAS-related activation would begin to increase starting at 45 ms (Fig. 2, point E), calculated as 20 ms following the presentation of the 25 ms-delayed SAS. Thus, normal voluntary initiation-related activation would occur from 35-45 ms, after which both voluntary and involuntary initiation activation would occur concurrently. This can be modelled by following the voluntary activation slope until 45 ms and then summing the voluntary and involuntary activation slopes (see Fig. 2, orange line and control + SAS slope triangles) until 100% threshold is achieved. Based on this calculation, initiation threshold would be predicted to be reached at 68 ms (Fig. 2, point F), resulting in a premotor RT of 93 ms, identical to the observed value of 93 ms. Using the same calculations, when the SAS was presented at 50 ms, startle-related initiation activation would not occur until 70 ms following the "go" signal, meaning that voluntary initiation would occur as normal from 35 to 70 ms, at which time an additive effect with startle-related activation would occur. In this case, the model predicts a premotor RT of 108 ms, again closely matching our observed result of 106 ms (Fig. 2, yellow line and symbols). Lastly, presenting the SAS at 75 ms provides little time for additive activation, with the model predicting a RT of 122 ms (green line), as compared to the observed value of 115 ms. As modelled here, motor commands are output to the muscles on non-startle trials once voluntary initiation reaches threshold at a mean time of 102 ms (Fig. 2 – point B). Thus, when the SAS is presented at 100 ms or later, startle-related initiation activation should have no effect on the response latency as there is no opportunity for additive activation. This is supported by our data as there was no significant RT reduction when the SAS was presented at 100 ms (M = 119 ms) or 125 ms (M = 127 ms) following the "go" signal, relative to control trials (M = 127 ms). Based on the above calculations, we argue that our data support a model of pre-response summation similar to that proposed by Siegmund et al. [10]. Although this experiment cannot definitively determine where summation occurs, we suggest that our timing data indicates that summation occurs prior to the output of task- or startle-related activation from central structures. Thus, we propose that a common response initiation structure underlies both normal voluntary and SAS-triggered responses.

Although an additive model predicts no change in RT when the SAS was delivered 75 and 100 ms following the "go" signal, we did observe a small but non-significant decrease in RT of 12 and 8 ms respectively, compared to control (e.g., see difference between green line and observed RT, Fig. 2). One possible explanation for these RT decreases is that the reported mean control RT value used in the model consists of a distribution of latencies that includes faster and slower values on individual trials. While the calculations associated with the mean value do not predict any RT shortening, individual trials with longer RTs would allow sufficient time for a short period of additive activation. This could result in a small decrease in mean RT associated with a change in RT distribution such that they are fewer long latency RTs. Although more research is required to examine this explanation in more detail, some evidence for this is provided by the RT distribution boundaries shown in Fig. 1 (e.g., SAS 75 vs. SAS 125).

In addition to finding the shortest RT values when the timeframe of additive activation was greatest (i.e., SAS presented at 0 ms or 25 ms), there was also a significantly greater incidence of a startle reflexive response at these times compared to when there would be little or no additive activation (i.e., SAS presented at ≥100 ms). This suggests the reflexive circuits associated with the startle response may have higher excitability prior to movement initiation as compared to when these processes are already well underway. Further support for additive activation is provided by the kinematics of the observed response. Numerous studies have found greater muscle burst amplitude and/or exaggerated kinematics during startle trials, a result typically attributed to additional activation of the nervous system due to the SAS [6,10]. In the current study, when the SAS was delivered coincident with or 25 ms following the "go" signal, peak displacement was significantly greater than in control trials, similar to previous data. These time periods coincide with the points at which participants were more likely to exhibit a reflexive startle response, which we have attributed to higher excitability in the startle-related pathways. It is also possible that for these earlier SAS presentations, startle-related activation contributes to movement initiation for a greater amount of time (Fig. 2, red and orange lines), leading to a greater likelihood that this increased activation would affect the movement kinematics.

Previous research has employed a loud acoustic stimulus during the RT interval and has shown results that are dissimilar to those found in present experiment. Kumru and Valls-Solé [5] presented a SAS in 20 ms intervals up to 100 ms following the visual "go" and reported a fairly linear rise in RT, increasing approximately 20 ms with each later SAS delivery. However, this study employed a visual "go" signal in contrast to the auditory "go" used here. Since more time is required to process the visual "go" signal [8], substantially longer control RTs were reported (mean of 188 ms compared to 127 ms in the current study), suggesting that voluntary initiation processes began later. A later onset of voluntary initiation would lead to the prediction that RTs would increase consistent with SAS delay for a longer time since activation would not be additive until much later than in the current experiment. Alternatively, it is also possible that voluntary activation related to visual processing of the go signal does not interact with the involuntary activation resulting from the SAS in the same manner as when both stimuli are auditory in nature.

To summarize, the current data provide compelling evidence that voluntary and startle-related initiation activation jointly contribute to the observed response latencies when a SAS is delivered after the "go" signal. We argue that a model in which voluntary response activation is additive at a cortical level with the involuntary activation provided by the SAS explains the observed short latency responses. This indirectly supports the hypothesis that the SAS acts as a faster and involuntary activation trigger for a common response initiation structure [1], as an additive model would be most consistent with initiation processes that ultimately occur through a similar corticospinal pathway, rather that the SAS triggering responses stored in subcortical structures through a reticulospinal pathway [12].

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